Current Trends in Immunology
Vol. 12, 2011

Review

Discovery of a novel inhibitor of the classical and lectin pathways of complement and its potential as a therapeutic modulator in preventing ischemia-reperfusion damage

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ABSTRACT

Inhibition or modulation of the human complement system has long been considered a target for therapeutic intervention given complement's central role in numerous inflammatory and autoimmune diseases. In particular, complement activation plays an essential role in ischemia-reperfusion (IR) injury in such conditions as myocardial infarction, stroke, organ transplantation rejection as well as other clinical disorders. While current complement inhibitory strategies have focused on downstream components C3, C5 and soluble complement regulators, inhibition of the initiator molecules of the classical and lectin pathways of complement, C1q and MBL (mannose binding lectin), respectively, have not been vigorously pursued. Previous studies have highlighted the critical role of C1 and MBL activation in initiating IR injury, thus inhibition of these molecules represents a viable therapeutic option for prevention of IR-mediated tissue damage. We have recently discovered a peptide originally derived from a viral capsid protein that has been demonstrated to potently inhibit C1 and MBL activation via a novel mechanism of action. In this review, we examine the role of the classical and lectin complement pathway activation in IR injury and the potential of this unique peptide inhibitor as an anti-complement therapeutic for use in IR disease. In view of the well-defined role of C1 and MBL activation in IR injury and the ability of this peptide to inhibit complement activation early at the point of initiation, this molecule provides a unique opportunity to halt complement activation in its tracks before amplification of the complement cascade and the commencing of inflammatory responses.

KEYWORDS: complement, C1q, MBL, ischemia, reperfusion, astrovirus, capsid protein, peptide

ABBREVIATIONS

IR, ischemia-reperfusion; MBL, mannose binding lectin; CLR, collagen-like region; GHR, globular-head regions, MASPs, MBL-associated serine proteases; MAC, membrane attack complex; C1-INH, C1 inhibitor; CABG, coronary artery bypass grafting; sCR1, Soluble human CR1; HAstV-1, human astrovirus 1; CP, coat protein; CPPs, coat protein peptides; HNP-1, human neutrophil peptide 1

INTRODUCTION

The complement system has traditionally been viewed as a host defense to bacterial and viral pathogens playing a "complementary" or subservient role to the humoral immune response. However, a number of studies in recent decades conclusively demonstrate that the complement system commands

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a dominant and essential position within the innate immune response as well as serves as a bridge between innate and adaptive immunity and maintains cellular homeostasis through clearance of apoptotic cellular debris and immune complexes. Thus, as part of the innate immune response, complement functions to recognize "danger" signals, that is, conserved motifs on pathogens as well as altered or damaged host cells [1]. Under normal circumstances, activation of the potent effector functions of complement (i.e., opsonization, pathogen lysis, cell activation, chemotaxis and inflammation) are precisely regulated by a series of membrane-bound and soluble protein regulators. However, upsetting this fragile equilibrium can ignite unwarranted complement activation resulting in inflammation and cellular lysis in a variety of autoimmune, neurodegenerative, inflammatory and infectious diseases. Identification of the pivotal role aberrant complement activation plays in disease has spawned renewed interest in dissecting the individual and overlapping functions of the various complement factors in each pathological condition and the development of specific complement inhibitors for therapeutic use. One such disease process is ischemia-reperfusion (IR) injury where numerous *in vitro* and *in vivo* studies have demonstrated a pivotal role for the classical and lectin pathways of complement in pathogenesis and the improved outcomes of specific and generalized complement inhibition on IR injury. The role of complement in IR disease and the potential for therapeutic intervention through complement inhibition has recently been the subject of an excellent review [2]. The purpose of this review is to spotlight the identification of a novel complement inhibitor of the classical and lectin pathways that may have potential as a therapeutic inhibitor for IR injury.

The complement system

The complement system comprises over 30 plasma and membrane-bound proteins which interact with each other through three pathways, the classical, lectin and alternative, to induce a regulated cascade of enzyme-mediated activation resulting in a variety of effector functions (Fig. 1). Complement activation initiates a rapidly escalating amplification cascade of extremely potent inflammatory responses that may result in tissue injury [3]. Many inflammatory diseases, including ischemia-reperfusion injury, are associated with dysregulated complement activation [4].

The classical pathway is initiated by C1, which is composed of the pattern recognition molecule C1q and the serine-protease tetramer, C1s-C1r-C1r-C1s [5]. C1q is composed of six stalks, or collagen-like regions (CLRs), attached to six globular-head regions (GHRs), forming a bouquet-like, quarternary structure [6]. The GHRs of C1q typically bind to IgM or clustered IgG initiating a conformational change in the CLRs [7]. The $C1r_2C1s_2$ complex is nested within the N-terminal region of the CLRs and undergoes cleavage and activation when the CLRs initiate their conformational change [8]. This activation of C1 will then initiate activation of the classical cascade pathway via C4 and then C2. C4 activation will generate the inflammatory peptide C4a and the opsonin C4b [9, 10].

The lectin pathway is initiated by the pattern recognition lectins in association with their cognate serine proteases, MASPs (MBL-associated serine proteases), which are homologous to the classical pathway serine protease C1s. The prototypical lectin is MBL, or mannose/mannan binding lectin. MBL has a similar ultrastructure to C1q and typically binds to foreign sugars (i.e. mannose) initiating a conformational change [11]. The nested MASPs will be cleaved and activated in response to this conformational change initiating activation of the lectin pathway leading to subsequent activation of C4 and then C2 [12].

The alternative pathway does not have a pattern recognition component, but does undergo spontaneous activation which is referred to as tick-over. The alternative pathway is very important in dramatically amplifying specific activation of the classical or lectin pathways, creating a potent positive-feedback loop. Humans deficient in alternative pathway components (i.e. do not have a competent alternative pathway) have a very high risk for severe bacterial infections [13, 14]. Thus, an intact alternative pathway is felt to be particularly critical for immune surveillance [15].

