Molecular Medicine 7(6): 367–382, 2001

Review Article

The Role of the Complement Cascade in Ischemia/Reperfusion Injury: Implications for Neuroprotection

Anthony L. D'Ambrosio, David J. Pinsky, and E. Sander Connolly

Columbia University Department of Neurological Surgery, The Neurological Institute, New York, New York, USA

Accepted March 20, 2001

Abstract

Background: The complement cascade plays a deleterious role in multiple models of ischemia/reperfusion (I/R) injury, including stroke. Investigation of the complement cascade may provide a critical approach to identifying neuroprotective strategies that can be effective at clinically relevant time points in cerebral ischemia. This review of the literature describes the deleterious effects of complement activation in systemic I/R models and previous attempts at therapeutic complement inhibition, with a focus on the potential role of complement inhibition in ischemic neuroprotection. Translation of these concepts into ischemic stroke models and exploration of related neuroprotective strategies are also reviewed.

Summary of Review: We performed a MEDLINE search to identify any studies published between 1966 and 2001 dealing with complement activation in the setting of I/R injury. We also searched for studies demonstrating up-regulation of any complement components within the central nervous system during inflammation and/or ischemia.

Conclusions: The temporal and mechanistic overlap of the complement cascade with other biochemical events occurring in cerebral I/R injury is quite complex and is only beginning to be understood. However, there is compelling evidence that complement is quite active in the setting of acute stroke, suggesting that anticomplement strategies should be further investigated through genetic analysis, nonhuman primate models, and clinical investigations.

Introduction

In an attempt to further develop our understanding of neuronal injury in cerebral ischemia, researchers have focused their efforts on investigating the pathophysiologic changes of leukocytes, cytokines, endothelial cells, adhesion molecules, nitric oxide, and cyclooxygenase as important contributing factors in ischemic brain injury (1). However, very little attention has been paid to one of the major elements of the inflammatory response, the complement cascade. The complement system is a component of the immune response comprised of multiple cascades that play an integrated role in the initiation and regulation of the inflammatory response. Furthermore, the complement cascade has been shown to play a critical role in a whole host of systemic organ ischemia/reperfusion (I/R)[1] models of tissue damage (2–8), and is believed to have deleterious effects in cerebral I/R injury. Of great interest is the fact that in these models, the mechanism of tissue injury appears to be predominantly vascular in origin.

On a macroscopic level, ischemic cerebral infarction is characterized by occlusion of a major artery after a thromboembolic event (Fig. 1). Cerebral blood flow changes in focal ischemia are dynamic. In theory, reduction of blood flow is extensive just after vessel occlusion. Collateral circulation may soon develop, leaving a core of severe ischemia and a peripheral area with a gradual decline of blood flow (penumbra) (9). Recanalization of the occluded artery often occurs spontaneously, or can be accomplished surgically or pharmacologically, thereby creating an environment of ischemia followed by reperfusion.

On a microscopic level, this vascular occlusion/reperfusion event causes damage to downstream neurons, glial cells, and endothelium resulting in

Address correspondence and reprint requests to: E. Sander Connolly, Jr, MD, Columbia University Department of Neurological Surgery, Neurological Institute Room 431, 710 West 168th Street, New York, NY 10032. Phone: (212) 305-0376; Fax: (212) 305-5544; E-mail:esc5@Columbia.edu

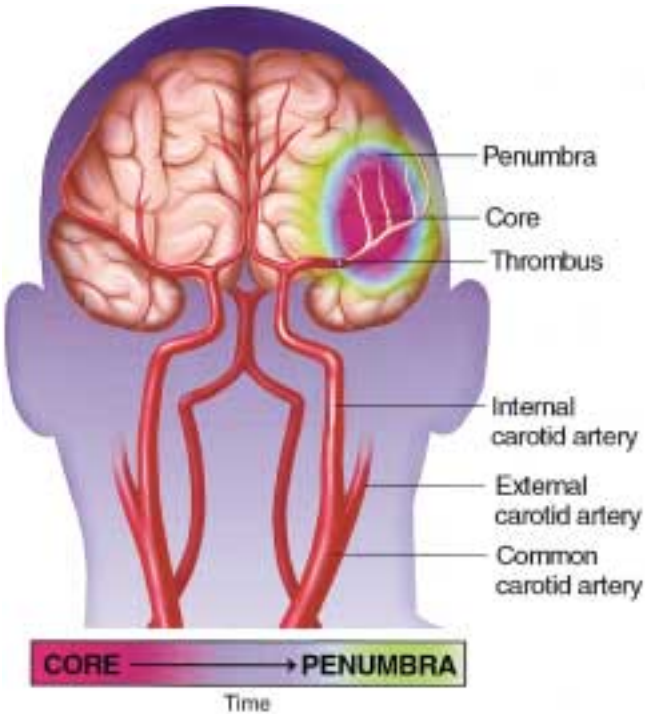

Fig. 1. Schematic of cerebral infarct. Ischemic cerebral infarction is initiated by a thrombus lodged in the middle cerebral artery. This results in a central core of severely ischemic tissue surrounded by a poorly perfused area of tissue ischemia known as the penumbra.

pan necrosis (9). This damage is a direct result of a complex overlapping of cascades leading to progressive cerebral injury. These inflammatory cascades are especially important in the setting of reperfused stroke where the mechanism of injury appears to be related to exacerbation of microvascular failure and release of reactive oxygen species (1). This inflammatory up-regulation occurs at clinically relevant time points in several I/R models, anywhere from minutes to days after the ischemic event. Clinical studies have shown that a large portion of patients reperfuse at least part of their ischemic stroke; therefore, investigation of the inflammatory response is critical in our attempts to develop neuroprotective strategies that can be effective at clinically relevant time points.

Stroke remains the third leading cause of death in the United States despite a decline in incidence over the last three decades. Unfortunately, there has been little progress in designing safe and effective treatment regimens in the management of acute stroke. Through extensive research efforts of multiple teams, our understanding of the pathophysiology of acute cerebral ischemia and subsequent neuronal cell death continues to grow. Investigators are continually testing novel neuroprotective strategies targeting multiple cellular cascades based on this newly acquired knowledge of the complicated mechanisms leading to the devastating outcomes seen clinically in stroke.

This review is focused on the complement cascade and its deleterious effects in the setting of acute cerebral I/R injury. In the setting of cerebral ischemia, a necrotic core is formed, which is surrounded by an apoptotic penumbral region (Fig. 1). In studying cerebral I/R injury, a distinction between these two regions is important, because molecular events are likely to differ between these regions. For the purpose of this review, when referenced studies specified this distinction, the terms "core" and "penumbra" will be used. When these regions were not specified, the terms "ischemic region" and "ischemic area" will be utilized. To discuss current neuroprotective strategies in cerebral ischemia, we review the deleterious effects of complement activation in I/R models of other organ systems and previous strategies aimed at attenuating tissue damage secondary to complement activation. Translation of these concepts into ischemic stroke models and exploration of novel neuroprotective strategies are also reviewed.

Inflammation and Stroke

Cerebral I/R brain injury evokes a profound up-regulation of the inflammatory response, which is initiated within the cerebral microvasculature (1). Within minutes of the vascular occlusion, multiple cascades of inflammatory events are initiated. These cascades include infiltration and accumulation of neutrophils and macrophages (10–13); platelet accumulation (1,13–15); adhesion molecule up-regulation, such as ICAM-1, P-selectin, and E-selectin (14,16–21); blood-brain barrier destruction (1,22,23); cytokine production including IL-1, IL-8, tumor necrosis factor-alpha (TNF-α), and platelet activating factor (PAF) (14,24–28); increased nitric oxide levels (1,21,29–34); and cyclooxygenase up-regulation (1,35–40).

Several temporal relationships have already been established regarding the activation of these cascades. For example, after reversible middle cerebral artery occlusion (MCAO) in the rat, P-selectin expression is seen on vascular endothelium within 0–90 minutes, ICAM-1 is present at 2–4 hours, and E-selectin is expressed at 7–24 hours (1). A similar time course is observed in mice (41,42). Also, the temporal up-regulation and expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) after cerebral I/R injury has been determined (1). Expression of these enzymes has been demonstrated to be up-regulated anywhere from 6 hours to 2 days after I/R injury (1).

One commonly held view is that reperfusion, following occlusion of a cerebral artery, increases cerebral injury (1,23). After the initial ischemic event, reperfusion of a major feeding artery following transient obstruction is accompanied by downstream microvascular perfusion defects as demonstrated in several models of I/R injury (11,43). One possible theory for neuronal injury following reperfusion is that microvascular reactivity in response to oxidative

