Complement activation following oxidative stress

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1. Introduction

The endothelium plays an integral role in the maintenance of vascular homeostasis. However, oxidative stress associated with reperfusion of ischemic tissues activates complement, resulting in a loss of vascular homeostasis and a pro-inflammatory state. Complement activation following ischemia-reperfusion (I/R) is associated with myocardial infarction (Hill and Ward, 1971), atherosclerosis (Vlaicu et al., 1985), intestinal ischemia (Fruchterman et al., 1998), hemorrhagic shock (Spain et al., 1999), sepsis (Lubbe et al., 1994) and pulmonary injury (Mulligan et al., 1996). However, the mechanisms of complement activation following oxidative stress have not been fully elucidated.

In this article we review the current knowledge regarding: (1) the organization and regulation of the complement system; (2) the role of complement depletion or inhibition in attenuating tissue injury; and (3) the mechanisms of complement activation following oxidative stress.

2. Organization of the complement system

Human complement is a cytotoxic defense system involved in the elimination of invading foreign cells and initiation of inflammation. The complement system is subdivided into three pathways: the classical, alternative and more recently described lectin complement pathway (LCP) (Fig. 1). Activation of these pathways occurs sequentially through the proteolytic cleavage and association of precursor molecules. Classical complement pathway activation occurs when antibody/antigen complexes interact with the first complement component, C1, leading to the generation of C1q. C1q can then bind to the Fc portion of complexed immunoglobulins resulting in activation of the C1r and C1s esterase sub-components of C1, and ultimately the formation of a C3 convertase. The alternative complement pathway is an antibody-independent pathway activated by a variety of mechanisms including yeast cell walls (zymosan), biomaterials (i.e., cardiopulmonary bypass and hemodialysis tubing), and tissue type plasminogen activator. The LCP is also an antibody-independent pathway activated by binding of mannose-binding lectin (also known as mannan/mannose binding protein; MBL) to carbohydrate structures present on the surface of bacteria, yeast, parasitic protozoa, and viruses (Turner, 1997). Associated with MBL are two serine proteases, MASP-1 and MASP-2, which cleave C2 and C4 to form the classical complement pathway C3 convertase (Thiel et al., 1997). MBL is structurally related to C1q, while MASP-1 and MASP-2 exhibit remarkable homology to the C1q-associated serine proteases, C1r and C1s (Ji et al., 1997). All three pathways merge at C3, where it is cleaved into C3a and C3b, and C5 is subsequently cleaved to form C5a and C5b. Addition of C6, C7, C8 and multiple C9 units to C5b results in formation of the terminal complement complex, C5b-9.
3. Regulation of the complement system

Complement activation in vivo is tightly regulated by both plasma and membrane-bound regulators. Plasma complement regulators play a major role in inhibition of excessive complement activation, while membrane-bound regulators protect cells against complement-mediated injury and participate in immune-complex clearance. Complement receptor type 1 (CR1; CD35) and membrane cofactor protein (MCP; CD46) are integral membrane proteins, while decay type accelerating factor (DAF; CD55) and protectin (CD59) are bound to the cell membrane phospholipid domain by a glycophosphatidylinositol anchor. CR1 plays a major role in opsonization and immune-complex clearance and along with MCP, acts as a co-factor for Factor I mediated cleavage of C3b. DAF accelerates the decay of the alternative complement pathway C3 convertase via dissociation of Bb. Thus, DAF, CR1 and MCP inhibit complement activation at the level of C3. Protectin is a 20 kDa glycoprotein that interacts with both C8 and C9 during C5b-9 assembly to limit C9 insertion into the cell membrane (Morgan and Meri, 1994; Meri, 1994).

Fluid-phase complement regulators include C1-inhibitor (C1 INH), C4-binding protein, properdin and Factors H and I. C1 INH prevents excessive amplification of the classical complement pathway by covalently complexing C1s and C1r, while still allowing free C1q to interact with its receptors (Ziccardi, 1983). The classical complement pathway is also regulated by C4-binding protein, which binds the C3 convertase, C4b2a, and accelerates its decay by dissociating C2a (Scharfstein et al., 1978). Factor I is a serine protease which regulates the classical and alternative complement pathway C3/C5 convertases by inactivating C4b and C3b by cleaving the C3b and C4b α-chains (Pangburn et al., 1977). Finally, Factor H accelerates the decay of the alternative complement pathway C3 convertase. Interestingly, properdin, which has historically been thought to stabilize the C3 convertase, has recently been demonstrated to promote Factor B binding to C3b and increase C3 convertase activity (Jelezarova et al., 1999). Thus, complement activation is regulated by multiple control mechanisms.