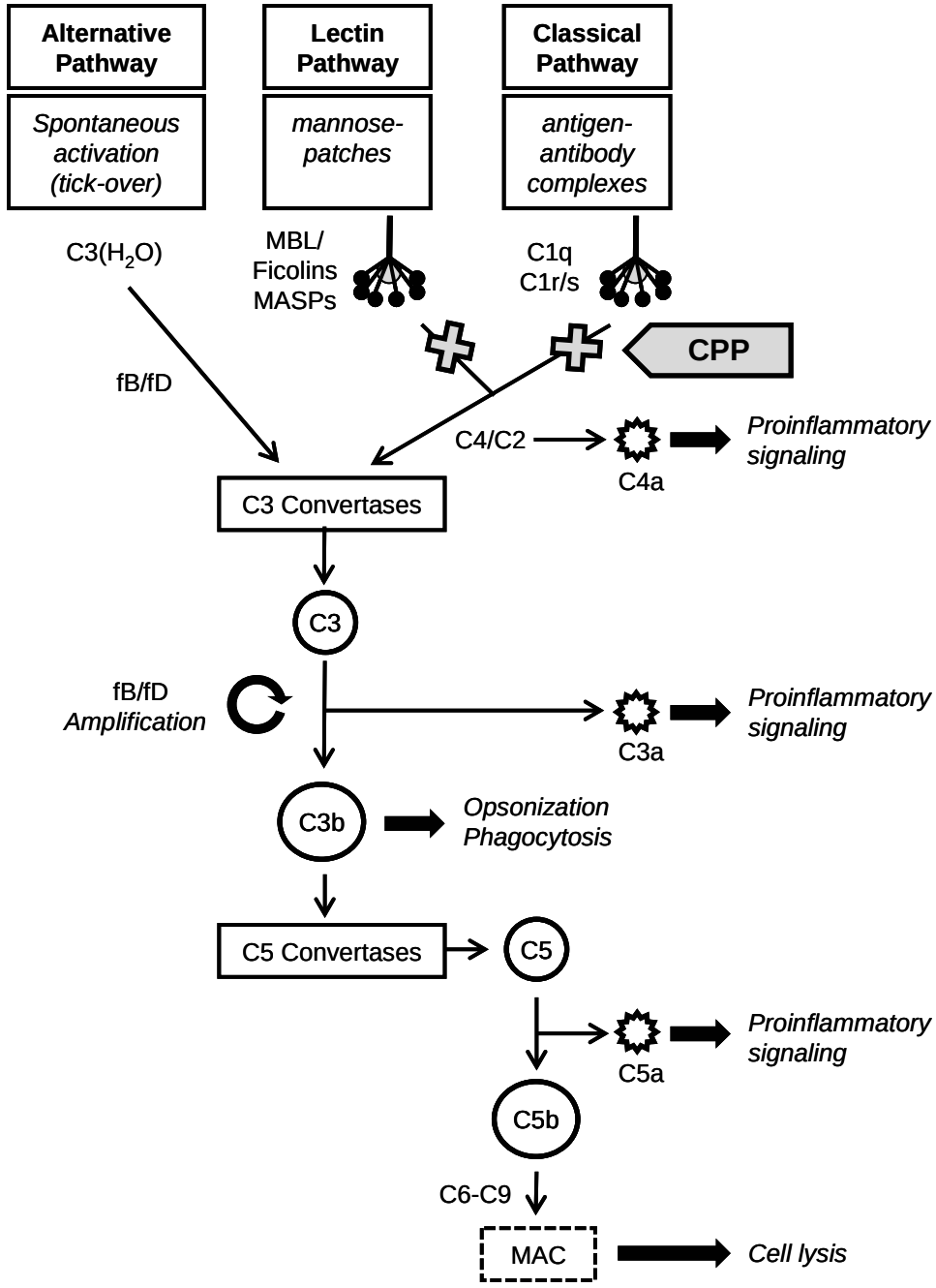

Fig. 1. Complement activation pathways and effectors. Coat protein peptides (CPPs) block activation of the classical and lectin pathways.

C3, the central component of the complement system, is activated to C3a and C3b by C3-convertases generated by the three initiating pathways. C3a induces chemotaxis and activates phagocytic cells in anticipation of impending battle. C3b is a critical opsonin marking cells for phagocytic cell attack. C3b combines with the previously activated enzymes to form C5-convertases and produce the potent anaphylatoxin C5a, which like C3a, also induces chemotaxis and activates phagocytic cells. C5-convertases also initiate the terminal complement cascade leading to membrane

attack complex (MAC) formation. MACs form pores in cell membranes leading to cellular damage and death.

Regulation of the complement system is mediated by soluble and cell membrane factors in order to prevent host tissue damage. Humans deficient in these regulatory factors commonly develop autoimmune diseases (e.g. acute macular degeneration, atypical hemolytic uremic syndrome, paroxysmal nocturnal hematuria) or infections due to consumption of complement components [16, 17]. Disruption of the delicate balance between activation and inhibition of complement activation (i.e. dysregulation) contributes to host tissue damage in a wide range of autoimmune and inflammatory diseases [18].

Role of complement in ischemia-reperfusion injury

IR injury is comprised of both ischemic and reperfusion-induced damage. Ischemic injury results from hypoxia-mediated cell death that typically has occurred before patients receive medical attention. Outside of organ transplantation or surgery-induced tissue hypoxia, ischemic cell death is usually unpreventable. Reperfusion injury is initiated by the return of oxygenated blood to the hypoxic tissue beds. This can occur spontaneously by removal of an occlusion (e.g. clot), but typically occurs at the time of fluid resuscitation (i.e. delivery of intravenous fluids), reestablishing cardiac circulation (e.g. reversal of ventricular fibrillation), or reestablishing oxygenation of the circulating blood (e.g. positive pressure ventilation). Because reperfusion injury typically occurs with the onset of medical intervention, it provides a golden opportunity to intervene and prevent further tissue damage. It has been shown in many settings that the tissue injury that occurs as the result of reperfusion can often exceed the damage caused by ischemia alone [19].