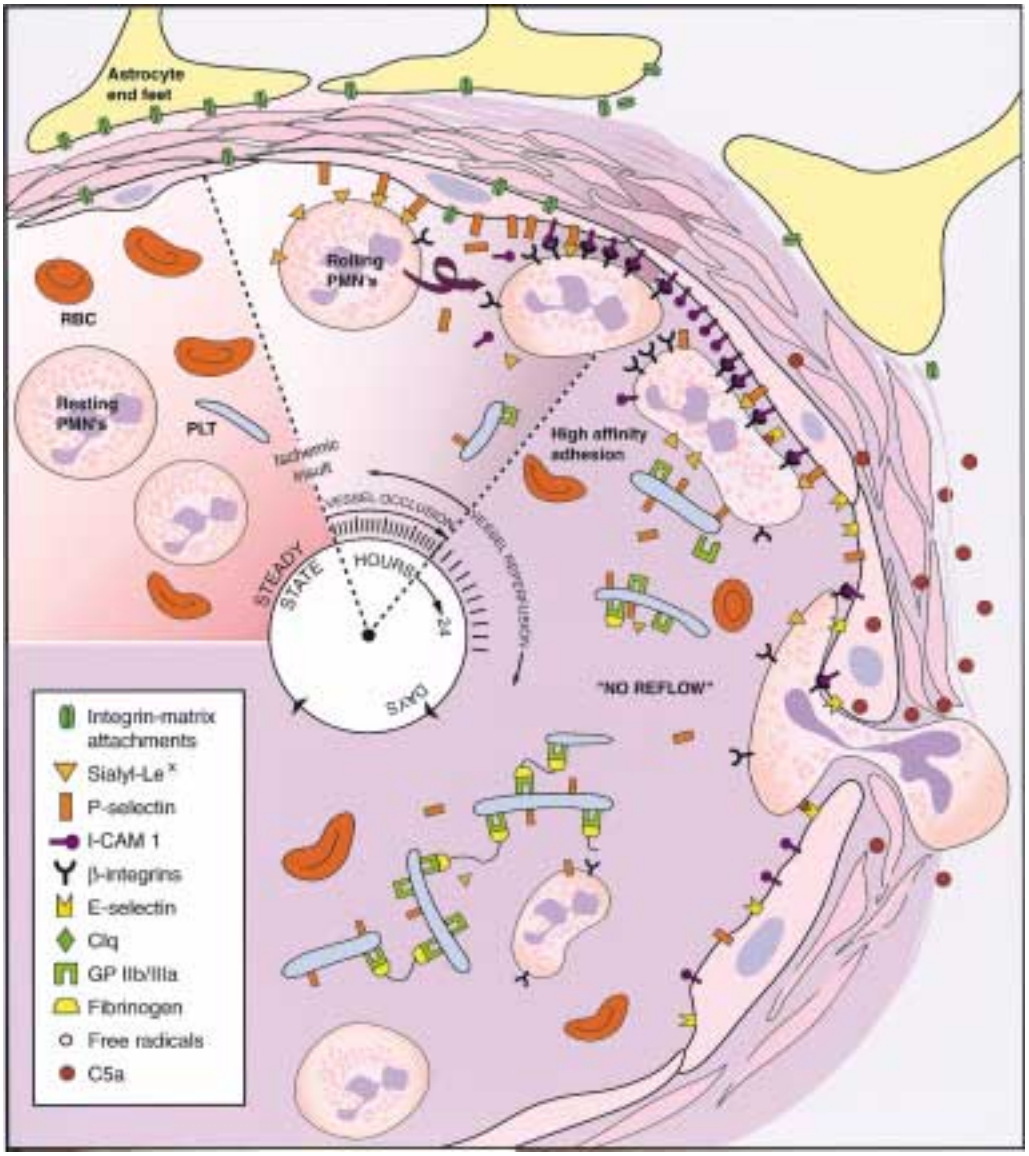

Fig. 2. Events occuring over time in the cerebral microvasculature and ischemic brain parenchyma in the setting of ischemic stroke. The temporal sequence of events proceeds in a clockwise rotation. In the *steady state,* blood components pass by the vascular endothelium with laminar flow and the PMNs are in a resting state. At the time of an *ischemic insult,* the cerebral microvasculature becomes occluded, and adhesion molecule expression is up-regulated on both the PMNs and the vascular endothelium. Within minutes to hours, PMNs begin to "roll" on the vascular endothelium. This process is dependent on the interaction of sialyl Le^x with P-selectin. Early changes in the cerebral microvessels include the loss of integrin-matrix attachments of endothelial cells and astrocyte end-feet that accompany loss of the basal lamina. Within several hours, high-affinity adhesion of rolling PMNs to the endothelium involves ICAM-1 binding to beta-integrin counterreceptors. Microvascular failure is exacerbated by platelet accumulation, activation, and GP IIb/IIIa upregulation. This leads to platelet crosslinking through fibrinogen and a *"no-reflow"* state within the microvasculature. E-selectin expression on the endothelium is up-regulated and activated PMN transmigration into the ischemic neurophil occurs through the pourous microvasculature. Within the ischemic parenchyma, the complement cascade is activated and the production of anaphylotoxins such as C5a further exacerbates PMN activating and accumulation. Activated neutrophils secrete cytotoxic factors such as inflammatory mediators (IL-8, LTB4), proteases, and free radicals into the neurophil. The neuronal response includes decreased energy production, increased expression of C1q on its surface, increases in intracellular Na^1, Cl^2, and $Ca^{1\ 1}$.

stress is both dynamic and rapid, and that a number of processes are initiated in the microvasculature early that are not recovered during reperfusion.

In response to the onset of inflammatory mediator up-regulation and oxidative stress, cerebral microvessels demonstrate increased permeability of the endothelial cell component of the blood–brain barrier, adhesion of polymorphonuclear leukocytes (PMNs) and platelets to the vascular endothelium, and loss of integrin-matrix attachments of endothelial cells and astrocytes that accompany loss of the basal lamina (1,44). These changes occur within minutes of proximal vessel occlusion and are not reversible with reperfusion (Fig. 2).

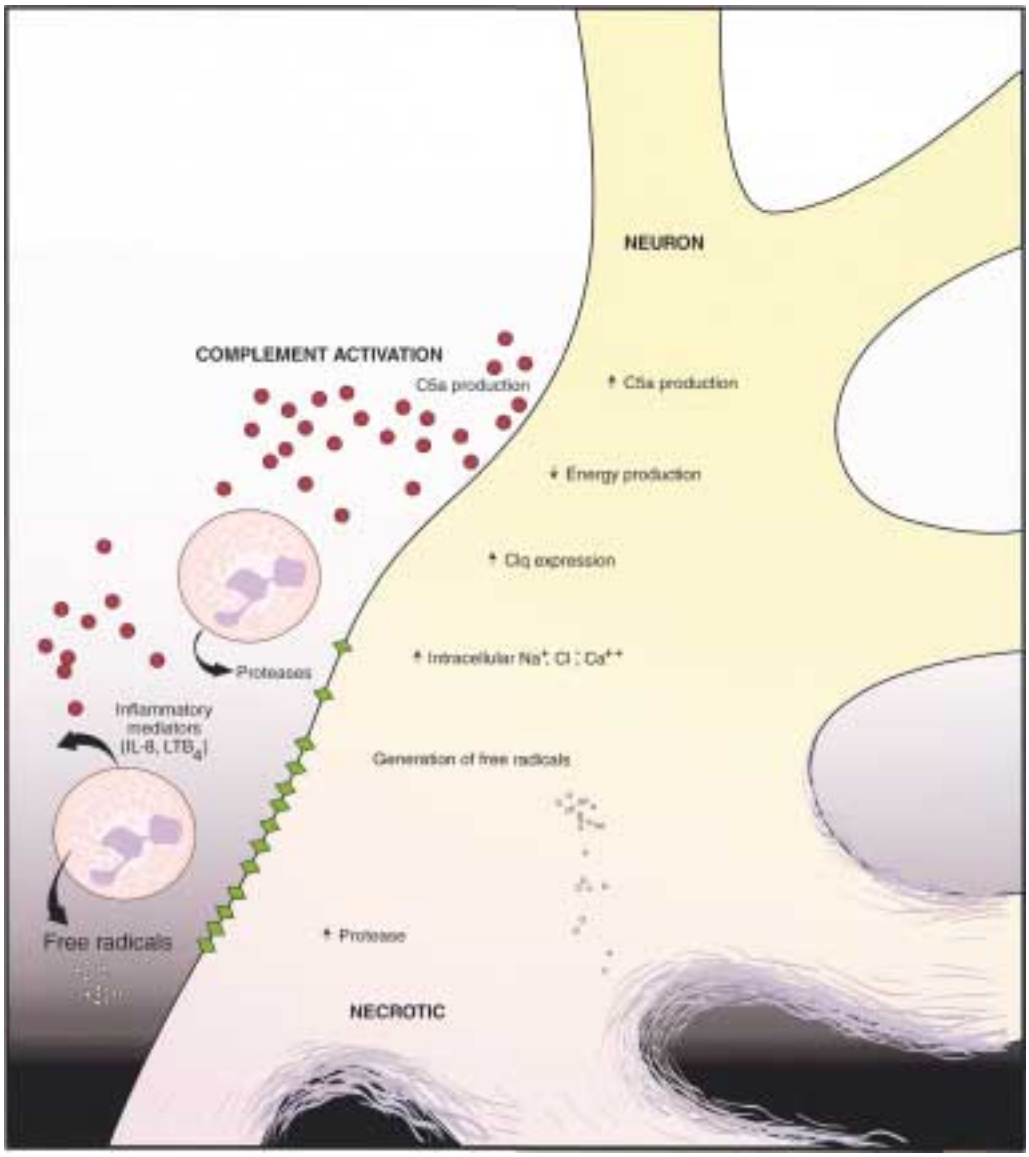

Fig. 2. (*Continued*)

Neutrophil activation and accumulation in the cerebral microvasculature play a critical role in neuronal injury in both permanent and reperfused cerebral ischemia (13,23,45–47); however, reperfusion greatly increases leukocyte accumulation (48). Experimental studies suggest that neutrophil activation and accumulation occurs within the first hours of central nervous system (CNS) I/R injury (24,49). Accumulation of neutrophils, together with red blood cells, fibrin deposition and platelets, can lead to capillary plugging and a reduction of microvascular blood flow, resulting in focal areas of stasis, and the "no-reflow" phenomenon (11,14,23,49,50).

As neutrophils accumulate and adhesion molecule expression is up-regulated, PMNs begin to extravasate into the brain parenchyma. Using a model of both transient (2 hours) and permanent MCAO in the rat, Zhang et al. (51) demonstrated an influx of neutrophils into ischemic brain parenchyma which peaked at 6–24 hours and 72 hours, respectively. The early influx of neutrophils in reperfused MCAO has been noted to occur during rapid expansion of the ischemic region. Zhang et al. state that the abrupt increase in the size of the ischemic lesion may be suggestive of the exacerbation of ischemic cell damage resulting from neutrophil influx (51). The detrimental effects of neutrophil influx has been further demonstrated by the significant neuroprotection observed after inhibition of neutrophil and cytokine actions (52). Figure 2 illustrates the proposed temporal and spatial relationships of PMNs, endothelial cells, platelets, cytokines, and adhesion molecules in focal cerebral I/R injury.

One very important cascade involved in the up-regulation of the inflammatory response and PMN activation and accumulation, and has received comparatively little attention, is the complement cascade. Nonetheless, there is compelling in vitro and in vivo evidence demonstrating that the complement cascade plays a vital and specific role in adhesion molecule up-regulation, PMN activation, chemotaxis, and accumulation (2,53–57), as well as platelet activation (55) in the setting of acute inflammation. Furthermore, there is evidence that the complement component, C5a, induces endothelial cell production of reactive oxygen species (ROS) (54). Using human umbilical vein endothelial cells (HUVEC), Kilgore et al. (58) demonstrated the ability of the terminal component of the complement cascade, the membrane attack complex (MAC), to induce endothelial cell expression of the cytokines IL-8 and monocyte chemoattractant protein-1 (MCP-1).

The complement cascade offers multiple opportunities for targeted blockade strategies that could prove very effective in inhibiting neuronal damage in stroke. In light of this possibility, it is imperative that investigators recognize the potential role of complement as a key cascade in the inflammatory response. To approach this endeavor in a systematic and logical manner, this article will review the basic mechanisms of the intact complement cascade, its different initiators, and its common components.

Complement Overview

The complement system was discovered many years ago as a heat labile component of normal plasma comprised of a set of proteins that work to eliminate microorganisms and other antigens from the body (59,60). More than half a century after its discovery, it became clear that complement played a major role in the initiation and control of inflammation in general (61). Previous research has shown that the complement system consists of three activation pathways: a single terminal pathway, regulatory proteins, and complement receptors (59). The effector functions of complement can be activated through three pathways: 1) the classical pathway, 2) the lectin pathway, and 3) the alternative pathway (Fig. 3). Each pathway has a different mechanism for cascade initiation. The classical pathway is commonly activated by antibodies binding to antigen; the lectin pathway is initiated by binding of serum lectin to mannose-containing carbohydrates on bacteria and viruses; and the alternative pathway can be initiated when a spontaneously activated complement component binds to the surface of a pathogen (60).

The complement cascade can be divided into "early" and "late" events (60). The early events consist of a series of proteolytic steps in which an inactive precursor protein is cleaved yielding an active fragment and a small peptide fragment. The active fragment binds to the surface of a pathogen and contributes to the next cleavage in the cascade. The smaller peptide fragment is released from the cell and often mediates inflammatory responses. The early events end with the production of a protease, C3 convertase, which is generated on the surface of a foreign substance. This enzyme cleaves C3 into C3a, a potent anaphylotoxin, and C3b. Multiple C3b molecules bind in clusters around the C3 convertase and act as strong ligands of complement receptor types 1 and 3 (CR1 and CR3) (59). The C3 convertase also generates a C5 convertase beginning the "late" cascade of events by cleaving C5 into C5a, a second potent anaphylotoxin, and C5b (60). The formation of C5b initiates a reaction sequence from the sixth component (C6) through the ninth component (C9), beginning the terminal pathway, that leads to the formation of the MAC complex, which damages the surface membrane of targeted cells.

