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The Complement System in Ischemia-Reperfusion Injuries

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Abstract

Tissue injury and inflammation following ischemia and reperfusion of various organs has been recognized for many years. Many reviews have been written over the last several decades outlining the role of complement in ischemia/reperfusion injury. This short review provides a current state of the art knowledge on the complement pathways activated, complement components involved and a review of the clinical biologics/inhibitors used in the clinical setting of ischemia/reperfusion. This is not a complete review of the complement system in ischemia and reperfusion injury but will give the reader an updated view point of the field, potential clinical use of complement inhibitors, and the future studies needed to advance the field.

Ischemia-Reperfusion Injuries

Ischemia-reperfusion injuries are characterized by an initial deprivation of blood flow to tissues/organs followed by restoration of blood flow. The initial ischemic period results in cellular changes that alter subsequent signaling pathways and molecular expression (Montalto et al., 2003; Wada et al., 2001; Liangos et al., 2010). Glycolysis provides adenosine triphosphate, which during ischemia becomes depleted, and results in increased lactic acid formation and a decrease in pH. As this process continues phospholipase A₂ converts phospholipids in the cell membrane into arachidonic acid, which functions as a precursor for the biosynthesis of leukotrienes and prostaglandins (Prasad et al., 1991; Goulet et al., 1994). Activation of polymorphonuclear leukocytes (PMN's), eicosanoids, cytokines, reactive oxygen species (ROS) and complement products have been shown by many groups to be involved in this initial phase (Arumugam et al., 2004). The intracellular and extracellular accumulation of these products triggers homeostatic pathways involving necrosis, apoptosis and possibly autophagy. The apoptotic response may then conclude with potential permanent tissue or end organ dysfunction.

A reduction in oxygen supply induces neutrophil adherence to endothelial cells (Riedemann and Ward, 2003). A hallmark of the reperfusion period is characterized by increased leukocyte adhesion to the vascular endothelium (Nolte et al., 1991). Expression of leukocyte adhesion molecules during the ischemic period allows for their increased anchoring to the

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vascular endothelium by P- and L-selectin (Lefer et al., 1994; Stotland and Kerrigan, 1997). Leukocyte accumulation during reperfusion induces significant increases in permeability of post capillary venues and toxic product deposition (Lehr et al., 1994).

The Complement System

The classical, lectin and alternative pathways interact and comprise the complement system (Figure 1). The initiation molecules of the classical, lectin and alternative pathways are C1q, mannose-binding lectin(MBL)/ficolins/Collectin-11 and C3b, respectively. All three pathways converge at the activation and cleavage of C3 into C3a and C3b, via separate biochemical processes. The classical pathway drives complement activation through a C1q associated C1r/C1s heterotetramer which is activated when C1q binds to its ligands (primarily Fc regions within immune complexes formed of immunoglobulins of the immunoglobulin classes IgM or IgG 1, 2, or 3). C1r cleaves its only so far known substrate C1s while activated C1s will cleave C4 or C4b-bound C2 to form the C3 convertase C4b2a. The lectin pathway initiation complexes use three different lectin pathway specific serine proteases, termed MBL-associated serine proteases (MASPs), which according to the sequence of their discovery are called MASP-1, MASP-2 and MASP-3. Of those, MASP-2 fulfils analogous activities to the classical pathway key enzyme C1s, as like C1s, MASP-2 can cleave C4 and C4b-bound C2 to form the lectin pathway C3 convertase C4b2a. In contrast to C1s, MASP-2 can drive lectin pathway activation in absence of any of the other two MASPs. Although MASP-1 can facilitate the process to form the C3 convertase complex by its ability to cleave C4b-bound C2, it is not capable to compensate for the loss of MASP-2 functional activity, since it cannot cleave C4 (Schwaebler et al., 2011). This underlines the essential role of MASP-2 in driving the lectin pathway of complement activation. Reports have indicated essential roles of the lectin pathway serine proteases MASP-1 and -3 in the initiation of the alternative pathway of complement activation (Fujita et al., 1999; Fujita, 2002; Fujita et al., 2004). The alternative pathway forms a feedback amplification loop of complement activation, as the alternative pathway C3 convertase complex is formed by C3b or C3b-like H₂OC3 and complement factor B, which is converted from its zymogen form C3bB into the alternative pathway C3 convertase C3bBb by factor D. The C3 convertase complexes of the classical and the lectin pathway, i.e. C4b2a and the alternative pathway, i.e. C3Bb, can switch their substrate specificities from C3 to C5 when multiple C3b molecules are covalently attached to close proximity to these complexes. These C5 convertase complexes, i.e. C4b2a (C3b)_n and C3bBb(C3b)_n finalize the enzyme mediated cascade activation events by the conversion of C5 into C5a and C5b. The anaphylatoxin, C5a, is a potent chemoattractant and triggers inflammation and activation of leukocytes, including PMNs. C5b assembles with terminal complement components C6, C7, C8, and C9 to form C5b-9. C5b-9 initiates cellular activation of nucleated cells, as well as lysis of anuclear cells (Walport, 2001a; Walport, 2001b).