IR injury is a multifaceted disease process involving cellular metabolism, radical oxygen species, and inflammation. The degree to which each of these mechanisms contribute to tissue damage appears to vary depending on the organ, mechanism of ischemia, and animal model. The cellular metabolism mechanisms appear to predominantly involve energy failure including loss of mitochondrial function. Energy failure consists of a fall in intracellular ATP, anaerobic respiration, accumulation of the products of anaerobic metabolism, and intracellular acidosis [20]. In brain hypoxia in particular (e.g. hypoxic ischemic encephalopathy), metabolic injury involves excitotoxic injury. This mechanism includes excessive activation of glutamate receptors contributing to cell death via excessive intracellular calcium leading to apoptosis or necrosis [21-23]. Mitochondrial damage can include organelle swelling and outer membrane rupture as well as loss of the ionic gradient uncoupling oxidative phosphorylation leading to ATP hydrolysis and the activation of degradative enzymes [24]. Free radicals are produced within minutes of reperfusion and can continue to be generated for several hours [25]. These compounds include: superoxide anion, hydrogen peroxide, hypochlorous acid, nitric oxide-derived peroxynitrite, and hydroxyl radical [26] which directly damage membrane proteins and phospholipids [27].

The inflammatory response in IR injury also appears to involve multiple components involving leukocytes, platelets, and complement. Neutrophils are felt to play a major role in cell-mediated inflammation. Neutrophils are stimulated by the generation of arachadonic acid as well as complement anaphylatoxins (C3a, C5a), which also contribute to neutrophil recruitment [28]. Neutrophils aggregate in the ischemia damaged tissues with subsequent release of proteases, elastases, and radical oxygen species, contributing to cellular damage as well as microvascular permeability [29]. Platelet activation also occurs during reperfusion, possibly a result of complement activation [30], and likely contributes to dysfunction in the microvasculature[31].

The role of complement in IR injury begins with the expression of ischemia-induced neoantigens [32, 33] on the surface of vascular endothelial cells (Fig. 2). Ischemic antigens are then bound by circulating natural antibodies (i.e. IgM) [34, 35]. Complement activation is initiated by the binding of IgM [36, 37] and then, depending of the tissue bed and animal model, proceeds via C1 and the classical pathway or MBL/MASPs [38] and the lectin pathway (Table 1). There is evidence for classical pathway, or lectin pathway, or both

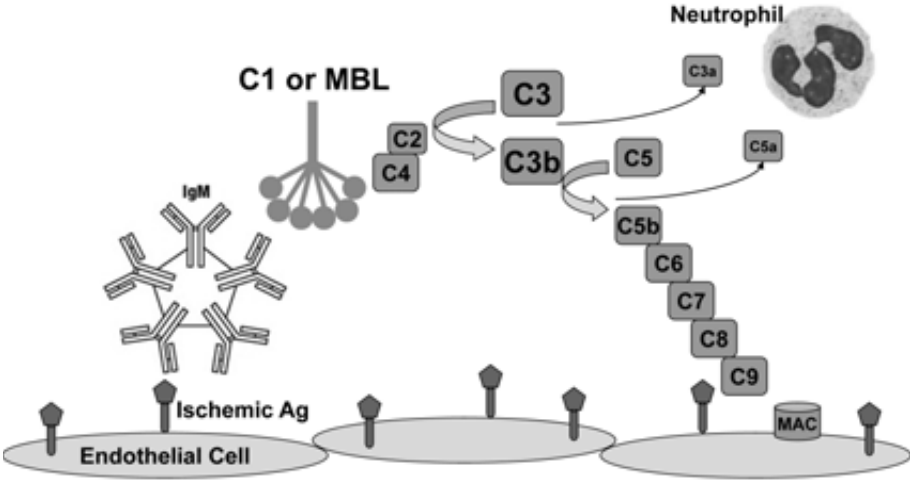

Fig. 2. Ischemic antigens expressed on endothelial cells are recognized by natural antibodies (IgM) initiating classical or lectin pathway activation leading to downstream inflammatory responses including opsonization (C3b), anaphylatoxins (C3a and C5a), and membrane attack complex (MAC) formation.

Table 1. Ischemia-reperfusion activation of complement by the classical and lectin pathways.

Classical pathway	Lectin pathway
Myocardial infarction [94]	Myocardial infarction [95]
Hypoxic ischemic encephalopathy [96]	Cerebral infarction [97]
Renal ischemia [98]	Renal ischemia [99]
Intestinal ischemia [100]	Intestinal ischemia [101]
Skeletal muscle ischemia [102]	Skeletal muscle ischemia [102]
Beta islet transplantation [103]	

contributing to IR injury for most organs, thus it remains unclear whether the involvement of one pathway or the other is dependent on the animal or the experimental circumstances of the ischemia and subsequent reperfusion [39]. Once either, or both, pathways are initially activated they follow a common amplification cascade to generation of C3-convertases and activation of the central component of complement C3 to C3b, which may covalently bind to damaged endothelium.

Once C3b is generated, the positive feedback loop of the alternative pathway will further amplify initial activation. The alternative pathway has been implicated as playing a role in renal reperfusion inflammation [40] and likely plays a role in accelerating complement activation regardless of the organ. Complement receptor 1 (CD35) and complement receptor 2 (CD21) are encoded by the same gene in mice and then alternatively spliced; thus, mice deficient for this gene (i.e. *CR2*) are null for both receptors [41]. CR1/2-deficient mice have shown decreased reperfusion damage in intestinal and myocardial infarction models [42, 43]. It is likely that these acute effects are mediated via CR1 on neutrophils interacting with the opsonins C3b and potentially C4b and iC3b on endothelial cells. There is evidence to suggest that CR2-deficient mice are unable to generate the natural antibodies to initiate complement-mediated reperfusion injury [44]. Subsequent terminal complement cascade activation results in C5a generation and eventual membrane attack complex. C5 has been shown to contribute to reperfusion injury in animal models evaluating

renal and skeletal muscle [40, 45]. The membrane attack complex also appears to contribute to reperfusion injury in liver transplantation [46, 47] and neonatal cerebral infarction [48] likely by inducing pores into the plasma membranes of endothelial cells.