The complement system itself has five proteins that inhibit the proteolytic enzymes that activate C3 and C5, and thereby, the C3 and C5 convertases of the alternative and classical pathways. These regulatory components are the two plasma proteins, factor H and C4-binding protein (C4-bp), and three membrane proteins, CR1, decay accelerating factor (DAF), and membrane cofactor protein (MCP) (62). Regulatory proteins of the complement cascade may be classified into three categories (59): 1) Inhibitors that prevent spontaneous or abortive activation in the fluid phase, 2) regulators that dampen or enhance the normal action of complement against target substances, and 3) inhibitors that protect host cells from the destructive action of complement. Although this classification system is fairly clear, some regulatory proteins do not fit neatly into one category. For example, a major substrate of CR1 is C3b bound to targets. Because CR1, in addition to acting as a receptor, inactivates C3/C5 convertases and prevents further convertase formation, it belongs to both the receptor category and the regulatory protein category.

Complement receptors function in the recognition of ligands, transduction of signals into cells, and induction of cellular responses, such as phagocytosis (59). Phagocytic cells express CR1, CR3, and C5a receptors (59,63,64). C5a induces margination and chemotaxis of neutrophils (59,63), and up-regulates surface expression of CR1 and CR3 (10,59,65), thus recruiting phagocytic cells and rendering them fully active.

Although the primary site of synthesis of complement components is the liver, numerous extrahepatic sources of complement have been found. Paramount to further discussion is the fact that complement biosynthesis in the CNS is now well established in microglia, astrocytes, and neurons (66). Furthermore, the components of this complement system have been shown to be both complete and functional (66). These data indicate that the complement system is considerably more complex than was

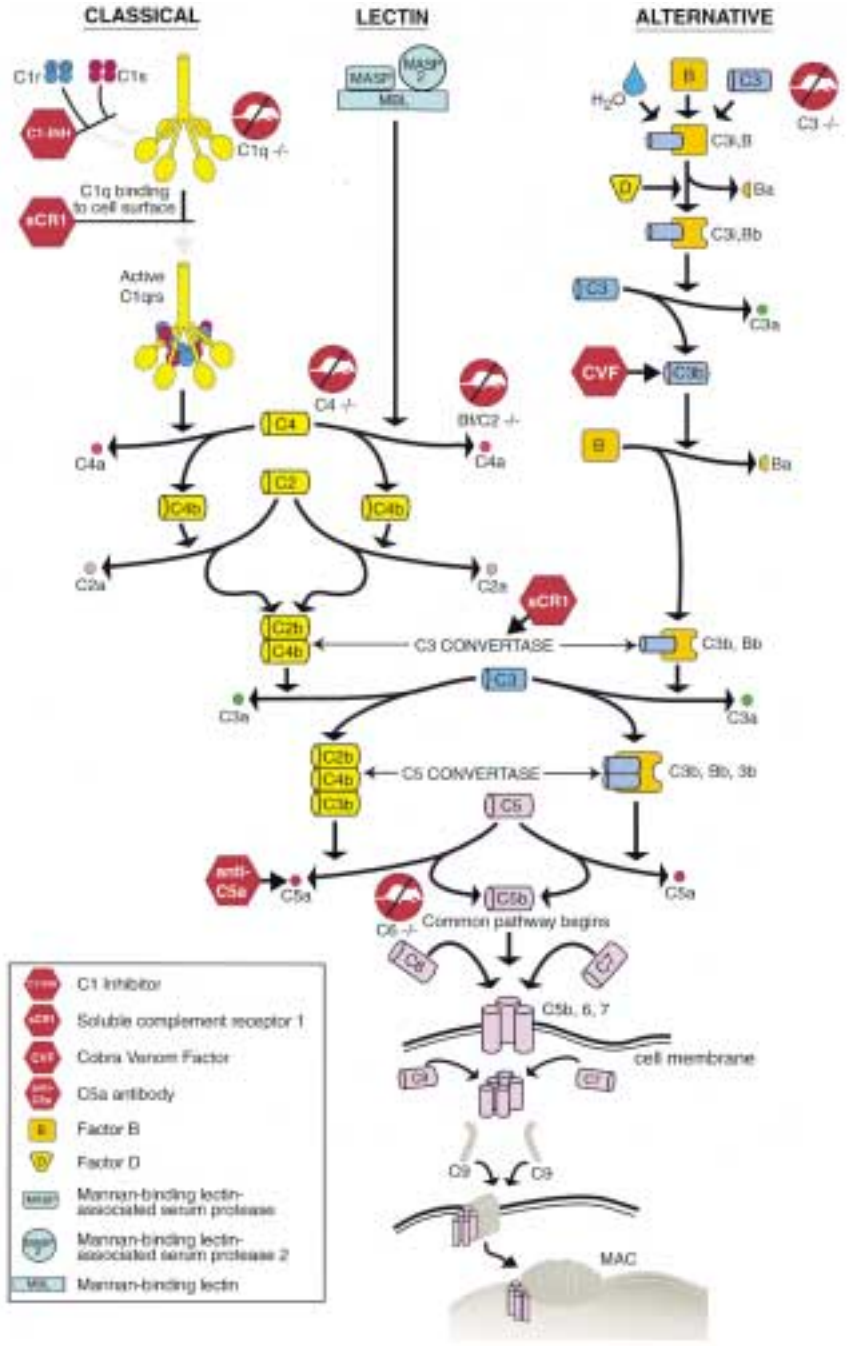

Fig. 3. Schematic of the reaction sequence of the entire complement cascade and current complement blockade strategies. Pharmacologic inhibition strategies are indicated by red stop signs. C1-INH blocks initiation of the classical complement cascade by inactivating the proteases C1r and C1s, thus restricting their effects on C1 binding and activation. Recombinant sCR1 blocks complement activation by both the alternative and classical pathways. CVF is a cobra C3b homologue, a potent specific activator of the alternative pathway of complement. CVF differs from human C3b in that it is not efficiently inactivated and as a result, CVF continues to activate the complement cascade leading to a rapid and prolonged depletion of complement activation potential. Humanized recombinant, single-chain antibody specific for C5 (h5G1.1—scFv) has been shown to reduce soluble C5b-9 formation. Genetically altered mice are indicated with the appropriate genetic deletion (2 /2).

previously envisioned. It is a system of rigorously controlled and highly integrated sets of reactions and interactions (59) present in multiple organ systems. Over the past several decades, researchers have rigorously investigated the role of the complement system in various models of tissue I/R injury.

Ischemia/Reperfusion Injury and Complement: Systemic Models

In 1970, Hill and Ward (8) published a seminal paper on the role of complement in ischemia. They postulated that, because complement played a role in leukotaxis (67,68) and produced anaphylotoxins (69), it represented a possible target for inhibition of the two primary features of the acute inflammatory response: accumulation of neutrophils and increased vascular permeability. In a model of myocardial ischemia in the rat, Hill and Ward demonstrated that complement depletion stopped the chemotactic activity of ischemic tissue and blocked neutrophil accumulation. This was a significant finding because complement had traditionally been studied based only on its role in immune hemolysis (70–73).

The implication of the complement cascade in acute inflammation sparked a long series of investigations into the role of complement in I/R injuries, of which the most thoroughly studied model system has been ischemic myocardium. Following Hill and Ward, Pinckard et al. (74) demonstrated the consumption of classical complement components in patients after acute myocardial infarction. A series of studies then localized complement components to myocardial tissue following regional ischemia and I/R injury when compared to tissue that was not subjected to ischemic stress (75–79). These findings demonstrate that complement activation exacerbates myocardial I/R injuries; however, they could not establish a clear mechanism for complement-induced tissue injury.

Several different animal models have been developed over the years to investigate the possible mechanisms of injury as a result of complement activation in acute ischemic states with subsequent tissue reperfusion. Complement has been shown to indirectly cause tissue damage in I/R by the generation of the anaphylotoxins C3a and C5a and subsequent up-regulation of endothelial cell adhesion molecules resulting in PMN accumulation/activation (57). The generation of C5a has also been implicated as playing a potential role in tissue damage in I/R models of rat myocardium (62), baboon myocardium (78), rat intestine (4,80,81), rat liver (6), organ transplantation (82), and murine skeletal muscle (3).

In a model of coronary artery I/R injury in the dog, Rossen et al. (83) investigated whether ischemic myocardium released molecules that react with the first component of complement, C1q. They found that mitochondria within ischemic myocardium release C1q-binding proteins, thereby activating the complement cascade. They hypothesized that a possible mechanism of complement activation in I/R is the release of subcellular constituents that bind C1q, thereby activating complement, leading to the release of anaphylotoxins, and thus the subsequent infiltration of inflammatory cells (75,83).

Direct deposits of different complement components have been demonstrated on ischemic tissue as well. For example, in a model of myocardial ischemia in the baboon, Pinckard et al. (78) demonstrated the deposition of C3 on swollen and infarcted tissue when compared to viable myocardium. In a model of rat liver I/R, Chavez-Cartaya et al. (6) demonstrated endothelial cell deposition of the complement components C3 and C9.

The final common pathway leading to the formation and deposition of the MAC complex has also been shown to occur in the setting of I/R tissue injury. Using antibodies to the human C5b-9 complement complex, Schafer et al. (79) demonstrated selective and exclusive labeling of myocardial cells located in "zones of infarction." They proposed that initial ischemia causes loss of the ability of myocardial cells to regulate complement turnover at the membrane level. The resulting deposition of the MAC complex on cell membranes may cause irreversible damage in the setting of acute ischemia and decreased blood flow (79,84). In support of this concept, Vakeva et al. (85) described the acquired loss of the MAC inhibitor, protectin (CD59), from infarcted myocardium. Protectin expression has been demonstrated on vascular endothelium as well (86). Alternatively, the identification of MAC complex deposition in I/R injury may be a natural sequelae of the activation of early, upstream complement cascade components (i.e., C1q). Direct tissue damage by deposition of the MAC complex in I/R injury has also been implicated in human myocardium (11), rabbit myocardium (87), rat myocardium (62), organ transplantation (82,84), and rat liver (6).

In addition, the MAC complex has been shown to be deposited on reperfused endothelial cells, which might be expected to lead to lytic endothelial cell damage. Alternatively, MAC deposition may initiate (in the sublytic role) coagulation and endothelial retraction thereby potentiating microvascular failure and tissue oxidant stress via leukocyte extravasation (88). Sublytic concentrations of the MAC complex can also up-regulate P-selectin expression (shared ability of C5a), E-selectin expression, and ICAM-1 expression, all of which are associated with the cerebrovascular "no-reflow" phenomenon and increased cerebral tissue injury (88).

To further support the role of complement in I/R injury, investigators have used different approaches to demonstrate the protective effect of complement inhibition on ischemic tissue.

Complement Inhibition: Systemic Models

Armed with the compelling evidence that complement plays an integral role in tissue damage in multiple I/R models, investigators have developed strategies targeting different aspects of the complement cascade attempting to alleviate its deleterious effects. The first generation of designed inhibitors was based on naturally occurring complement receptors and regulatory molecules (89). More recently, humanized antibodies and synthetic molecules that block the activation of complement have been developed (89).