4. Pro-inflammatory vascular effects of complement

Complement activation results in the formation of several pro-inflammatory mediators that may alter vascular homeostasis. Biologically active complement components include C3a, C5a, iC3b and C5b-9. In addition to stimulating white blood cell activation and chemotaxis, C5a may further amplify the inflammatory response by inducing production of macrophage inflammatory protein (MIP)-2, cytokine-induced neutrophil chemoattractant (CINC), MIP-1α, MIP-1β, monocyte chemoattractant protein (MCP)-1, tumor necrosis factor (TNF), interleukin (IL)-1 and IL-6 (Czermak et al., 1999).

C5b-9 and iC3b may also alter vascular homeostasis. iC3b is formed following C3b cleavage and is a specific ligand for leukocyte adhesion to the vascular endothelium via CD18/CD11b (Marks et al., 1989). Along these same lines, C5b-9 may activate endothelial nuclear factor (NF)-κB to increase leukocyte adhesion molecule transcription and expression (Kilgore et al., 1997). Endothelial leukocyte adhesion molecules influenced by complement include vascular cell adhesion molecule (VCAM)-1 (Tedesco et al., 1997), intercellular adhesion molecule (ICAM)-1, E-selectin (Kilgore et al., 1995) and P-selectin (Foreman et al., 1994). C5b-9 may also promote leukocyte activation and chemotaxis by inducing endothelial IL-8 and MCP-1 (Kilgore et al., 1996) secretion. Finally, C5b-9 may alter vascular tone by inhibiting endothelium-dependent relaxation (Stahl et al., 1995) and decreasing endothelial cyclic guanosine monophosphate (Collard et al., 1999a). Thus, complement may alter vascular homeostasis and
blood flow by inhibiting endothelium-dependent relaxation, and stimulating leukocyte activation and adhesion.

5. Myocardial ischemia-reperfusion injury: role of complement

I/R injury is a complex phenomenon involving multiple cell types and bioactive molecules. Despite more than two decades of study, the role of complement in I/R injury is not fully understood. Initial observations by Hill and Ward in a rat model of permanent coronary artery occlusion demonstrated that the ischemic heart produced a protease that cleaved C3 and stimulated leukocyte activation and chemotaxis (Hill and Ward, 1971). Other investigators have shown in human and animal experimental models of myocardial ischemia/infarction that C1q (Rossen et al., 1985), C3 (Pinckard et al., 1980), C4, C5 (Crawford et al., 1988) and C5b-9 (Schafer et al., 1986) deposit within the myocardium. Additionally, the human heart contains mRNA for all of the complement components, and myocardial ischemia/infarction increases myocardial complement mRNA and protein expression (Yasojima et al., 1998). Thus, local complement protein production may also contribute to myocardial ischemic injury. Together, these studies demonstrate that myocardial I/R activates and deposits complement which, in addition to causing direct tissue injury and cellular activation, stimulates leukocyte activation and chemotaxis (Dreyer et al., 1992).

6. Ischemia-reperfusion injury: role of anti-complement therapy

Excessive complement activation resulting in organ injury is associated with a variety of human pathological conditions. Thus, a number of anti-complement therapeutic strategies are currently in pre-clinical or clinical development (Persidis, 1998). The effectiveness of complement inhibition or depletion in preventing tissue injury following oxidative stress has been established in many animal models.

6.1. Complement depletion using cobra venom factor

Numerous studies have used cobra venom factor (CVF) to study the role of complement in myocardial infarction. CVF activates the alternative complement pathway leading to consumption of C3 and the terminal complement components (C5–C9). In a rat model of myocardial infarction, Hill and Ward (1971) demonstrated that decompensation with CVF prior to ischemia markedly reduced serum C3 levels and tissue chemotactic activity following ischemia. Further, leukocyte infiltration into the infarcted areas was significantly reduced despite the absence of systemic leukopenia. CVF administration has also been shown to have long-term protective effects as demonstrated by reduced myocardial infarct size following 21 days of ligation in dogs (Maroko et al., 1978). A similar reduction in myocardial creatine kinase depletion and C3 deposition after CVF-mediated complement depletion has been observed in a baboon model of myocardial infarction (Pinckard et al., 1980). Interestingly, CVF decreases myocardial infarct size, leukocyte infiltration and complement deposition even if CVF is administered after the induction of ischemia (Crawford et al., 1988). CVF has also been shown to reduce complement-mediated tissue injury in experimental models of cerebral ischemia (Vasthare et al., 1998), shock (Ikai et al., 1996) and xenotransplantation (Candinas et al., 1996). Thus these studies demonstrate that complement depletion attenuates I/R injury.