It is curious to note that there is very little data published with respect to complement-mediated ischemia reperfusion injury for transplanted solid organs. This would appear to be an important issue particularly for cadaveric organs with prolonged intervals of cold ischemia time prior to re-implantation. The lack of investigation in this area may be due to the difficulty in distinguishing ischemic antigen-mediated IR injury in allotansplantation from complement activation caused by natural antibodies binding to non-self-antigens. Despite the potential for multiple triggers of complement-mediated injury in allotransplanted organs, there appears to be a potentially important role for preventing complement-mediated IR injury to these organs, possibly extending viable cold ischemia times and improving function of the graft.

Complement inhibitors of ischemia-reperfusion injury

Inhibiting activation of the classical and lectin pathways has been attempted with several compounds in a variety of organ IR models. The most extensively tested compound is human C1 inhibitor (C1-INH) which is purified from the blood of many human donors [49]. C1-INH inhibits the classical, lectin, and alternative pathways, as well having effects on the kinin and coagulation cascades [50]. C1-INH has been tested in animal models of IR injury involving the heart, brain, liver, intestines, and kidneys in a variety of animal species [51-55]. It has also been shown to be beneficial in a variety of human trials for acute myocardial infarction, emergency coronary artery bypass grafting (CABG), and emergency CABG after percutaneous transluminal coronary angioplasty [56-59]. There has also been a report of using C1-INH in newborns receiving extracorporeal membrane oxygenation after cardiac surgery resulting in multiple infants suffering fatal thromboembolic events [60]. The major limitation of using C1-INH as a therapeutic agent is the cost, because the compound is expensive to purify in significant quantities to achieve supraphysiological concentrations in the blood. As with any product purified from human blood, there is an inherent risk for the transmission of human pathogens despite screening and cleaning procedures.

In limited animal model testing, there has been some success in blocking IgM binding and subsequent complement activation. In a murine model, myocardial infarction was improved using either a synthetic peptide mimetic epitope or monoclonal antibodies against non-muscle myosin heavy chain II [61]. Additional strategies that have shown some promise in inhibiting early complement activation in animal models of IR injury include the use of low molecular weight dextran [62], as well as heparin derivatives and glycosaminoglycan analogs [63-65]. All of these compounds have the potential to increase the risk for bleeding due to anti-coagulant properties. A more targeted strategy of blocking classical pathway activation using a novel small molecule inhibitor has also been described. This molecule (C1s-INH-248) has shown some promise in a rabbit model of myocardial infarction [66, 67].

A number of additional strategies have been used to block IR injury mediated by downstream effectors including C3-mediated effects and anaphylatoxin-mediated effects. Soluble human CR1 (sCR1) and subsequent derivatives (i.e. sCR1-sLE^x) bind to C3b and other complement opsonins blocking their interaction with membrane-bound CR1 (CD35) and accelerating the degradation of C3b by factor I. sCR1 and derivatives have shown promise in rodent models of myocardial infarction and cerebral infarction [68-70]. In clinical trials, sCR1 has shown the ability to block complement activation in patients receiving cardiac surgery with cardiopulmonary bypass and patients receiving lung transplantation [71, 72]. However, improvements in survival were not demonstrated in either group, except when analyzing a male-only subpopulation in the heart surgery trial.

Inhibition of anaphylatoxin effects has shown promise in several animal models of IR injury using a variety of strategies. Anti-anaphylatoxin strategies attempted in animals include: monoclonal

anti-C5a antibody, anti-C5 minibody, C5a receptor antagonists, RNA-interference mediated inhibition of C5a receptor expression, and a C3a receptor antagonist [73-79]. Humanized anti-C5 antibody has shown some promise in several clinical trials of IR injury. These trials include: evaluating cognitive deficits after cardiopulmonary bypass, myocardial infarction treated with percutaneous angioplasty, and coronary artery bypass grafting with cardiopulmonary bypass [80-84]. However, when evaluated in comparison with currently available therapies in clinical trials of myocardial infarction, the benefits of anti-C5 antibody is less clear [85]. Although a promising strategy, it is inherently limited to effectors downstream of C3 activation and will not affect inflammation potentially mediated by C4a, C4b, C3b, or C3a.

Coat protein peptide inhibition of classical and lectin pathway activation

Astrovirus coat protein inhibition of the classical and lectin pathways

Human astroviruses are a major cause of diarrhea in human infants [86]. The virus replicates in intestinal epithelial cells, but produces no inflammation in the intestinal tissues [87]. We showed in hemolytic complement assays that lysates made from cell culture infected with HAstV serotypes 1, 2, 3, and 4 all strongly inhibited serum complement activation, >84% [88]. Human astrovirus 1 (HAstV-1) is the most common serotype worldwide [89]. Recombinantly expressed HAstV-1 coat protein (CP) was able to inhibit complement-mediated hemolysis >90%. Subsequent testing showed that CP was able to inhibit the activation of C3, C5, and C5b-9 in serum, demonstrating that the generation of downstream effectors was inhibited [88]. CP strongly inhibited activation of the classical pathway and antibody-initiated complement activation in a variety of systems, but had no significant effects on the alternative pathway. CP inhibited activation of C1, as assayed by C1s activation, preventing the activation of C4 in serum. Moreover, complement activation could be restored with the addition of exogenous C1. CP bound to both the globular head regions and collagen-like regions (CLR) of C1q, but had higher affinity for the CLR, where the C1s-C1r-C1r-C1s tetramer is nestled. C1s was displaced from intact C1 by CP, suggesting the displacement of the cognate serine protease(s) is the likely mechanism of inhibiting C1 activation [90]. CP also inhibited mannan activation of MBL/MASP-2 in human serum [90]. MBL with a point mutation that prevents binding to MASP-2 (i.e. Lys55Gln substitution) cannot bind CP, suggesting that CP competes for the cognate serine protease binding site of MBL, similar to C1.

Coat protein peptide inhibition of antibody-initiated complement activation

Within the amino acid sequence of the CP molecule, we identified a 60-residue region of homology corresponding to human neutrophil peptide 1 (HNP-1). HNP-1 has been identified by other investigators as an inhibitor of C1 and MBL [91, 92]. From the region of homology, two synthetic peptides were generated of 30 residues each.