In the 1960s, Klein and Nelson purified a specific protein from the snake *Naja naja kaouthia* that activated complement, cobra venom factor (CVF). CVF is a cobra C3b homologue, a potent specific activator of the alternative pathway of complement that functions like mammalian C3b (90). CVF differs from human C3b in that it is not efficiently inactivated by factors H and I, and therefore cannot be down-regulated. As a result, CVF continues to activate the complement cascade leading to a rapid and prolonged depletion of complement activation potential (Fig. 3). In a model of permanent coronary

artery ligation in the rat, Hill and Ward showed that with CVF, myocardial infarcts failed to develop leukotactic activity and had little neutrophil accumulation (8). Since then, CVF has been shown to significantly reduce myocardial infarct size in several other I/R studies (91–93). CVF is also protective against complement-mediated pulmonary injury (94,95), shock (5), and xenotransplantation injury (96).

CR1 (CD35) is a member of a large family of C3 and C4 binding proteins known as the regulators of complement activation (RCA) family. The molecule itself is a single chain membrane-bound glycoprotein. CR1 is a potent regulator of C3 and C5 activation because it is a high-affinity receptor for C3b and C4b. It also has the capacity to accelerate decay of both the classical and alternative pathways because it exerts cofactor activity for factor-I-mediated proteolytic degradation. By removing the transmembrane and cytoplasmic domains of CR1, Weisman et al. (62) were able to synthesize a soluble form of the CR1 receptor, soluble complement receptor 1 (sCR1). Recombinant sCR1 blocked complement activation in human serum by both the alternative and classical pathways (Fig. 3). sCR1 has also been shown to significantly reduce complement-mediated tissue damage in numerous animal models of I/R injury such as xenotransplantation (96), myocardial (62,97,98), pulmonary (84), intestinal (4), skeletal (3), and hepatic (6,99) injury.

Monoclonal antibodies to C5a have shown protective effects in myocardial ischemia (100,101) and, in the setting of septic shock, have improved tissue oxygen extraction (102). In patients undergoing cardiopulmonary bypass, a humanized recombinant, single chain antibody specific for C5 (h5G1.1—scFv) was recently found to significantly reduce soluble MAC complex formation, leukocyte CD11b expression, postoperative myocardial injury, cognitive defects, and blood loss (103) (Fig. 3).

Congenital or genetically engineered deficiencies of specific complement components have also been used to study the role of complement in various disease states. In a model of hind limb I/R in the mouse, Weiser et al. (104) demonstrated 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 (Fig. 3). Furthermore, C6-deficient rabbits have been shown to be protected against myocardial I/R injury (105) and to have decreased incidence of both fatal arrhythmias and capillary plugging (106).

C1, the first component of the classical complement cascade, is a multimolecular complex composed of the protein C1q, and the catalytic subunit C1s-C1r-C1r-C1s, the latter of which is a calcium-dependent tetramer composed of two different serine proteases (107,108) (Fig. 3). C1 esterase inhibitor (C1-INH), a soluble glycoprotein, inhibits the classical complement pathway. Specifically, it stops initiation of the classical complement cascade by inactivating the proteases C1r and C1s, thus restricting their effects on C1 binding and activation (Fig. 3). C1-INH has been used in a model of myocardial infarction in the cat by Buerke et al. (109), who demonstrated that C1-INH administration prior to reperfusion significantly decreased myocardial necrosis and myeloperoxidase (MPO) activity. These findings were confirmed in a model of myocardial I/R in the rat (110). Furthermore, C1-INH administration in rats decreased P-selectin and ICAM-1 expression following myocardial ischemia reperfusion (111).

Complement inhibition has opened an entire world of potential novel protective strategies to attenuate tissue damage in the setting of acute I/R injury. A summary of the complement inhibition strategies discussed above is listed in Table 1. As our understanding of the biology of the complement cascade continues to grow, so does our interest in the development of more precise complement inhibitors. More importantly, the mechanisms of tissue damage via complement activation are continually being investigated. Essential to clinical stroke is the question of whether or not these mechanisms translate into benefit in the setting of cerebral I/R injury.

Cerebral Ischemia AND Complement: Stroke Models

It has become clear that inflammation and tissue destruction in CNS disease is due, at least in part, to the activation of complement (112). There has been a shift in the paradigm that the CNS is immunologically privileged and that complement, in the setting of an intact blood–brain barrier, is not present in the CNS. Biosynthesis of complement components is the CNS is now well established (66). Furthermore, there is abundant evidence suggesting that complement plays a direct role in neuronal cell death in the setting of CNS inflammation (112,113). As a matter of fact, the deleterious effects of the complement system have been implicated in multiple disease processes including subarachnoid hemorrhage (114–117), trauma (118), Alzheimer's disease (119–121), multiple sclerosis (122,123), Guillain–Barré syndrome (124), subacute sclerosing panencephalitis (125), and stroke (15,126). Studies in the last decade have demonstrated that essentially all of the activation components, regulatory molecules, and receptors of the complement system are produced by astrocytes, microglia, and neurons (66,127–135) (Table 2).

Acute I/R brain injury initiates a complex and dynamic cascade of inflammatory events leading to microvascular failure and neuronal cell death. In light of the fact that complement is intrinsically expressed in brain tissue, it seems logical that many of the same mechanisms that caused I/R injury in systemic models may also be at play in cerebral I/R injury. In an attempt to elucidate the possible mechanisms by which complement may exacerbate the

Table 1. Complement inhibition strategies and their effects in different models of tissue ischemia/reperfusion

	Study	Year/ Anticomplement Strategy	Model System	Species	Result
Systemic I/R					
Models	Maroko et al. (91)	1978 CVF	Cardiac ischemia	Dog	Decreased infarct size
	Maclean et al. (93)	1978 CVF	Cardiac ischemia	Rat	Decreased infarct size
	Crawford et al. (92)	1988 CVF	Cardiac ischemia	Baboon	Decreased infarct size
	Weisman et al. (62)	1990 sCR1	Cardiac ischemia	Rat	Decreased infarct size
	Hill et al. (4)	1992 sCR1	Intestinal ischemia	Rat	Decreased intestinal injury
	Pemberton et al. (3)	1993 sCR1	Skeletal muscle ischemia	Mouse	Increased muscle cell viability and improved circulation
	Buerke et al. (109)	1995 C1-INH	Cardiac ischemia	Cat	Improved contractility and vascular function
	Murohara et al. (110)	1995 C1-INH, sCR1	Cardiac ischemia	Rat	Attenuation of neutrophil accumulation and myocardial injury
	Amsterdam et al. (100)	1995 C5a MAb	Cardiac ischemia	Pig	Inhibition of neutrophil cytotoxic activity
	Candinas et al. (96)	1996 sCR1, CVF	Xenotransplantation/heart	Pig/Rat	Decrease inflammatory response
	Hopken et al. (102)	1996 CVF, C5a MAb	Septic shock	Pig	Decreased serum IL-6 bioactivity (75%)
	Weiser et al. (104)	1996 C3 −/−, C4 −/−	Skeletal muscle ischemia	Mouse	Protection against reperfusion injury
	Naka et al. (84)	1997 sCR1	Isogenic lung transplantation	Rat/Rat	Improved outcomes
	Lazar et al. (97)	1998 sCR1	Cardiac ischemia	Pig	Significant limitation of ischemic damage
	Vakeva et al. (101)	1998 C5a MAb	Cardiac ischemia	Rat	Decreased infarct size
	Kilgore et al. (105)	1998 C6 −/−	Cardiac ischemia	Rabbit	Decreased infarct size; decrease neutrophil influx
	Fitch et al. (103)	1999 Human C5 Ab	Cardiopulmonary bypass	Human	Attenuation of postoperative myocardial injury, cognitive deficits, and blood loss
Cerebral I/R					
Models	Vasthare et al. (140)	1998 CVF	B CCA occlusion	Rats	Improved cerebral blood flow and outcom
	Lew et al. (141)	1999 CVF	ICA autologous clot	Rabbit	No change in infarct volume
	Huang et al. (15)	1999 sCR1	MCA occlusion/ reperfusion	Mouse	Inhibition of platelet and neutrophil accumulation; reduced infarct volumes

Abbreviation: I/R, ischemia/reperfusion; CVF, cobra venom factor; sCR1, soluble complement receptor 1; C1-INH, C1 inhibitor; Mab, monoclonal antibody; (−/−), gene deletion; B, bilateral; CCA, common carotid artery; ICA, internal carotid artery; MCA, middle cerebral artery.

inflammatory response and subsequent neuronal cell death, researchers have designed different model systems of cerebral I/R injury.

Using a model of permanent MCAO in the mouse, Van Beek et al. (136) examined the expression of anaphylatoxin C3a and C5a receptors (C3aR and C5aR) at both the mRNA and protein levels. They observed a significant increase in the expression of C3aR and C5aR mRNAs in the ischemic cortex. Furthermore, C3aR and C5aR was detected by immunohistochemistry on both neurons and astrocytes. The up-regulation of C3aR and C5aR on glial cells and endothelial cells in response to permanent focal brain ischemia was found to be correlated with reactive gliosis, suggesting that C3a and C5a may mediate pro-inflammatory reactions in response to focal cerebral ischemia (136). As demonstrated in the systemic models of I/R injury, C3a and C5a may play an active and indirect role in the accumulation and activation of neutrophils, microvascular failure, and neuronal cell damage in the setting of ischemic stroke.

Table 2. Central nervous system production of complement components

Cell Type	Complement Component
Astrocyte	C1C9, factors B, D, H, I, C1INH, C4BP, DAF, MCP, CD59, clusterin, C3aR, C5aR, CR2
Microglia	C1, C1qB, C3, C4, clusterin, C1INH, CD59, CR1, CR3, CR4, C1qR, C3aR, C5aR
Neuron	C1C9, factor B, CD59, DAF, C1INH, clusterin, CR1, C3aR, C5aR
Oligodendrocyte	CD59, DAF, C3aR, C5aR
Ependymal	C3, C5aR
Endothelial	C1qB, C1r, Cls, C2, C3, C4, C5, C7, C8 gamma-subunit, C9

Lindsberg et al. (116) demonstrated complement deposition in brain samples from patients who died after ischemic stroke. Using anti–C5b-9 neoantigen monoclonal antibody, they were able to show complex deposition within areas of infarction necrosis. This group concluded that positive staining for the C5b-9 neoantigen confirmed that the terminal complement pathway had been activated and resulted in MAC assembly in discrete infarct regions (116). Furthermore, they proposed the mechanistic possibility that a portion of the complement proteins demonstrated within the infarcted tissue may be produced locally in the brain parenchyma after an ischemic insult.

Using a model of transient MCAO in the rat, Nishino et al. (22) used immunohistochemistry to demonstrate the presence of complement factor C3 and IgG in the core area of cerebral ischemia at both 1 and 3 days following reperfusion. From these data, the authors suggest that, in transient cerebral ischemic injury, especially in the core region, dysfunction of the blood–brain barrier follows I/R injury. This breakdown may seriously impair the protective mechanisms of the brain against destructive inflammatory processes as demonstrated by IgG and C3 immunoreactivity (22). Furthermore, the authors theorized that C3 could potentially bind to the Fc fragment of IgG forming a complex which interacts with receptors on phagocytes (such as CR1) and could potentially act as a superopsonin and superlysin, which could "draw out" neurons in the ischemic area (22).