Historical Perspectives of Complement Research in I/R Injuries

Initial documented complement mediated injury studies during ischemia were reported in a myocardial ischemia model in which C3 cleavage was associated with chemotaxis and leukocyte activation (Hill and Ward, 1971). Myocardial ischemia-reperfusion models would become a prominent model used in the following years to investigate complement activation and its role in I/R (MacLean et al., 1978; Pinckard et al., 1980; Weisman et al., 1990; Burke et al., 1992; Vakeva et al., 1998; Amsterdam et al., 1995; Jordan et al., 2001; Walsh et al., 2005). Complement activation in I/R is observed in multiple organs. Ischemia/reperfusion models have studied complement's involvement in the gastrointestinal system (Wada et al., 2001; Fruchterman et al., 1998; Williams et al., 1999; Karpel-Massler et al., 2003; Zhao et al., 2002; Tofukuji et al., 2000), skeletal muscle (Weiser et al., 1996; Toomayan et al., 2003;

Brock et al., 2001; Wong et al., 1999; Kyriakides et al., 1999; Chan et al., 2006), kidneys (Zhou et al., 2000; Thurman et al., 2003), brain (Tofukuji et al., 1999), systemic shock (Spain et al., 1999) and lungs (Peng et al., 2005).

There is some degree of controversy as to what molecules and pathways are involved in the initiation of complement activation following I/R in the literature. For example, early studies using RAG1^{-/-} mice (Williams et al., 1999) or wild type mice treated with sCR1 compromised the identification of complement involvement (Hill et al., 1992). C4^{-/-} and IgM^{-/-} mice studied in models of gastrointestinal ischemia/reperfusion (GI/R) presented with a decreased deposition of complement C3 in reperfused ischemic tissues suggesting an involvement of antibody-mediated classical pathway activation (Williams et al., 1999). The absence of C4 would necessarily inhibit complement activation via the classical pathway as would the absence of C1q (Williams et al., 1999), while C4 deficient mouse and human serum and plasma could still maintain a residual lectin pathway activation route (Schwaebble et al., 2011). The physiological importance of a C4 independent bypass activation of the lectin pathway is underlined by independent recent reports showing that C4 deficient mice are not protected from I/R following renal transplantation (Lin et al., 2006), whilst MASP-2 deficient mice are (Farrar et al., 2009). Factor D (fD) ^{-/-} mice undergoing GI/R were protected against GI and pulmonary injury, but C3 deposition was still observed in the lungs and GI tract (Stahl et al., 2003). C2/fB^{-/-} mice were also protected from GI/R injury. However, reconstitution of C2/fB ^{-/-} mice with human C2 resulted in intestinal and pulmonary injury (Hart et al., 2005). These studies suggested that complement was activated by a C2-dependent mechanism and injury was amplified by alternative pathway activation.

Skeletal muscle I/R models also examined the role of complement. C3^{-/-} or C4^{-/-} mice were protected from vascular permeability following skeletal muscle I/R. RAG2^{-/-} mice were also protected from injury, whereas reconstitution with WT sera restored a WT phenotype following I/R (Weiser et al., 1996). Thus, another animal model revealed an antibody-dependent mediated complement activation following I/R, which would suggest initiation of the classical pathway.

As explanations for these previous studies using RAG1^{-/-} and RAG2^{-/-} mice were being postulated, additional reports in renal models of I/R were investigated. C4^{-/-} mice undergoing renal I/R were not protected from injury. In contrast, C3^{-/-}, C5^{-/-} or C6^{-/-} mice were afforded protection from renal I/R injury. In the absence of the knowledge that lectin pathway can operate via a C4-bypass activation route it was suggested that initiation of complement activation is primarily driven by the alternative pathway. Furthermore, C6^{-/-} mice treated with anti-C5 monoclonal antibody (mAb) were not further protected from I/R injury. The data suggested that C5b-9 mediated renal tubular injury following I/R (Zhou et al., 2000).