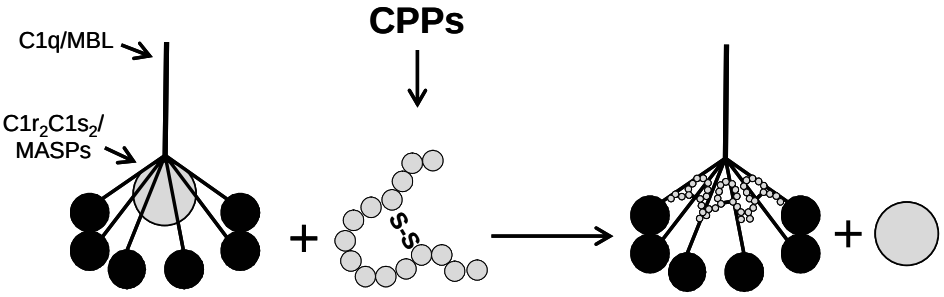

Fig. 3. A model of coat protein peptides (CPPs) interfering with the normal association of C1q/MBL with their cognate serine proteases (C1r2C1s2/MASPs). CPP functionally displace the C1r2C1s2/MASPs by binding to the CLR of C1q/MBL, respectively.

Coat protein peptide 1 (CPP1) inhibited the binding of CP to C1q and inhibited C1 activation, as assayed by C1s cleavage, suggesting a similar mechanism of action as the parent compound [93]. Coat protein peptide 2 did not demonstrate either of these properties, demonstrating that the active region of CP could be successfully narrowed to a small peptide construct. Additional truncations demonstrated that removing the central region of CPP1 (Δ8-22) yielded a functional peptide of 15 residues [93]. The Δ8-22 construct inhibited antibody-initiated complement activation to a greater degree than CPP1, as demonstrated in hemolytic as well as C4 activation assays and similar to the parent CP, the Δ8-22 construct still had minimal effect on the alternative pathway [93]. These efforts have yielded a 15-residue peptide construct that is specific for inhibiting antibody-initiated complement activation. A model of the mechanism by which CP and the CPP derivatives inhibit C1 and MBL activation is presented in Fig. 3.

CONCLUSIONS

IR injury is a very appealing potential application for complement inhibition strategies. Intervention is possible in most situations of IR injury where the reperfusion injury occurs after or during medical treatment. Thus, therapeutic compounds can be instilled along with resuscitation fluids. Because anti-IR compounds will be delivered intravenously with resuscitation fluids, oral bioavailability is not critical. Much of the pathogenic process of reperfusion injury involves the endothelial cells of the vascular bed where resuscitation fluids and therapeutic compounds are delivered. Additionally, there is potential for directed treatment of organs in settings such as angioplasty and organ transplantation. Reperfusion injury occurs in minutes to hours, such that prolonged complement inhibition is not required.

To date, there is ample evidence in a variety of animal models that complement activation occurs during the ischemia-reperfusion process and contributes to IR injury. It is fairly well established, at this point, that the mechanism of complement-mediated IR injury begins with the display of ischemic antigens on endothelial cells leading to the binding of natural antibodies and activation of C1 and the classical pathway, or MBL/MASPs and the lectin pathway, or both. Downstream effectors that are likely to play a role are C3b/CR1 interactions, anaphylatoxins (i.e. C3a and C5a), and membrane attack complex (MAC) formation. Thus far, clinical trial results of inhibiting complement-mediated IR injury have shown some promising results, but pronounced improvements in clinical outcomes (i.e. death) have not been definitive. Clinical trials have either attempted to prevent initial complement activation with C1-INH or inhibit downstream effectors using sCR1 or anti-C5 antibodies. At this point in their current formulations, none of these strategies appear to embody an optimal combination of efficacy, safety, and cost.

A strategy for inhibiting complement-mediated IR injury that encompasses several appealing properties is targeting classical and lectin pathway inhibition at the first components of each pathway, C1 and MBL/MASPs. Inhibiting complement at the initial components of activation would prevent downstream generation of all the important complement effectors of inflammation including complement opsonins, anaphylatoxins, and membrane attack complexes. Ideally, the immune surveillance activities of the alternative pathway would be maintained intact decreasing the risk of severe infections by microbial pathogens. Coat protein peptides (CPPs) are small peptide inhibitors that specifically inhibit the classical and lectin pathways (Fig. 1) and would appear to be ideal for inhibiting complement-mediated IR injury. These CPPs are easily and inexpensively synthesized and do not carry the risk of potentially transmitting blood borne pathogens. Given that they are derived from astrovirus, which does not demonstrate cytotoxicity, they are likely to have a favorable safety profile. However, these properties need to be demonstrated to occur in animal models of IR injury. It remains likely that whatever anti-complement strategy appears to be ideal for inhibiting complement-mediated IR injury, it will need to be combined with other therapies that protect against metabolic mechanisms of pathogenesis and reactive oxygen species, in order to yield optimal clinical outcomes.