CR3 binds the intermediate C3 cleavage product C3bi (Fig. 3, C3i,Bb of the alternative pathway) produced during complement activation (137). Using a monoclonal antibody to CR3 (OX42), Kato et al. (16) investigated the temporal and spatial relationships of complement up-regulation in a model of transient MCAO in the rat. In this study, Kato et al. analyzed three distinct brain regions: 1) "ischemic area" (ischemic core), 2) "transitional zone" (penumbra), and 3) "surrounding areas" (16). Furthermore, they analyzed brain sections at 4 hr, and 1, 3, 7, and 14 days after ischemia. They reported OX42 immunoreactivity in both microglia and invading leukocytes. After 1 day, they observed fragmented OX42-positive debris (destroyed microglial cells) and scattered OX42-positive small, round cells (leukocytes, most likely neutrophils) in the ischemic core. After 3 days, they demonstrated accumulation of OX42-positive leukocytes within the infarct. After 7 days, the infarct core was covered by OX42-positive larger round cells (monocytes/macrophages), and they demonstrated a narrow rim of high cellularity, OX42-positive microglia in the penumbra. Finally, at 14 days, the number of OX42-positive cells had decreased. It would be premature to interpret the mechanistic significance of these findings without further investigation. However, the spatiotemporal up-regulation of CR3 demonstrated in this study adds evidence for the local generation of complement components in the setting of cerebral I/R injury.

In a recent in vitro study, Tohgi et al. (138) used PC12 rat cell culture as a model system for studying the expression of C1q, a critical initiator of the classical complement cascade (Fig. 3), in the setting of hypoxia. PC12 cells are of neuroectodermal origin, and have been used previously as a model system for studying neuronal cell death under diverse conditions, including hypoxia (138). In this study, cultured cells were subjected to 9 hr of hypoxia and then returned to a standard normoxic atmosphere for subsequent analysis at 0, 6, 12, 24, 48, and 72 hr after hypoxia (138). Using reverse transcriptase polymerase chain reaction (RT-PCR), Toghi et al. showed that, prior to hypoxia, PC12 cells did not express C1q mRNA. After hypoxia, however, C1q mRNA was expressed at 0 hr posthypoxia and continually expressed thereafter (138). Furthermore, C1q expression was demonstrated to continue up to 72 hr, with a peak expression at 24 hr posthypoxia (138). This in vitro study demonstrated an up-regulation of C1q after hypoxia in PC12 cultured cells in the absence of other cellular components, but did not identify mechanisms by which C1q mRNA is up-regulated in response to hypoxia. Nevertheless, these findings provide compelling support for complement up-regulation by neuronal cells in the setting of ischemic stress.

Using an in vivo model of transient MCAO in the mouse, our group was recently able to demonstrate the presence of C1q on neurons following cerebral I/R injury (15). After subjecting mice to 45 min of MCAO followed by 23 hr of reperfusion, prominent neuronal staining for C1q was seen in the ipsilateral cerebral cortex. Barely detectable levels of C1q were demonstrated in contralateral cortical neurons, and nonischemic control brain did not demonstrate any detectable C1q (15). The presence of C1q

in ischemic cerebral tissue was also confirmed by protein immunoblot analysis. This study lends further evidence for the up-regulation of early complement components in the setting of cerebral I/R injury. The pathophysiologic effects of the presence of C1q on ischemic neurons was further investigated in this study through the administration of soluble complement receptor-1 (sCR1), a potent inhibitor of early complement activation, and will be discussed in detail in the following section.

In a model of transient global ischemia in the rat, Schafer et al. (126) demonstrated the up-regulation of C1q expression in ischemic microglia. Using in situ hybridization, they found C1q mRNA levels to be dramatically increased in microglial cells at 72 hr postischemia. Interestingly, however, C1q mRNA expression was not demonstrated in neuronal cells (126). Using immunostaining techniques, C1q protein expression was found exclusively on microglial cells throughout all brain areas and at all time points following I/R injury (126). Astrocytic cells, endothelial cells, and neuronal cells were not found to express C1q by this technique (126). The pathophysiologic significance of C1q up-regulation by microglial cells after I/R injury were not investigated in this study. However, the authors propose that elevated levels of C1q may promote CNS inflammation through local complement activation, or C1q receptor binding and inflammatory cell activation (126).

In a more recent study, Van Beek et al. (139) further characterized the glial response and expression of complement factors using a model of permanent MCAO in the mouse. Through semiquantitative RT-PCR, they found elevated levels of C1qB and C4 mRNA in the ischemic cortex at 1 day post MCAO, which peaked at day 3 post MCAO. Using in situ hybridization, they found that C1qB and C4 mRNA was greatly up-regulated at 3 days after MCAO in the perifocal area, forming a rim around the infarction. This finding was confirmed through photoemulsion autoradiography, which clearly demonstrated strong expression of C1qB in cells surrounding the infarcted core. This study helps to establish local complement activation occuring within the CNS in response to ischemia. The authors suggest that local complement activation may contribute to secondary brain damage after a focal cerebral ischemic event (139).

Although the mechanisms by which complement produces its deleterious effects on neurons in I/R injury are not well understood, it is increasingly apparent that the complement system is present and active in the setting of both permanent and reperfused cerebral ischemia. The belief that complement is not intrinsically expressed in the intact CNS no longer appears to be true. Complement components are actively expressed by every cell type in the brain and their up-regulation in the setting of inflammation now appears well established (66,112). Given this, investigators have begun to move forward in attempts to inhibit complement activation in a variety of disease states in the CNS. Researchers have taken data from systemic I/R models and have begun to apply them to cerebral I/R models in an attempt to further elucidate the role of complement in the pathophysiology of cerebral ischemia.

Complement Inhibition and Stroke

To develop novel neuroprotective strategies in the setting of cerebral I/R injury, researchers have turned to the complement system for potential targets. Following much of what has proved successful in systemic models, many of the same complement inhibition strategies have been investigated in the brain.

In an attempt to determine if removal of the complement system could provide protection for the brain during I/R injury, Vasthare et al. (140) pretreated a cohort of rats with CVF 1 day prior to temporary cerebral ischemia. Reversible forebrain ischemia was then performed by bilateral common carotid artery occlusion for 15 min followed by a reduction in blood pressure to 50 mmHg by hemorrhage (140). Cerebral blood flow (CBF) and somatosensory evoked potentials (SSEPs) were monitored throughout the study period (140). To determine complement depletion in animals pretreated with CVF, a CH33 assay was used, which demonstrated a marked depletion in complement hemolytic activity in the experimental group (140). These data showed that CBF in complement-depleted animals was significantly higher during the reperfusion period when compared to controls (140). Furthermore, SSEPs could not be detected in any of the control animals during the first 10 min of reperfusion and in only one control animal after 15 min of reperfusion (140). On the contrary, SSEPs were present in all of the CVF-treated animals after 5 min of reperfusion (140).

The data reported by Vasthare et al. (140) show that complement depletion, prior to cerebral I/R injury, may have neuroprotective effects. Unfortunately, by depleting the complement system prior to ischemia, a separate analysis of the effects of complement depletion during I/R injury cannot be performed. Furthermore, the effects of complement depletion may disrupt other cellular events not analyzed in this study. The effects of CVF on the coagulation system, for example, might account for improved CBF and significant return of SSEPs in the reperfusion period. Despite the weaknesses of this early study, the results obtained the hypothesis that complement activation may exacerbate cerebral I/R injury.

Using a model thromboembolic stroke in the rabbit either with or without concomitant tissue plasminogen activator thrombolysis, Lew et al. (141) studied the effects of complement depletion using CVF on infarct size, CBF, and intracranial pressure (ICP). Contrary to the findings by Vasthare et al. (140) and despite evidence of systemic complement depletion in the CVF treatment groups, Lew et al. found no significant differences in CBF, infarct size,

or ICP between the complement-depleted animals and their appropriate controls. This study has similar weaknesses with respect to elucidating the effects of CVF on other cellular events as described above. Furthermore, complement may have a role in I/R injury that is dependent on significant reperfusion. Lew et al. state that in previous studies using this same model, the benefit seen with suppression of neutrophil function is related to restoration of CBF. A similar conclusion was also drawn by Prestigiacomo et al. (48), where antineutrophil strategies proved cerebroprotective in reperfused stroke, but not in animals subjected to permanent stroke. In the study by Lew et al., only a modest return of CBF following clot embolization was seen. This may have minimized any potentially detrimental effect of neutrophil activation, and thus, of the complement cascade.

To address some of the weaknesses of the studies previously discussed, Huang et al. (15) used a model of MCAO and reperfusion in the mouse in which they administered sCR1, a potent inhibitor of complement activation (Fig. 3), just prior to I/R injury. C1q is a critical initiator of the classical complement pathway (Fig. 3) and a ligand for C1qRp, a receptor on myeloid cells that enhances phagocytosis (142,143). The presence of C1q on neurons subjected to I/R injury may target these neurons for opsonization or complement-mediated cell death (15). If this mechanism in fact occurs and has detrimental effects on neurologic outcome, then pharmacologic blockade of this process could potentially improve outcome after I/R injury. Therefore, Huang et al. (15) analyzed the effects of sCR1 administration prior to cerebral I/R on infarct volume, neurologic deficit, PMN accumulation, platelet accumulation, CBF, and intercerebral hemorrhage. They demonstrated that sCR1 colocalized to neurons expressing C1q. Furthermore, sCR1 administration prior to I/R injury was reported to be moderately cerebroprotective in the setting of reperfused stroke as demonstrated by a significant reduction in neurologic deficit, PMN accumulation, and platelet accumulation. Trends were also recognized toward smaller infarct volumes and improved CBF in sCR1-treated animals (15). No significant bleeding risk was noted with sCR1 administration.

Together, these studies suggest a potential place for anticomplement strategies in the treatment of reperfused stroke, but further studies are certainly needed to determine that exact role.

Future Work With Anticomplement Strategies in the Setting of Acute Cerebral Ischemia: Cocktail Strategies and the Use of Translational Models

The temporal and mechanistic overlap of the complement cascade with other biochemical events occurring in cerebral I/R injury is quite complex and as yet only beginning to be understood. However, in light of the fact that multiple cascading events are simultaneously activated in stroke, Huang et al. (15,144) sought to wed an anticomplement strategy, sCR1, to an antileukocyte adhesion and antithrombotic strategy, sialyl Lewis^x (sLe^x), by covalent modification of sCR1 by sLe^x glycosylation.

In vitro experiments using this glycoprotein product, sCR1sLe^x, convincingly demonstrated binding of cell surface E-selectin and also blockade of P-selectin–mediated cellular adhesion (144). In vivo work subsequently demonstrated that the fusion protein inhibited selectin-mediated events in addition to the complement cascade, thereby significantly protecting the reperfused brain against leukocyte and platelet recruitment and neuronal injury (15). What is interesting about the nature of this protection is that the degree of pro-inflammatory microvascular failure observed with the fusion protein was dramatically less than with either a solitary anticomplement strategy, sCR1, or a solitary anti-inflammatory strategy with sLe^x (unpublished observations). This led to the hypothesis that anticomplement strategies were indeed working, at least in part, through protection of the microvascular bed. Further studies, however, showed that this was probably not the only mechanism of protection; although immunohistochemical studies showed marked accumulation of the fusion protein and sCR1 in the cerebral vascular lumen, presumably binding leukocytes and platelets, there was also marked binding to the neuronal cell bodies. When this was further investigated with double staining, it became clear that the sCR1 and the fusion protein were both binding to C1q expressed on ischemic neurons. Perhaps, as alluded to earlier, C1q expression in this context might be marking injured but not dead neurons for deletion by the immune system and that binding by a systemically administered agent might hide these neurons from destruction. Whether hiding them is ultimately beneficial is as yet not entirely clear as animals surviving these experiments were only examined out to 72 hr. Nonetheless, it appears that animals were neurologically improved to the degree that would be expected given the fairly crude neurologic assessments used and the degree of infarct volume reductions seen on pathologic review. More important, however, is the fact that this bifunctional glycoprotein offers one of very few examples of the use of a combined or "cocktail" strategy in the treatment of experimental cerebral I/R injury.