6.2. C1 esterase inhibition

C1 INH inhibits classical complement pathway activation. Lefer and colleagues in a feline model of myocardial I/R demonstrated that C1 INH administration prior to reperfusion significantly decreased myocardial necrosis and myeloperoxidase (MPO) activity, while preserving myocardial contractility and coronary endothelial function (Buerke et al., 1995). These findings were later confirmed in a rat model of myocardial I/R (Murohara et al., 1995). Additionally, C1 INH administration in rats decreased P-selectin and ICAM-1 expression following myocardial I/R (Buerke et al., 1998). Treatment with C1 INH has also been shown in a porcine model of myocardial I/R to decrease serum troponin-T levels and preserve regional myocardial contractility (Horstick et al., 1997). Thus, classical complement pathway inhibition following I/R attenuates tissue injury and preserves myocardial function.

6.3. Classical and alternative complement pathway convertase inhibition

Weisman et al. (1990) demonstrated in a rat model of myocardial I/R that recombinant soluble CR1 (sCR1) administration reduced myocardial infarction, C5b-9 deposition, and leukocyte infiltration. Additionally, sCR1 has been shown to reduce tissue injury in other experimental models of myocardial I/R (Lazar et al., 1998), skeletal muscle ischemia (Pemberton et al., 1993) and hyperacute rejection (Gralinski et al., 1996). Although these studies effectively demonstrate that sCR1 reduces tissue injury, they do not delineate the relative contribution of early
(C3a, C3b) vs late (C5a, C5b-9) complement components to organ injury.

6.4. C5a Inhibition

C5 and its cleavage product, the anaphylatoxin C5a, have been extensively studied with regard to their role in I/R injury. Amsterdam and colleagues demonstrated a significant reduction in myocardial infarct size in pigs treated with monoclonal anti-porcine C5a antibody (Amsterdam et al., 1995). Similarly, porcine C5a inhibition has been shown to decrease impairment of endothelium-dependent vasorelaxation and myocardial MPO activity following CPB and cardioplegia-induced myocardial arrest (Tofukuji et al., 1998). Treatment with anti-rat C5a antibody in a rat model of hindlimb I/R decreased lung vascular permeability, MPO, and cytokine-induced neutrophil chemoattractant (Bless et al., 1999). Further, C5a inhibition in a porcine model of septic shock has been shown to improve tissue oxygen extraction and attenuate IL-6 and lactate levels (Hopken et al., 1996). Thus, inhibition of the anaphylatoxin, C5a, reduces organ injury following I/R.

6.5. C5 Inhibition

In recent years, a number of animal and human studies have been conducted using monoclonal anti-C5 antibodies to inhibit C5 cleavage, thereby blocking C5a and C5b-9 formation. C5 inhibition was recently shown to significantly decrease myocardial infarct size, apoptosis and leukocyte infiltration in a rat model of I/R (Vakeva et al., 1998). Additionally, monoclonal anti-human C5 therapy has been used to inhibit C5a and C5b-9 generation, serum complement hemolytic activity, neutrophil CD11b upregulation, and platelet P-selectin expression in an extracorporeal circulation model (Rinder et al., 1995). A humanized, recombinant, single chain antibody specific for C5 (h5G1.1-scFv) was recently found to significantly reduce soluble C5b-9 (sC5b-9) formation, leukocyte CD11b expression, postoperative myocardial injury, cognitive defects, and blood loss in patients undergoing cardiopulmonary bypass (Fitch et al., 1999). Anti-human C5 therapy has also been shown to reduce the incidence of myocardial injury in an ex vivo model of cardiac xenograft rejection (Kroshus et al., 1995). Thus, inhibition of terminal complement activation significantly reduces complement-mediated tissue injury following oxidative stress.

6.6. Depletion or ‘knock out’ of specific complement components

Congenital or genetically-engineered deficiencies of specific complement components have also been used to study the role of complement in various disease states. Weiser et al. (1996) demonstrated in a murine model of hindlimb I/R that vascular injury was significantly decreased in C3 and C4 knock out mice, suggesting that classical and/or lectin complement pathway inhibition reduces tissue injury. Further, C6-deficient rabbits have been shown to be protected against myocardial I/R injury (Kilgore et al., 1998) and to have a decreased incidence of fatal arrhythmias and capillary plugging (no-reflow) (Ito et al., 1996). Thus, these data further suggest a role for complement, especially the terminal complement components, in mediating reperfusion injury.

7. Mechanisms of complement activation following oxidative stress

Although I/R is known to activate complement, the mechanisms of complement activation at the endothelial surface are not fully known. The location of the endothelium at the blood/tissue interface makes it a potential target for oxidants produced by the endothelial cells themselves (Zweier et al., 1988) or by leukocytes recruited by the inflammatory response (Ward, 1991). Reactive oxygen species, such as hydrogen peroxide (H$_2$O$_2$), have been shown to directly activate C5 via a nonenzymatic mechanism (Vogt et al., 1989). Further, inhibition of reactive oxygen species formation decreases complement activation and deposition (Kilgore et al., 1994; Collard et al., 1998). Thus, one mechanism of complement activation following I/R may involve the formation of reactive oxygen species.