REFERENCES

1. Kohl, J. 2006, Adv. Exp. Med. Biol., 586, 71.
2. Banz, Y. and Rieben, R. 2011, Ann. Med., Early Online, 1.
3. Sarma, J. V. and Ward, P. A. 2011, Cell. Tissue Res., 343, 227.
4. Ricklin, D., Hajishengallis, G., Yang, K., and Lambris, J. D. 2010, Nat. Immunol., 11, 785.
5. Cooper, N. R. 1985, Adv. Immunol., 37, 151.
6. Poon, P. H., Schumaker, V. N., Phillips, M. L., and Strang, C. J. 1983, J. Mol. Biol., 168, 563.
7. Gaboriaud, C., Thielens, N. M., Gregory, L. A., Rossi, V., Fontecilla-Camps, J. C., and Arlaud, G. J. 2004, Trends Immunol., 25, 368.
8. Gaboriaud, C., Teillet, F., Gregory, L. A., Thielens, N. M., and Arlaud, G. J. 2007, Immunobiology, 212, 279.
9. Gorski, J. P., Hugli, T. E., and Muller-Eberhard, H. J. 1981, J. Biol. Chem., 256, 2707.
10. Law, S. K. and Dodds, A. W. 1997, Protein Sci., 6, 263.
11. Bohlson, S. S., Fraser, D. A., and Tenner, D. A. 2007, Mol. Immunol., 44, 33.
12. Wallis, R., Mitchell, D. A., Schmid, R., Schwaeble, W. J., and Keeble, A. H. 2010, Immunobiology, 215, 1.
13. Figueroa, J. E. and Densen, P. 1991, Clin. Microbiol. Rev., 4, 359.
14. Ram, S., Lewis, L. A., and Rice, P. A. 2010, Clin. Microbiol. Rev., 23, 740.
15. Daha, M. R. 2010, Crit. Rev. Immunol., 30, 47.
16. Zipfel, P. F. and Skerka C. 2009, Nat. Rev. Immunol., 9, 729.
17. Song, W. C. 2006, Autoimmunity, 39, 403.
18. Markiewski, M. M. and Lambris, J. D. 2007, Am. J. Pathol., 171, 715.
19. Kumar, V., Abbas, A. K., Fausto, N., and Astereds, J. 2010, Robbins and Cotran Pathologic Basis of Disease. 8 (Ed.) Saunders Elsevier: Philadelphia.
20. Jennings, R. B., Murry, C. E., Steenbergen, C. Jr., and Reimer, K. A. 1990, 82 (3 Suppl.), II2.
21. Jensen, F. E. 2005, J. Child Neurol., 20, 950.
22. Leuchtmann, E. A., Ratner, A. E., Vijitruth, R., Qu, Y., and McDonald, J. W. 2003, Neurobiol. Dis., 14, 336.
23. Ankarcrona, M., Dypbukt, J. M., Bonfoco, E., Zhivotovsky, B., Orrenius, S., Lipton, S. A., and Nicotera, P. 1995, Neuron, 15, 961.
24. Halestrap, A. P., Clarke S. J., and Javadov, J. A. 2004, Cardiovasc. Res., 61, 372.
25. Bolli, R., Jeroudi, M. O., Patel, B. S., DuBose, C. M., Lai, E. K., Roberts, R., and McCay, P. B. 1989, Proc. Natl. Acad. Sci. USA., 86, 4695.
26. Verma, S., Fedak, P. W., Weisel, R. D., Butany, J., Rao, V., Maitland, A., Li, R. K., Dhillon, B., and Yau, T. M. 2002, Circulation, 105, 2332.
27. Toyokuni, S. 1999, Pathol. Int., 49, 91.
28. Vinten-Johansen, J. 2004, Cardiovasc. Res., 61, 481.
29. Rodrigues, S. F. and Granger, D. N. 2010, Cardiovasc. Res., 87, 291.
30. Peerschke, E. I., Yin, W., and Ghebrehiwet, B. 2010, Mol. Immunol., 47, 2170.
31. Gawaz, M. 2004, Cardiovasc. Res., 61, 498.
32. Zhang, M., Takahashi, K., Alicot, E. M., Vorup-Jensen, T., Kessler, B., Thiel, S., Jensenius, J. C., Ezekowitz, R. A., Moore, F. D., and Carroll, M. C. 2006, J. Immunol., 177, 4727.
33. Kulik, L., Fleming, S. D., Moratz, C., Reuter, J. W., Novikov, A., Chen, K., Andrews, K. A., Markaryan, A., Quigg, R. J., Silverman, G. J., Tsokos, G. C., and Holers, V. M. 2009. J. Immunol., 182, 5363.
34. Zhang, M., Alicot, E. M., and Carroll, M. C. 2008. Mol. Immunol., 45, 4036.
35. Keith, M. P., Moratz, C., Egan, R., Zacharia, A., Greidinger, E. L., Hoffman, R. W., and Tsokos, G. C. 2007, Autoimmunity, 40, 208.
36. Fleming, S. D. 2006, Autoimmunity, 39, 379.
37. Chan, R. K., Ding, G., Verna, N., Ibrahim, S., Oakes, S., Austen, W. G. Jr., Hechtman, H. B., and Moore, F. D. Jr. 2004, J. Surg. Res., 122, 29.