For future strategies in cerebral protection to provide direct clinical benefit to humans, scientists and clinicians will need to collaborate to develop effective models of I/R injury that will translate into the clinical arena. This will require investigators to no longer study therapeutic strategies in isolation, for what may be ineffective when used alone may significantly improve an alternate therapy. Similarly, what may be effective when used in isolation may be rendered ineffective when used in the setting of

other agents that are routinely utilized (e.g., tissue plasminogen activator [tPA]).

More extensive research is also necessary in nonhuman primate models to accurately determine the temporal relationships of complement up-regulation with respect to other inflammatory cascades. Studies in primates will not only confirm the importance of these cascades in a markedly different cerebrovascular bed, but will help to guide the design of rational clinical trials, especially in terms of primary endpoints. For instance, it is very difficult to obtain meaningful neurologic outcome data for small rodents. Motor scales, even complex ones that evaluate the time to remove stickers from paws, do not necessarily translate to the human condition where the ability to recover from neurologic injury is considerably more tenuous. Finally, the pathophysiology of cerebral I/R injury may be better understood if we begin to investigate changes in gene expression during and long after an acute ischemic injury. There have been great advances in our understanding of the human genome of late, and the application of these technologies to cerebral I/R injury may provide a better understanding of the precise mechanisms, compensatory and otherwise, that currently elude our understanding and thereby impair our ability to treat the devastation that is human stroke.

Acknowledgments

We would like to thank Ryan King and James McKinnell for help in compiling the extensive database of articles reviewed. We would also like to thank Nancy Heim for her dedication and hard work creating the illustrations for this manuscript. Portions of the results reviewed in this manuscript were funded by NIH grant support under Dr. David J. Pinsky (RO1 NS41460 and RO1 HL59488) and Dr. E. Sander Connolly, Jr. (KO8 NS02038 AND RO1 NS40409).

References

1. del Zoppo G, Ginis I, Hallenbeck JM, Iadecola C, Wang X, Feuerstein GZ. (2000) Inflammation and stroke: putative role for cytokines, adhesion molecules and iNOS in brain response to ischemia. *Brain Pathol.* **10:** 95–112.
2. Foreman KE, Glovsky MM, Warner RL, Horvath SJ, Ward PA. (1996) Comparative effect of C3a and C5a on adhesion molecule expression on neutrophils and endothelial cells. *Inflammation* **20:** 1–9.
3. Pemberton M, Anderson G, VetVicka V, Justus DE, Ross GD. (1993) Microvascular effects of complement blockade with soluble recombinant CR1 on ischemia/reperfusion injury of skeletal muscle. *J. Immunol.* **11:** 5104–5113.
4. Hill J, Lindsay TF, Ortiz F, Yeh CG, Hechtman HB, Moore, FD Jr. (1992) Soluble complement receptor type 1 ameliorates the local and remote organ injury after intestinal ischemia–reperfusion in the rat. *J. Immunol.* **149:** 1723–1728.
5. Ikai M, Itoh M, Joh T, Yokoyama Y, Okada N, Okada H. (1996) Complement plays an essential role in shock following intestinal ischemia in rats. *Clin. Exp. Immunol.* **106:** 156–159.
6. Chavez-Cartaya RE, DeSola GP, Wright L, Jamieson NV, White DJ. (1995) Regulation of the complement cascade by soluble complement receptor type 1. Protective effect in experimental liver ischemia and reperfusion. *Transplantation* **59:** 1047–1052.
7. Zamora MR, Davis RD, Keshavjee SH, et al. (2000) Complement inhibition attenuates human lung transplant reperfusion injury: a multicenter trial. *Chest* **116:** 46S.
8. Hill JH, Ward PA. (1971) The phlogistic role of C3 leukotactic fragments in myocardial infarct of rats. *J. Exp. Med.* **133:** 885–900.
9. Kato H, Kogure K. (1999) Biochemical and molecular characteristics of the brain with developing cerebral infarction. [Review]. *Cell. Mol. Neurobiol.* **19:** 93–108.
10. Dawson DA, Ruetzler CA, Carlos TM, Kochanek PM, Hallenbeck JM. (1996) Polymorphonuclear leukocytes and microcirculatory perfusion in acute stroke in the SHR. *Keio J. Med.* **45:** 248–252.
11. del Zoppo GJ, Schmid-Schonbein GW, Mori E, Copeland BR, Chang C. (1991) Polymorphonuclear leukocytes occlude capillaries following middle cerebral artery occlusion and reperfusion in baboons. *Stroke* **22:** 1276–1283.
12. Matsuo Y, Onodera H, Shiga Y, et al. (1994) Correlation between myeloperoxidase-quantified neutrophil accumulation and ischemic brain injury in the rat. Effects of neutrophil depletion. *Stroke* **25:** 1469–1475.
13. Bednar MM, Raymond S, McAuliffe T, Lodge PA, Gross CE. (1991) The role of neutrophils and platelets in a rabbit model of thromboembolic stroke. *Stroke* **22:** 44–50.
14. Winquist RJ, Kerr S. (1997) Cerebral ischemia-reperfusion injury and adhesion. *Neurology* **49**(suppl 4)**:** S23–S26.
15. Huang J, Kim LJ, Mealey R, et al. (1999) Neuronal protection in stroke by an sLe^x-glycosylated complement inhibitory protein. *Science* **285:** 595–599.
16. Kato H, Kogure K, Liu XH, Araki T, Itoyama Y. (1996) Progressive expression of immunomolecules on activated microglia and invading leukocytes following focal cerebral ischemia in the rat. *Brain. Res.* **734:** 203–212.
17. Okada Y, Copeland BR, Mori E, Tung MM, Thomas WS, del Zoppo GJ. (1994) P-selectin and intercellular adhesion molecule-1 expression after focal brain ischemia and reperfusion. *Stroke* **25:** 202–211.
18. Hallenbeck JM. (1996) Significance of the inflammatory response in brain ischemia. *Acta Neurochir. Suppl.* **66:** 27–31.
19. Zhang RL, Chopp M, Zaloga C, et al. (1995) The temporal profiles of ICAM-1 protein and mRNA expression after transient MCA occlusion in the rat. *Brain Res.* **682:** 182–188.
20. Lindsberg PJ, Carpen O, Paetau A, Karjalainen-Lindsberg ML, Kaste M. (1996) Endothelial ICAM-1 expression associated with inflammatory cell response in human ischemic stroke. *Circ. Res.* **94:** 939–945.
21. Kuroda S, Siesjo BK. (1997) Reperfusion damage following focal cerebral ischemia: pathophysiology and therapeutic windows. *Clin. Neurosci.* **4:** 199–212.
22. Nishino H, Czurko A, Fukuda A, et al. (1994) Pathophysiological process after transient ischemia of the middle cerebral artery in the rat. *Brain Res. Bull.* **35:** 51–56.
23. Jean WC, Spellman SR, Nussbaum ES, Low WC. (1998) Reperfusion injury after focal cerebral ischemia: the role of inflammation and the therapeutic horizon. *Neurosurgery* **43:** 1382–1397.
24. Bednar MM, Gross CE, Howard DB, Lynn M. (1997) Neutrophil activation in acute human central nervous system injury. *Neurol. Res.* **19:** 588–592.
25. Wang X, Yue TL, Barone FC, White RF, Gagnon RC, Feuerstein GZ. (1994) Concomitant cortical expression of TNF-alpha and IL-1 beta mRNAs follows early response gene expression in transient focal ischemia. *Molecular and Chemical Neuropathology* **23:** 103–114.
26. Kostulas N, Pelidou SH, Kivisakk P, Kostulas V, Link H. (1999) Increased IL-1beta, IL-8, and IL-17 mRNA expression in blood mononuclear cells observed in a prospective ischemic stroke study. *Stroke* **30:** 2174–2179.