A pathophysiological relationship between oxidative stress, expression of neo-epitopes on endothelial cells and subsequent activation of immunological components, including the complement system existed in the literature (Ogawa et al., 1991). Early studies, using a reductionist model of low oxygen tension (hypoxia) and endothelial cells, demonstrated the need for C2 in the activation of complement on human endothelial cells in vitro and deposition of C3b (Collard et al., 1997). These results were further confirmed by an independent study showing C3b deposition on stressed human endothelial cells in the absence of immune complexes that could have driven the classical activation pathway of complement (Vakeva and Meri, 1998).

The specific role of MBL and complement activation following oxidative stress in vitro and also in vivo were studied in multiple models (Montalto et al., 2001; Lekowski et al., 2001;

Collard et al., 1999; Collard et al., 2000; Collard et al., 2001; Jordan et al., 2001) and were the logical extension of these previous in vitro studies (Vakeva and Meri, 1998; Collard et al., 1997). Monoclonal antibodies that recognized MBL were developed and characterized for their ability to inhibit MBL binding and lectin complement pathway activation. Functional inhibition of MBL binding to stressed endothelium inhibited complement activation and subsequent C3b deposition (Collard et al., 2000; Montalto et al., 2001; Lekowski et al., 2001). Reperfusion of ischemic myocardium was necessary for not only MBL binding but also C3 deposition in the rat (Collard et al., 1997). Preservation of rat myocardium from myocardial ischemia/reperfusion (MI/R) injury, inflammation and complement activation was observed with anti-MBL-A mAb treatment (Jordan et al., 2001). These early studies led to continued research on the role of classical, lectin and alternative pathways in I/R injury, as well as additional models of human disease.

Knowledge Discovery in Current Models

Research into complement's role in the immunological response to I/R has produced many elegant studies in the literature. These early studies have also contributed to additional gains in our knowledge of complement's role in I/R, as well as its interaction with hyperglycemia and MBL-dependent lectin pathway complement activation following MI/R, skeletal I/R and gastrointestinal ischemia/reperfusion (GI/R) (Chan et al., 2006; Pavlov et al., 2012; Hart et al., 2005; Walsh et al., 2005; Murata et al., 2007; La Bonte et al., 2008; Busche et al., 2008; La Bonte et al., 2009; Busche et al., 2009; Schwaebler et al., 2011; Busche and Stahl, 2010). These scientific advances have also aided in the clarification and confirmation that the classical pathway does not initiate complement activation following I/R nor does the absence of its initiation molecule (e.g., C1q) prevent I/R injuries of the primary ischemic organ (Hart et al., 2005; Walsh et al., 2005). Murata et al (Murata et al., 2007) examined the role of MBL in humoral rejection of B10A hearts transplanted into immunoglobulin deficient (Ig-KO) mice. Ig-KO mice given monoclonal antibody to MHC class 1 antigens demonstrated no C4d deposition. Further, reconstitution with IgG1 or low dose IgG2b also did not deposit C4d. In contrast, IgG1 and IgG2b reconstitution demonstrated complement activation and C4d deposition on the coronary endothelium. Additional in vitro studies demonstrated that this complement activation was MBL-dependent and raised a novel hypothesis of non-complement activating antibodies and MBL in humoral rejection (Murata et al., 2007).

La Bonte et al (La Bonte et al., 2008) using a model of Type 2 DM demonstrated increased inflammation, infarct size and tissue injury following MI/R compared to controls that was significantly inhibited by the non-specific serine protease inhibitor, FUT-175 (La Bonte et al., 2008). The role of complement in hyperglycemia was also examined in a model of Type 1 DM in mice (Busche et al., 2008). Hyperglycemic MBL null mice were protected from the significant dilated cardiomyopathy and increased tissue injury and inflammation compared to hyperglycemic WT mice (Busche et al., 2008). La Bonte et al (La Bonte et al., 2009) further evaluated the role of MBL in MI/R in the Type 2 DM model by treatment with anti-MBL-A mAb or FUT-175. Both treatment groups revealed similar inhibition of injury and inflammation, revealing an important role of MBL in hyperglycemic MI/R (La Bonte et al., 2009). Pavlov et al (Pavlov et al., 2012) demonstrated a critical role of MBL in acute hyperglycemia-induced arteriole dysfunction (e.g., loss of nitric oxide) and cardiomyopathy. These basic science findings confirm the clinical observations in Type 1 DM and point to the importance of functional MBL levels and vascular homeostasis in DM (Hansen et al., 2004; Hansen et al., 2003; Hovind et al., 2005). Further, MBL binds to fructosamines and provides potential important binding sites for the resulting complement activation in DM (Fortpiet et al., 2010). These studies have revealed an important role of MBL in DM and an

additional therapeutic strategy for preventing cardiovascular morbidity and mortality associated with hyperglycemic states.