38. McMullen, M. E., Hart, M. L., Walsh, M. C., Buras, J., Takahashi, K., and Stahl, G. L. 2006, Immunobiology, 211, 759.
39. Diepenhorst, G. M., van Gulik, T. M., and Hack, C. E. 2009, Ann. Surg., 249, 889.
40. Zhou, W., Farrar, C. A., Abe, K., Pratt, J. R., Marsh, J. E., Wang, Y., Stahl, G. L., and Sacks, S. H. 2000, J. Clin. Invest., 105, 1363.
41. Chen, Z., Koralov, S. B., Gendelman, M., Carroll, M. C., and Kelsoe, G. 2000, J. Immunol., 164, 4522.
42. Fleming, S. D., Egan, R. P., Chai, C., Girardi, G., Holers, V. M., Salmon, J., Monestier, M., and Tsokos, G. C. 2004, J. Immunol., 173, 7055.
43. Zhang, M., Michael, L. H., Grosjean, S. A., Kelly, R. A., Carroll, M. C., and Entman, M. L. 2006, J. Mol. Cell Cardiol., 41, 62.
44. Woods, K. M., Pope, M. R., Hoffman, S. M., and Fleming, S. D. 2011, J. Immunol., 186, 1755.
45. Kyriakides, C., Austen, W., Jr., Wang, Y., Favuzza, J., Kobzik, L., Moore, F. D., Jr., and Hechtman, H. B. 1999, Am. J. Physiol., 277(6 Pt 1), C1263.
46. Fondevila, C., Shen, X. D., Tsuchihashi, S., Uchida, Y., Freitas, M. C., Ke, B., Busuttil, R. W., and Kupiec-Weglinski, J. W. 2008, Liver Transpl., 14, 1133.
47. Bellamy, M. C., Gedney, J. A., Buglass, H., Gooi, J. H. and Leeds Liver Group. 2004, Liver Transpl., 10, 273.
48. Imm, M. D., Feldhoff, P. W., Feldhoff, R. C., and Lassiter, H. A. 2002, Neurosci. Lett., 325, 175.
49. Wagner, E. and Frank M. M. 2010, Nat. Rev. Drug Discov., 9, 43.
50. Jiang, H., Wagner, E., Zhang, H., and Frank, M. M. 2001, J. Exp. Med., 194, 1609.
51. Buerke, M., Murohara, T. and Lefer, A. M. 1995, Circulation, 91, 393.
52. Gesuete, R., Storini, C., Fantin, A., Stravalaci, M., Zanier, E. R., Orsini, F., Vietsch, H., Mannesse, M. L., Ziere, B., Gobbi, M., and De Simoni, M. G. 2009, Ann. Neurol., 66, 332.
53. Heijnen, B. H., Straatsburg, I. H., Padilla, N. D., Van Mierlo, G. J., Hack, C. E., and Van Gulik, T. M. 2006, Clin. Exp. Immunol., 143, 15.
54. Karpel-Massler, G., Fleming, S. D., Kirschfink, M., and Tsokos, G. C. 2003. J. Surg. Res., 115, 247.
55. Castellano, G., Melchiorre, R., Loverre, A., Ditonno, P., Montinaro, V., Rossini, M., Divella, C., Battaglia, M., Lucarelli, G., Annunziata, G., Palazzo, S., Selvaggi, F. P., Staffieri, F., Crovace, A., Daha, M. R., Mannesse, M., van Wetering, S., Paolo Schena, F., and Grandaliano, G. 2010, Am. J. Pathol., 176, 1648.
56. Bauernschmitt, R., Bohrer, H., and Hagl, S. 1998, Intensive Care Med., 24, 635.
57. de Zwaan, C., van Dieijen-Visser, M. P., and Hermens, W. T. 2003. Am. J. Cardiovasc. Drugs, 3, 245.
58. Thielmann, M., Marggraf, G., Neuhäuser, M., Forkel, J., Herold, U., Kamler, M., Massoudy, P., and Jakob, H. 2006. Eur. J. Cardiothorac. Surg., 30, 285.
59. Fattouch, K., Bianco, G., Speziale, G., Sampognaro, R., Lavalle, C., Guccione, F., Dioguardi, P., and Ruvolo, G. 2007, Eur. J. Cardiothorac. Surg., 32, 326.
60. Arzneimittelkommission der Deutschen Aerzteschaft. Schwerwiegende Thrombenbildung nach Berinert HS. Deutsches Aerzteblatt, 2000, 97, 1016.
61. Haas, M. S., Alicot, E. M., Schuerpf, F., Chiu, I., Li, J., Moore, F. D., and Carroll, M. C. 2010, Cardiovasc. Res., 87, 618.
62. Johansson, H., Goto, M., Dufrane, D., Siegbahn, A., Elgue, G., Gianello, P., Korsgren, O., and Nilsson, B. 2006, Am. J. Transplant., 6, 305.
63. Strunk, R. and Colten, H. R. 1976, Clin. Immunol. Immunopathol., 6, 248.
64. Tanhehco, E. J., Kilgore, K. S., Naylor, K. B., Park, J. L., Booth, E. A., and Lucchesi, B. R. 1999, J. Cardiovasc. Pharmacol., 34, 153.
65. Lauver, D. A., Booth, E. A., White, A. J., Poradosu, E., and Lucchesi, B. R. 2005, J. Pharmacol. Exp. Ther., 312, 794.

66. Buerke, M., Schwertz, H., Seitz, W., Meyer, J., and Darius, H. 2001, J. Immunol., 167, 5375.
67. Buerke, M., Schwertz, H., Längin, T., Buerke, U., Prondzinsky, R., Platsch, H., Richert, J., Bomm, S., Schmidt, M., Hillen, H., Lindemann, S., Blaschke, G., Müller-Werdan, U., Werdan, K. 2006, Biochim. Biophys. Acta., 1764, 1536.
68. Weisman, H. F., Bartow, T., Leppo, M. K., Marsh, H. C. Jr., Carson, G. R., Concino, M. F., Boyle, M. P., Roux, K. H., Weisfeldt, M. L., and Fearon, D. T. 1990, Science, 249, 146.
69. Huang, J., Kim, L. J., Mealey, R., Marsh, H. C., Jr., Zhang, Y., Tenner, A. J., Connolly, E. S. Jr., and Pinsky, D. J. 1999, Science, 285, 595.
70. Zacharowski, K., Otto, M., Hafner, G., Marsh, H. C. Jr., and Thiemermann, C. 1999, Br. J. Pharmacol., 128, 945.
71. Lazar, H. L., Keilani, T., Fitzgerald, C. A., Shapira, O. M., Hunter, C. T., Shemin, R. J., Marsh, H. C., Jr., Ryan, U. S., and TP10 Cardiac Surgery Study Group. 2007, Circulation, 116(11 Suppl.), I83.
72. Keshavjee, S., Davis R. D., Zamora, M. R., de Perrot, M., and Patterson, G. A. 2005, J. Thorac. Cardiovasc. Surg., 129, 423.
73. Amsterdam, E. A., Stahl, G. L., Pan, H. L., Rendig, S. V., Fletcher, M. P., and Longhurst, J. C. 1995, Am. J. Physiol., 268(1 Pt 2), H448.
74. Ferraresso, M., Macor, P., Valente, M., Della Barbera, M., D'Amelio, F., Borghi, O., Raschi, E., Durigutto, P., Meroni, P., and Tedesco, F. 2008, Transplantation, 86, 1445.
75. Arumugam, T. V., Woodruff, T. M., Stocks, S. Z., Proctor, L. M., Pollitt, S., Shiels, I. A., Reid, R. C., Fairlie, D. P., and Taylor, S. M. 2004, J. Hepatol., 40, 934.
76. Kim, G. H., Mocco, J., Hahn, D. K., Kellner, C. P., Komotar, R. J., Ducruet, A. F., Mack, W. J., and Connolly, E. S., Jr. 2008, Neurosurgery, 63, 122.
77. Zheng, X., Zhang, X., Feng, B., Sun, H., Suzuki, M., Ichim, T., Kubo, N., Wong, A., Min, L. R., Budohn, M. E., Garcia, B., Jevnikar, A. M., and Min, W. P. 2008, Am. J. Pathol., 173, 973.
78. Proctor, L. M., Arumugam, T. V., Shiels, I., Reid, R. C., Fairlie, D. P., and Taylor, S. M. 2004, Br. J. Pharmacol., 142, 756.
79. Rynkowski, M. A., Kim, G. H., Garrett, M. C., Zacharia, B. E., Otten, M. L., Sosunov, S. A., Komotar, R. J., Hassid, B. G., Ducruet, A. F., Lambris, J. D., and Connolly, E. S. 2009, J. Cereb. Blood Flow Metab., 29, 98.
80. Mathew, J. P., Shernan, S. K., White, W. D., Fitch, J. C., Chen, J. C., Bell, L., and Newman, M. F. 2004, Stroke, 35, 2335.
81. Granger, C. B., Mahaffey, K. W., Weaver, W. D., Theroux, P., Hochman, J. S, Filloon, T. G., Rollins, S., Todaro, T. G., Nicolau, J. C., Ruzyllo, W., Armstrong, P. W. and COMMA Investigators. 2003, Circulation, 108, 1184.
82. Mahaffey, K. W., Granger, C. B., Nicolau, J. C., Ruzyllo, W., Weaver, W. D., Theroux, P., Hochman, J. S., Filloon, T. G., Mojcik, C. F., Todaro, T. G., Armstrong, P. W., and COMPLY Investigators. 2003, Circulation, 108, 1176.
83. Smith, P. K., Shernan, S. K., Chen, J. C., Carrier, M., Verrier, E. D., Adams, P. X., Todaro, T. G., Muhlbaier, L. H., Levy, J. H., and PRIMO-CABG II Investigators. 2011, J. Thorac. Cardiovasc. Surg., 142, 89.
84. Haverich, A., Shernan, S. K., Levy, J. H., Chen, J. C., Carrier, M., Taylor, K. M., Van de Werf, F., Newman, M. F., Adams, P. X., Todaro, T. G., van der Laan, M., and Verrier, E. D. 2006, Ann. Thorac. Surg., 82, 486.
85. Testa, L., Van Gaal, W. J., Bhindi, R., Biondi-Zoccai, G. G., Abbate, A., Agostoni, P., Porto, I., Andreotti, F., Crea, F., and Banning, A. P. 2008, J. Thorac. Cardiovasc. Surg., 136, 884.
86. Shastri, S., Doane, A. M., Gonzales, J., Upadhyayula, U., and Bass, D. M. 1998, J. Clin. Microbiol., 36, 2571.
87. Koci, M. D., Moser, L. A., Kelley, L. A., Larsen, D., Brown, C. C., Schultz-Cherry, S. 2003, J. Virol., 77, 11798.
88. Bonaparte, R. S., Hair, P. S., Banthia, D., Marshall, D. M., Cunnion, K. M., and Krishna, N. K. 2008, J. Virol., 82, 817.