27. Relton JK, Rothwell NJ. (1992) Interleukin-1 receptor antagonist inhibits ischaemic and excitotoxic neuronal damage in the rat. *Brain Res. Bull.* **29:** 243–246.
28. Casale TB, Carolan EJ. (1999) Combination of IL-8 plus TNF alpha induces additive neutrophil migration. *Allergy Asthma Proc.* **20:** 361–363.
29. Nathan C. (1997) Inducible nitric oxide synthase: what difference does it make? [Review]. *J. Clin. Invest.* **100:** 2417–2423.
30. Iadecola C. (1997) Bright and dark sides of nitric oxide in ischemic brain injury. [Review]. *Trends Neurosci.* **20:** 132–139.
31. Grandati M, Plotkine M, Verrecchia C, Revaud ML, Allix M, Boulu RG. (1997) Calcium-independent NO-synthase activity and nitrites/nitrates production in transient focal cerebral ischaemia in mice. *Br. J. Pharmacol.* **122:** 625–630.
32. Iadecola C, Zhang F, Casey R, Clark HB, Ross ME. (1996) Inducible nitric oxide synthase gene expression in vascular cells after transient focal cerebral ischemia. *Stroke* **27:** 1373–1380.
33. Iadecola C, Zhang F, Xu S, Casey R, Ross ME. (1995) Inducible nitric oxide synthase gene expression in brain following cerebral ischemia. *J. Cereb. Blood Flow Metab.* **15:** 378– 384.
34. Iadecola C, Xu X, Zhang F, el-Fakahany EE, Ross ME. (1995) Marked induction of calcium-independent nitric oxide synthase activity after focal cerebral ischemia. *J. Cereb Blood Flow Metab.* **15:** 52–59.
35. Miettinen S, Fusco FR, Yrjanheikki J, et al. (1997) Spreading depression and focal brain ischemia induce cyclooxygenase-2 in cortical neurons through N-methyl-D-aspartic acid-receptors and phospholipase A2. *Proc. Natl. Acad. Sci. U.S.A.* **94:** 6500–6505.
36. Nogawa S, Forster C, Zhang F, Nagayama M, Ross ME, Iadecola C. (1998) Interaction between inducible nitric oxide synthase and cyclooxygenase-2 after cerebral ischemia. *Proc. Natl. Acad. Sci. U.S.A.* **95:** 10966–10971.
37. Nogawa S, Zhang F, Ross ME, Iadecola C. (1997) Cyclo-oxygenase-2 gene expression in neurons contributes to ischemic brain damage. *J. Neurosci.* **17:** 2746–2755.
38. Planas AM, Soriano MA, Justicia C, Rodriguez-Farre E. (1999) Induction of cyclooxygenase-2 in the rat brain after a mild episode of focal ischemia without tissue inflammation or neural cell damage. *Neurosci. Lett.* **275:** 141–144.
39. Iadecola C, Forster C, Nogawa S, Clark HB, Ross ME. (1999) Cyclooxygenase-2 immunoreactivity in the human brain following cerebral ischemia. *Acta Neuropathol.* **98:** 9–14.
40. Nakayama M, Uchimura K, Zhu RL, et al. (1998) Cyclooxygenase-2 inhibition prevents delayed death of CA1 hippocampal neurons following global ischemia. *Proc. Natl. Acad. Sci. U.S.A.* **95:** 10954–10959.
41. Connolly ES, Winfree CJ, Springer TA, et al. (1996) Cerebral protection in homozygous null ICAM-1 mice after middle cerebral artery occlusion: role of neutrophil adhesion in the pathogenesis of stroke. *J. Clin. Invest.* **97:** 209–216.
42. Choudhri TF, Hoh BL, Zerwes H, et al. (1998) Reduced microvascular thrombosis and improved outcome in acute murine stroke by inhibiting GP IIb/IIIa receptor-mediated platelet aggregation. *J. Clin. Invest.* **102:** 1301–1310.
43. Ames A, Wright RL, Kowada M, Thurston JM, Majno G. (1968) Cerebral ischemia: the no-reflow phenomenon. *Am. J. Pathol.* **52:** 437–453.
44. del Zoppo GJ, Hallenbeck JM. (2000) Advances in the vascular pathophysiology of ischemic stroke. *Thromb. Res.* **98:** 73–81.
45. Kochanek PM, Hallenbeck JM. (1992) Polymorphonuclear leukocytes and monocytes/macrophages in the pathogenesis of cerebral ischemia and stroke. *Stroke* **23:** 1367–1379.
46. Matsuo Y, Onodera H, Shiga Y, et al. (1994) Role of cell adhesion molecules in brain injury after middle cerebral artery occlusion in the rat. *Brain Res.* **656:** 344–352.
47. Hartl R, Schurer L, Schmid-Schonbein GW, del Zoppo GJ. (1996) Experimental antileukocyte interventions in cerebral ischemia. *J. Cereb. Blood Flow Metab.* **16:** 1108–1119.
48. Prestigiacomo CJ, Kim SC, Connolly ES, Liao H, Yan S, Pinsky DJ. (1999) CD18-mediated neutrophil recruitment contributes to the pathogenesis of reperfused but not nonreperfused stroke. *Stroke* **30:** 1110–1117.
49. del Zoppo GJ. (1997) Microvascular responses to cerebral ischemia/inflammation. *Ann. NY Acad. Sci.* **823:** 132–147.
50. del Zoppo GJ, von Kummer R, Hamann GF. (1998) Ischaemic damage of brain microvessels: inherent risks for thrombolytic treatment in stroke [editorial]. *J. Neurol. Neurosurg. Psychiatry* **65:** 1–9.
51. Zhang RL, Chopp M, Chen H, Garcia JH. (1994) Temporal profile of ischemic tissue damage, neutrophil response, and vascular plugging following permanent and transient (2H) middle cerebral artery occlusion in the rat. *J. Neurol. Sci.* **125:** 3–10.
52. Phillips JB, Williams AJ, Adams J, Elliot PJ, Tortella FC. (2000) Protease inhibitor PS519 reduces infarction and attenuates leukocyte infiltration in a rat model of focal cerebral ischemia. *Stroke* **31:** 1686–1693.
53. Czermak BJ, Friedl HP, Ward PA. (1998) Complement, cytokines, and adhesion molecule expression in inflammatory reactions. [Review]. *Proceedings of the Association of American Physicians* **110:** 306–312.
54. Murphy HS, Shayman JA, Till GO. (1991) Superoxide responses of endothelial cells to C5a and TNF-alpha: divergent signal transduction pathways. *Am. J. Physiol.* **263:** L51–L59.
55. Peerschke EI, Reid KB, Ghebrehiwet B. (1993) Platelet activation by C1q results in the induction of alpha IIb/beta 3 integrins (GPIIb-IIIa) and the expression of P-selectin and procoagulant activity. *J. Exp. Med.* **178:** 579–587.
56. Williams FM. (1996) Neutrophils and myocardial reperfusion injury. [Review]. *Pharmacol. Ther.* **72:** 1–12.
57. Foreman KE, Vaporciyan AA, Bonish BK, et al. (1994) C5a-induced expression of P-selectin in endothelial cells. *J. Clin. Invest.* **94:** 1147–1155.
58. Kilgore KS, Schmid E, Shanley TP. (1997) Sublytic concentrations of the membrane attack complex (MAC) of complement induce endothelial interleukin 8 (IL-8) and monocyte protein 1 (MCP-1) through nuclear factor-kB activation. *Am. J. Pathol.* **150:** 2019–2031.
59. Kinoshita T. (1991) Biology of complement: the overture. *Immunol. Today* **12:** 291–295.
60. Janeway C. (1999). *Immunobiology: The immune system in health and disease*. New York, Garland Publishing, pp. 339–358.
61. Frank MM, Fries LF. (1991) The role of complement in inflammation and phagocytosis. *Immunol. Today* **12:** 322–326.
62. Weisman HF, Bartow T, Leppo MK, et al. (1990) Soluble human complement receptor type 1: in vivo inhibitor of complement suppressing post-ischemic myocardial inflammation and necrosis. *Science* **249:** 146–151.
63. Bednar MM, Gross CE, Russell SR, Short D, Giclas PC. (2000) Activation of complement by tissue plasminogen activator, but not acute cerebral ischemia, in a rabbit model of thromboembolic stroke. *J. Neurosurg.* **86:** 139–142.
64. Hays SJ. (1998) Therapeutic approaches to the treatment of neuroinflammatory diseases. [Review]. *Current Pharmaceutical Design* **4:** 335–348.
65. Bartko D, Lesicky O, Buc M. (1997) [Cerebral infarct and the immune response]. [Slovak]. *Bratisl Lek Listy* **98:** 321–329.
66. Thomas A, Gasque P, Vaudry D, Gonzalez B, Fontaine M. (2000) Expression of a complete and functional complement system by human neuronal cells in vitro. *Int. Immunol.* **12:** 1015–1023.
67. Keller HU, Sorkin E. (1968) Chemotaxis of leukocytes. *Experientia* **24:** 641–752.
68. Ward PA. (1970) Neutrophil chemotactic factors and related clinical disorders. *Arthritis Rheum.* **13:** 181–186.
69. Muller-Eberhard HJ. (1968). Chemistry and reaction mechanisms of complement. In: Dixon FJ Jr, Kunkel HG, eds. *Advances in Immunology.* New York, Academic Press, pp. 1–80.
70. Ballow M, Cochrane CG. (1969) Two anticomplementary factors in cobra venom: hemolysis of guinea pig erythrocytes by one of them. *J. Immunol.* **103:** 944–952.
71. Klein PG, Wellensiek HJ. (1965) Multiple nature of the third component of guinea-pig complement. *Immunology* **8:** 590–603.

72. Flexner S, Noguchi H. (1903) Snake venom in relation to hemolysis, bacteriolysis, and toxicity. *J. Exp. Med.* **6:** 277–301.
73. Hill JH, Ward PA. (1969) C3 leukotactic fragments produced by tissue protease. *J. Exp. Med.* **130:** 505.
74. Pinckard RN, Olson MS, Giclas PC, Terry R, Boyer JT, O'Rourke RA. (1975) Consumption of classical complement components by heart subcellular membranes in vitro and in patients after acute myocardial infarction. *J. Clin. Invest.* **56:** 740–750.
75. Rossen RD, Swain JL, Michael LH, Weakley S, Giannini E, Entman ML. (1985) Selective accumulation of the first component of complement and leukocytes in ischemic canine heart muscle: a possible initiator of an extra myocardial mechanism of ischemic injury. *Circ. Res.* **57:** 119–130.
76. Vakeva A, Morgan BP, Tikkanen I, Helin K, Laurila P, Meri S. (1994) Time course of complement activation and inhibitor expression after ischemic injury of rat myocardium. *Am. J. Pathol.* **144:** 1357–1368.
77. McManus LM, Kolb WP, Crawford MH, O'Rourke RA, Grover FL, Pinckard RN. (1983) Complement localization in ischemic baboon myocardium. *Lab. Invest.* **48:** 436–447.
78. Pinckard RN, O'Rourke RA, Crawford MH, et al. (1980) Complement localization and mediation of ischemic injury in baboon myocardium. *J. Clin. Invest.* **66:** 1050–1056.
79. Schafer H, Mathey D, Hugo F, Bhakdi S. (1986) Deposition of the terminal C5b-9 complement complex in infarcted areas of human myocardium. *J. Immunol.* **137:** 1945–1949.
80. Gibbs SA, Weiser MR, Kobzik L, Valeri CR, Shepro D, Hechtman HB. (1996) P-selectin mediates intestinal ischemic injury by enhancing complement deposition. *Surgery* **119:** 652–656.
81. Kimura T, Andih A, Fujiyama Y, Saotome T, Bamba T. (1998) A blockade of complement activation prevents rapid intestinal ischemia-reperfusion injury by modulating mucosal mast cell degranulation in rats. *Clin. Exp. Immounol.* **111:** 484–490.
82. Dong J, Pratt JR, Smith RAG, Dodd I, Sacks SH. (1999) Strategies for targeting complement inhibitors in ischaemia/reperfusion injury. *Mol. Immunol.* **36:** 957–963.
83. Rossen RD, Michael LH, Kagiyama A, et al. (1988) Mechanism of complement activation after coronary artery occlusion: evidence that myocardial ischemia in dogs causes release of constituents of myocardial subcellular origin that complex with human C1q in vivo. *Circ. Res.* **62:** 572–584.
84. Naka Y, Marsh HC, Scesney SM, Oz MC, Pinsky DJ. (1997) Complement activation as a cause for primary graft failure in an isogeneic rat model of hypothermic lung preservation and transplantation. *Transplantation* **64:** 1248–1255.
85. Vakeva A, Laurila R, Meri S. (1992) Loss of expression of protectin (CD59) is associated with complement membrane attack complex deposition in myocardial infarction. *Lab. Invest.* **67:** 608–616.
86. Hamilton KK, Ji Z, Rollins S, Stewart BH, Sims PJ. (1990) Regulatory control of the terminal component proteins at the surface of the human endothelial cells-neutralization of a C5b-9 inhibitor by antibody to CD59. *Blood* **76:** 2572–2577.
87. Homeister JW, Satoh P, Lucchesi BR. (1992) Effects of complement activation in the isolated heart: role of the terminal complement components. *Circ. Res.* **71:** 303–319.
88. Hattori R, Hamilton KK, McEver RP, Sims PJ. (1989) Complement proteins C5b-9 induce secretion of high molecular weight multimeters of endothelial von Willebrand factor and translocation of granule membrane protein GMP-140 to the cell surface. *J. Biol. Chem.* **264:** 9053–9060.
89. Marsh JE, Pratt JR, Sacks SH. (1999) Targeting the complement system. *Curr. Opin. Nephrol. Hypertens.* **8:** 557–562.
90. Vogel CW, Smith CA, Muller-Eberhard HJ. (1984) Cobra venom factor: structural homology with the third component of human complement. *J. Immunol.* **133:** 3235–3241.
91. Maroko PR, Carpenter CB, Chariello M. (1978) Reduction by Cobra Venom Factor of myocardial necrosis after coronary artery occlusion. *J. Clin. Invest.* **61:** 661–670.
92. Crawford MH, Grover FL, Kolb WP, et al. (1988) Complement and neutrophil activation in the pathogenesis of ischemic myocardial injury. *Circulation* **78:** 1449–1458.
93. Maclean D, Fishbein MC, Braunwald E, Maroko PR. (1978) Long-term preservation of ischemic myocardium after experimental coronary artery occlusion. *J. Clin. Invest.* **61:** 541– 551.
94. Ren XD, Huang SJ, Sun JJ, Zhu ZG. (1994) Protective effect of cobra venom factor on pulmonary injury induced by oleic acid. *Int. J. Immunopharmacol.* **16:** 969–975.
95. Dehring DJ, Steinberg SM, Wismar BL, Lowery BD, Carey LC, Cloutier CT. (1987) Complement depletion in a porcine model of septic acute respiratory disease. *J. Trauma* **27:** 615–625.
96. Candinas D, Lesnikoski BA, Robson SC, et al. (1996) Effect of repetitive high dose treatment with soluble complement receptor 1 and cobra venom factor on discordant xenograft survival. *Transplantation* **62:** 336–342.
97. Lazar HL, Hamasaki T, Bao Y, Rivers S, Bernard SA, Shemin RJ. (1998) Soluble complement receptor type I limits damage during revascularization of ischemic myocardium. *Ann. Thorac. Surg.* **65:** 973–977.
98. Homeister JW, Lucchesi BR. (1994) Complement activation and inhibition in myocardial ischemia and reperfusion injury. *Annu. Rev. Pharmacol. Toxicol.* **34:** 17–40.
99. Jaeschke H, Farhood A, Bautista AP, Spolarics Z, Spitzer JJ. (1993) Complement activates Kupffer cells and neutrophils during reperfusion after hepatic ischemia. *Am. J. Physiol.* **264:** G801–G809.
100. Amsterdam EA, Stahl GL, Pan H-L, Rendig SV, Fletcher MP, Longhurst JC. (1995) Limitation of reperfusion injury by a monoclonal antibody to C5a during myocardial infarction in pigs. *Am. J. Physiol. Heart Circ. Physiol.* **268:** H448–H457.
101. Vakeva A, Agah A, Rollins SA, Matis LA, Li L, Stahl GL. (1998) Myocardial infarction and apoptosis after myocardial ischemia and reperfusion. Role of terminal complement components and inhibition by anti-C5 therapy. *Circulation* **97:** 2259–2267.
102. Hopken U, Mohr M, Struber A, et al. (1996) Inhibition of interleukin-6 synthesis in an animal model of septic shock by anti-C5a monoclonal antibodies. *Eur. J. Immunol.* **26:** 1103–1109.
103. Fitch JC, Rollins S, Matis L, et al. (1999) Pharmacology and biological efficacy of a recombinant, humanized, single-chain antibody C5 complement inhibitor in patients undergoing coronary artery bypass graft surgery with cardiopulmonary bypass . *Circulation* **100:** 2499–2506.
104. Weiser MR, Williams JP, Moore FD Jr, et al. (1996) Reperfusion injury of ischemic skeletal muscle is mediated by natural antibody and complement. *J. Exp. Med.* **183:** 2343– 2348.
105. Kilgore KS, Park JL, Tanheco EJ, Booth EA, Marks RM, Lucchesi BR. (1998) Attenuation of interleukin-8 expression in C6-deficient rabbits after myocardial ischemia/reperfusion. *J. Mol. Cell. Cardiol.* **30:** 75–85.
106. Ito W, Schafer HJ, Bhakdi S, et al. (1996) Influence of the terminal complement-complex on reperfusion injury, no-reflow and arrythmias: a comparison between C6-competent and C6-deficient rabbits. *Cardiovasc. Res.* **32:** 294–305.
107. Thielens NM, Bersch B, Hernandez J, Arluad GJ. (1999) Structure and functions of the interaction domains of C1r and C1s: keystones of the architecture of the C1 complex. *Immunopharmacology* **42:** 3–13.
108. Lorincz Z, Gal P, Dobo J, et al. (2000) The cleavage of two C1s subunits by a single active C1r reveals substantial flexibility of the C1s-C1r-C1r-C1s tetramer in the C1 complex. *J. Immunol.* **165:** 2048–2051.
109. Buerke M, Murohara T, Lefer AM. (1995) Cardioprotective effects of a C1 esterase inhibitor in myocardial ischemia and reperfusion. *Circulation* **91:** 393–402.
110. Murohara T, Guo JP, Delyani JA, Lefer AM. (1995) Cardioprotective effects of selective inhibition of the two complement activation pathways in myocardial ischemia and reperfusion injury. *Methods Find. Exp. Clin. Pharmacol.* **17:** 499–507.