The role of MASP-2 in the MBL complex in I/R injuries has recently been examined (Schwaeble et al., 2011). Novel MASP-2 $-/-$ mice or an inhibitory antibody against MASP-2 were used in mouse models of GI/R and MI/R. MASP-2 $-/-$ mice were protected from I/R injury of the gastrointestinal tract and myocardium compared to WT mice. Similarly, functional inhibition of MASP-2 with mAb AbyD04211 (1 mg/kg, 12 hr before GI/R) significantly protected the gastrointestinal tissue two-fold better than an irrelevant isotype control mAb. In an experimental mouse model of stroke, MASP-2 deficient mice showed a significant degree of protection with significantly lower infarct sizes than their wildtype controls. Likewise, application of the therapeutic MASP-2 inhibitor mAb AbyD04211 revealed significant protection for cerebral IRI (Chrysanthou et al., unpublished). This finding is in good agreement with the recent report demonstrating that MBL null mice also show a significant degree of protection in an experimental mouse model of stroke (Cervera et al., 2010).

An additional line of evidence, which underlines a critical role of the lectin pathway component MASP-2 in human I/R comes from a recent clinical study showing that MASP-2 levels are significantly decreased in acute MI patients compared to control (Zhang et al., 2011). Further, coronary circulation of MASP-2 is reduced following global ischemia in patients and correlates with plasma cardiac troponin I levels (Zhang et al., 2011). These data confirm the important role of the MBL complex in MI/R and GI/R and suggest that functional inhibition of MASP-2 or MBL affords tissue protection following I/R. Moreover, a recent paper by Schwaeble et al. points to the importance of re-investigating previous studies using C4 deficient mouse models which have lead to an exclusion of the involvement of the lectin pathway since it is now evident that C4 deficient mice are not necessarily deficient of lectin pathway functional activity (Schwaeble et al., 2011).

Previous studies in gastrointestinal or skeletal I/R models demonstrated that ischemic tissues offered novel binding sites for IgM, which in theory offered a molecular mechanism for classical complement pathway activation (Weiser et al., 1996; Williams et al., 1999). Further, CR2 $-/-$ mice are protected from GI/R injury, whereas reconstitution of this genotype with IgM and IgG restores I/R injuries (Fleming et al., 2002). IgM and complement components are co-localized in human infarcted myocardium (Krijnen et al., 2005). However, other studies using C1q $-/-$ mice suggest that I/R injury was not classical pathway dependent (Hart et al., 2005; Walsh et al., 2005). While MBL and IgM do bind, the J-chain interferes with MBL binding when IgM engages its ligand (Nevens et al., 1992; Arnold et al., 2005). However, compared to the pentameric IgM, the hexameric form of IgM is a more efficient complement activator (Collins et al., 2002; Hughey et al., 1998).

Clarification of the controversy surrounding the role of IgM, MBL and C1q in activation of complement in I/R models has recently been described in several models. In vitro, IgM binds MBL and activates complement on human endothelial cells and sensitized human RBCs (McMullen et al., 2006). In a model of GI/R, using mice that lack circulating IgM (sIgM) and MBL-A and -C, complement activation and tissue injury were present when both sIgM and MBL were reconstituted, in preference each alone. Similarly, findings were also observed in a mouse model of MI/R using the sIgM/MBL null mouse line and reconstitution with MBL and/or IgM (Busche et al., 2009). These data were also confirmed in a similar GI/R model using Rag1 $-/-$ and confirmed co-localization of IgM and MBL, whereas C1q $-/-$ mice were not protected from GI/R injury (Zhang et al., 2006). Combined, these findings suggest that IgM binds to the neo-epitope presented during I/R with subsequent MBL binding and complement activation.