89. Matsui, S. M., and Greenberg, H. B. Astroviruses in Fields Virology, Knipe, D. M. and Howley, P. M. Editors. 2001, Lippincott Williams & Wilkins: Philadelphia. p. 875-893.
90. Hair, P. S., Gronemus, J. Q., Crawford, K. B., Salvi, V. P., Cunnion, K. M., Thielens, N. M., Arlaud, G. J., Rawal, N., and Krishna, N. K. 2010, Mol. Immunol., 47, 792.
91. van den Berg, R. H., Faber-Krol, M. C., van Wetering, S., Hiemstra, P. S., and Daha, M. R. 1998, Blood, 92, 3898.
92. Groeneveld, T. W., Ramwadhdoebé, T. H., Trouw, L. A., van den Ham, D. L., van der Borden, V., Drijfhout, J. W., Hiemstra, P. S., Daha, M. R., and Roos, A. 2007, Mol. Immunol., 44, 3608.
93. Gronemus, J. Q., Hair, P. S., Crawford, K. B., Nyalwidhe, J. O., Cunnion, K. M., and Krishna, N. K. 2010, Mol. Immunol., 48, 305.
94. Halas, Y. A., Rahal, E., Abdelnoor, A. M., Haddad, R., and Abchee, A. 2005, Immunopharmacol. Immunotoxicol., 27, 405.
95. Busche, M. N., Walsh, M. C., McMullen, M. E., Guikema, B. J., and Stahl, G. L. 2008, Diabetologia, 51, 1544.
96. Ten, V. S., Sosunov, S. A., Mazer, S. P., Stark, R. I., Caspersen, C., Sughrue, M. E., Botto, M., Connolly, E. S. Jr., and Pinsky, D. J. 2005, Stroke, 36, 2244.
97. Cervera, A., Planas, A. M., Justicia, C., Urra, X., Jensenius, J. C., Torres, F., Lozano, F., and Chamorro, A. 2010, PLoS One, 5, e8433.
98. van der Pol, P., Roos, A., Berger, S. P., Daha, M. R., and van Kooten, C. 2011, Am. J. Physiol. Renal Physiol., 300, F932.
99. Moller-Kristensen, M., Wang, W., Ruseva, M., Thiel, S., Nielsen, S., Takahashi, K., Shi, L., Ezekowitz, A., Jensenius, J. C., and Gadjeva, M. 2005, Scand. J. Immunol., 61(5), 426-34.
100. Lee, H., Green, D. J., Lai, L., Hou, Y. J., Jensenius, J. C., Liu, D., Cheong, C., Park, C. G., and Zhang M. 2010, Mol. Immunol., 47, 972.
101. Hart, M. L., Ceonzo, K. A., Shaffer, L. A., Takahashi, K., Rother, R. P., Reenstra, W. R., Buras, J. A., and Stahl, G. L. 2005, J. Immunol., 174, 6373.
102. Chan, R. K., Ibrahim, S. I., Takahashi, K., Kwon, E., McCormack, M., Ezekowitz, A., Carroll, M. C., Moore, F. D., Jr., and Austen, W. G. Jr. J. 2006. J. Immunol., 177, 8080.
103. Tjernberg, J., Ekdahl, K. N., Lambris, J. D., Korsgren, O., and Nilsson, B. 2008, Transplantation, 85, 1193.