A second possible mechanism of complement activation following I/R involves activation of the classical complement pathway by natural antibody binding to a neo-epitope. Support for this hypothesis comes in part from studies using mice deficient in C3, C4 or serum immunoglobulin (Williams et al., 1999). Immunoglobulin-deficient mice were protected against vascular injury following hindlimb ischemia. Reconstitution of immunoglobulin reversed this protective effect suggesting antibody-dependent activation of the classical complement pathway (Williams et al., 1999). These data support the role of a neo-epitope induced by I/R that activates complement via the classical complement pathway.

7.1. Possible role of the LCP

In recent years, antibody-independent activation of the classical complement pathway at the level of C2 and C4 via the LCP has been described following binding of MBL to cell surface carbohydrates (Turner, 1996). MBL is a calcium-dependent C-type mamma-
liant lectin involved in innate immunity and is specific for mannose and N-acetylglucosamine (GlcNAc). Human MBL circulates in the blood (1–2 μg/ml plasma) as large homo-oligomers (200–650 kDa) consisting of 9–18 32 kDa monomers. Each monomer consists of four distinct domains: (1) a NH2-terminal region, rich in cysteine residues that form disulfide bonds to stabilize the oligomers; (2) a collagen-like domain composed of 18–20 tandem repeats of a Gly-X-Y sequence similar in overall structure to C1q; (3) a neck region; and (4) a COOH-terminal carbohydrate recognition domain that recognizes and binds to mannose and GlcNAc residues (Kozutsumi et al., 1980). Human MBL is encoded by a single gene on the long arm of chromosome 10 and exists in a single form (Sastry et al., 1989). MBL gene transcription is augmented by IL-6, dexamethasone and heat shock and is down regulated by IL-1 (Arai et al., 1993).

Associated with MBL are two novel C1r2C1s2-like serine proteases, MASP-1 and MASP-2 (Thiel et al., 1997), that cleave C2 and C4 to form the classical C3 convertase following MBL binding to carbohydrate ligands. Thus, unlike the classical complement pathway, activation of the LCP does not require antibody, C1, or C1q deposition. Further, the contribution of MBL and the LCP following I/R is unclear as C1 INH also inhibits MASP-1 and MASP-2 (Wong et al., 1998), and activation of the LCP is also inhibited in C4-deficient animals.

We have previously shown that reoxygenation of hy-
poxic human endothelial cells activates complement via a C2-dependent pathway requiring new protein synthesis (Collard et al., 1997, 1998). Although MBL does not normally recognize the body’s own tissues (Malhotra et al., 1995), cellular hypoxia and oxygen-derived free radical formation may alter endothelial surface protein expression (Ogawa et al., 1991) and glycosylation (Weinhouse et al., 1993). Thus, oxidative stress may alter cell surface membrane glycosylation leading to increased MBL deposition and activation of the LCP. To test this hypothesis, we designed functionally inhibitory monoclonal antibodies (mAbs) against human MBL to demonstrate that oxidative stress of human endothelial cells results in antibody-independent (i.e., classical complement pathway-independent) activation of the LCP (Collard et al., 1999b).

Immunofluorescent confocal microscopy was performed on human umbilical vein endothelial cells (HUVECs) subjected to 0 or 24 h of hypoxia (1% O₂) and then reoxygenated (21% O₂) for 3 h in the presence of 30% human sera or 30% human sera treated with anti-human MBL mAbs 3F8 (5 μg/ml) or 1C10 (50 μg/ml). MBL (blue) and C3 (green) staining were significantly increased on hypoxic/reoxygenated HUVECs compared to normoxic cells (Fig. 2). Additionally, MBL and C3 co-localized (white) on the endothelial cells. Treatment of human sera with mAb 3F8, but not 1C10 (a non-functionally inhibitory anti-human MBL control mAb), significantly attenuated MBL and C3 staining following oxidative stress (Fig. 2). Thus, these data demonstrate that endothelial oxidative stress leads to MBL deposition and activation of the LCP. Further, these data suggest that anti-MBL therapy may represent a novel therapeutic strategy for preventing complement activation following oxidative stress in humans.

8. Summary

It is clear that complement plays an important role in the inflammatory process following oxidative stress in cellular and animal models. Clinical trials underway with novel complement inhibitors will establish the potential therapeutic benefit of complement inhibition in human disease. For as much as we understand about the role of complement in disease states, many questions remain. How is complement activated on endothelial cells following oxidative stress? What is the ligand for MBL on endothelial cells following oxidative stress? Will inhibition of MBL provide tissue protection to the extent observed with other complement inhibitors such as sCR1 or anti-C5 mAbs? These questions and more will undoubtedly be answered in the next millennium.

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