111. Buerke M, Prufer D, Dahm M, Oelert H, Meyer J, Darius H. (1998) Blocking of classical complement pathway inhibits endothelial adhesion molecule expression and preserves ischemic myocardium from reperfusion injury. *J. Pharmacol. Exp. Ther.* **286:** 429–438.
112. Barnum SR. (1999) Inhibition of complement as a therapeutic approach in inflammatory central nervous system (CNS) disease. [Review]. *Mol. Med.* **5:** 569–582.
113. Gasque P, Singhrao SK, Neal JW, Gotze O, Morgan BP. (1997) Expression of the receptor for Complement C5a (CD88) is up-regulated on reactive astrocytes, microglia, and endothelial cells in the inflamed human central nervous system. *Am. J. Pathol.* **150:** 31–41.
114. Ostergaard JR, Kristensen BO, Svehag S, Teisner B, Miletic T. (1987) Immune complexes and complement activation following rupture of intracranial saccular aneurysms. *J. Neurosurg.* **66:** 891–897.
115. Hidetoshi K, Takashi S. (1989) Activated complement components C3a and C4a in cerebrospinal fluid and plasma following subarachnoid hemorrhage. *J. Neurosurg.* **71:** 741–746.
116. Lindsberg PJ, Ohman J, Lehto T, et al. (1996) Complement activation in the central nervous system following blood-brain barrier damage in man. *Ann. Neurol.* **40:** 587–596.
117. German JW, Gross CE, Giclas P, Watral W, Bednar MM. (1996) Systemic complement depletion inhibits experimental cerebral vasospasm. *Neurosurgery* **39:** 141–15.
118. Kaczorowski SL, Schiding JK, Toth CA, Kochanek PM. (1995) Effect of soluble complement receptor-1 on neutrophil accumulation after traumatic brain injury in rats. *J. Cereb. Blood Flow Metab.* **15:** 860–864.
119. Velazquez P, Cribbs DH, Poulos TL, Tenner AJ. (1997) Aspartate residue 7 in amyloid beta-protein is critical for classical complement pathway activation: implications for Alzheimer's disease pathogenesis. *Nat. Med.* **3:** 77–79.
120. Afagh A, Cummings BJ, Cribbs DH, Cotman CW, Tenner AJ. (1996) Localization and cell association of C1q in Alzheimer's disease brain. *Exp. Neurol.* **138:** 22–32.
121. McGeer PL, Akiyama H, Itagaki S, McGeer EG. (1989) Activation of the classical complement pathway in brain tissue of Alzheimer patients. *Neurosci. Lett.* **107:** 341–346.
122. Wren DR, Noble M. (1989) Oligodendrocytes and oligodendrocyte/type-2 astrocyte progenitor cells of adult rats are specifically susceptible to the lytic effects of complement in absence of antibody. *Proc. Natl. Acad. Sci. USA* **86:** 9025–9029.
123. Cyong J, Witkin SS, Rieger B, Barbarese E, Good RA, Day NK. (1982) Antibody-independent complement activation by myelin via the classical complement pathway. *J. Exp. Med.* **155:** 587–598.
124. Sanders ME, Koski CL, Robbins D, Shin ML, Frank MM, Joiner KA. (1986) Activated terminal complement in cerebrospinal fluid in Guillain-Barre syndrome and multiple sclerosis. *J. Immunol.* **136:** 4456–4459.
125. Albrecht P. (1978) Immune control in experimental subacute sclerosing panencephalitis. *Am. J. Clin. Pathol.* **170:** 175.
126. Schafer MK, Schwaeble WJ, Post C, et al. (2000) Complement C1q is dramatically up-regulated in brain microglia in response to transient global cerebral ischemia. *J. Immunol.* **164:** 5446–5452.
127. Spiegel K, Emmerling MR, Barnum SR. (1997). *Inflammatory mechanisms and management of neurodegeneration*. Totowa, NJ, Humana Press, pp. 129–176.
128. Morgan BP, Gasque P, Singhrao S, Piddlesden SJ. (1997) The role of complement in disorders of the nervous system. *Immunopharmacology* **38:** 43–50.
129. Nataf S, Stahel PF, Davoust N, Barnum SR. (1999) Complement anaphylatoxin receptor expression on neurons: new tricks for old receptors? *Trends Neurosci.* **22:** 397–402.
130. McGeer PL, McGeer EG. (1999) Inflammation of the brain in Alzheimer's disease: implications for therapy. *J. Leukoc. Biol.* **65:** 409–415.
131. Scolding NJ, Morgan BP, Compston DA. (1998) The expression of complement regulatory proteins by adult human oligodendrocytes. *J. Neuroimmunol.* **84:** 69–75.
132. Gasque P, Singhrao SK, Neal JW. (1998) The anaphylatoxin receptor for complement C3a is expressed by rat oligodendrocytes. Role in cell chemotaxis and cell activation. *J. Neuroimmunol.* **90:** 21A.
133. Klegeris A, Bissonnette CJ, Dorovini-Zis K, McGeer PL. (2000) Expression of complement messenger RNAs by human endothelial cells. *Brain Res.* **871:** 1–6.
134. Johnson SA, Lampert-Etchells M, Pasinetti GM, Rozovsky I, Finch CE. (1992) Complement mRNA in the mammalian brain: responses to Alzheimer's disease and experimental brain lesioning. *Neurobiol. Aging* **13:** 641–648.
135. Pasinetti GM, Johnson SA, Rozovsky I, et al. (1992) Complement C1qB and C4 mRNAs responses to lesioning in rat brain. *Exp. Neurol.* **118:** 117–125.
136. Van Beek J, Bernaudin M, Petit E, et al. (2000) Expression of receptors for complement anaphylatoxins C3a and C5a following permanent focal cerebral ischemia in the mouse. *Exp Neurol* **161:** 373–382.
137. Robinson AP, White TM, Mason DW. (1986) Macrophage heterogeneity in the rat as delineated by two monoclonal antibodies MRC Ox-41 and MRC Ox-42, the later recognizing complement receptor type 3. *Immunology* **57:** 239–247.
138. Tohgi H, Utsugisawa K, Nagane Y. (2000) Hypoxia-induced expression of C1q, a subcomponent of the complement system, in cultured rat PC12 cells. *Neurosci. Lett.* **291:** 151–154.
139. Van Beek J, Chan P, Bernaudin M, Petit E, Mackenzie ET, Fontaine M. (2000) Glial responses, clusterin, and complement in permanent focal cerebral ischemia in the mouse. *Glia* **31:** 39–50.
140. Vasthare US, Barone FC, Sarau HM, et al. (1998) Complement depletion improves neurological function in cerebral ischemia. *Brain Res. Bull.* **45:** 413–419.
141. Lew SM, Gross CE, Bednar MM, et al. (1999) Complement depletion does not reduce brain injury in a rabbit model of thromboembolic stroke. *Brain Res. Bull.* **48:** 325–331.
142. Webster SD, Park M, Fonseca MI, Tenner AJ. (2000) Structural and functional evidence for microglial expression of C1qR(P), the C1q receptor that enhances phagcytosis. *J. Leukoc. Biol.* **67:** 109–116.
143. Nepomuceno RR, Henschen-Edman AH, Burgess WH, Tenner AJ. (1997) cDNA cloning and primary structure analysis of C1qR(P), the human C1q/MBL/SPA receptor that mediates enhanced phagocytosis in vitro. *Immunity* **6:** 119–129.
144. Rittershaus CW, Thomas LJ, Miller DP, et al. (1999) Recombinant glycoproteins which inhibit complement activation and also bind the selectin adhesion molecule. *J. Biol. Chem.* **274:** 11237–11244.