Inhibition of MBL-A also protects kidney function following renal I/R (van de Pol et al., 2012). An interesting aspect of this particular renal I/R model, was that inhibition of complement C5 was ineffective in protecting the kidney from I/R injury. Further, C3 depletion with cobra venom factor was also ineffective in protecting the kidney from I/R injury. These data suggested a direct action of the MBL complex on renal injury following I/R. Using isolated primary human proximal tubular epithelial cells (PTEC) or the HK-2 PTEC line, MBL induced cytotoxicity, which was not apparent on human endothelial cells. Along these same lines, Renner et al (Renner et al., 2010) depleted peritoneal B cells, which prevented IgM deposition in renal glomeruli and attenuated renal injury after I/R. While the glomerular IgM activated the classical pathway, little C3 was deposited. However, neither MBL^{-/-}, C4^{-/-} nor C1q^{-/-} mice were protected from renal I/R injury. Along these lines, factor B (fB) ^{-/-} mice or inhibition of fB protects the kidney from I/R injury (Thurman et al., 2006; Thurman et al., 2003). These studies suggest that that alternative pathway initiates complement activation. Why there appears to be confusion about the importance of MBL in similar renal I/R models remains to be addressed.

Complement and Drug-Target Elucidation in I/R Injuries

Complement drug-target elucidation has attempted to either completely deplete complement components or to inhibit specific pathway components. Few anti-complement therapeutics however have been approved for clinical use. Early MI/R studies used cobra venom factor (CVF) to deplete complement to reduce inflammation and tissue injury (Hill and Ward, 1971; MacLean et al., 1978; Maroko et al., 1978; Pinckard et al., 1980). However, because of immunogenic responses, CVF has not been used clinically. A humanized recombinant form of CVF, HC3-1496, was developed (Vogel and Fritzinger, 2007) and depletes C3, but does not form a functional C5 convertase. In a mouse MI/R model, HC3-1496 was equi-effective to CVF in reducing infarct size and preserving cardiac function following MI/R (Gorsuch et al., 2009).

The first complement specific inhibitor developed for future clinical use was complement receptor type one, sCRI (e.g., TP10). TP10 has been used in many models of I/R and was found efficacious in the vast majority (Xiao et al., 1997; Weisman et al., 1990; Rioux, 2001; Pratt et al., 1996; Mulligan et al., 1992; Kyriakides et al., 2001; Eror et al., 1999). However, the clinical use of TP10 has not met important endpoints to advance into further clinical trials (Lazar et al., 2004; Li et al., 2004).

C1INH complexes with C1 to inhibit C1 and components of the contact system proteases, including factors XIIa, XIa and kallikrein, as well as the lectin complement pathway (Cedzynski et al., 2008; Rossi et al., 2001; Rossi et al., 2005; Davis, III, 2004). Preclinical studies demonstrated the cardioprotective effects of C1INH in GI/R and MI/R injury (Buerke et al., 1995; Horstick et al., 1997; Horstick et al., 2001b; Horstick et al., 2001a). Clinically, C1INH preserved hemodynamic performance and resulted in lower serum troponin levels compared with placebo treated patients undergoing CABG (Fattouch et al., 2007). Additionally, C1INH was used as “rescue therapy” for the treatment of MI/R injury in patients following failed percutaneous transluminal angioplasty (Bauernschmitt et al., 1998). A recombinant form of human C1INH has been made and approved for clinical use in Europe, which also binds and inhibits MBL suggesting that this form of C1INH may have additional advantages over plasma derived C1INH (Gesuele et al., 2009).

There are tremendous numbers of antibodies and proteins with inhibitory actions against various complement components in the literature and this review will not include all of them (Ricklin and Lambris, 2007). Because of the many inflammatory and cellular activating properties of C5a and C5b-9, many biologics have been designed against C5 to inhibit

formation of C5a and C5b-9 or to inhibit C5a function (e.g., the anaphylatoxin or its receptor). These biologics include but are not limited to the following: eculizumab, pexelizumab, Mubodina, Ergidina, TNX-558, and Neutrazumab (Woodruff et al., 2011).

Administration of a pexelizumab decreased overall patient mortality associated with acute myocardial infarction in the COMMA and COMPLY trials, but failed to meet the primary endpoint (Armstrong et al., 2006; Granger et al., 2003; Theroux et al., 2004). Similarly, patients requiring concomitant CABG plus CPB and treated with pexelizumab showed reduced myocardial injury and accompanying disorders during a phase IIa clinical trial (Shernan et al., 2004). Although the primary endpoint for this study was not reached, the study demonstrated an overall reduction in post-operative patient morbidity and mortality and resulted in another clinical trial, PRIMO-CABG II. PRIMO-CABG II did not meet the primary composite endpoint of death or MI (Smith et al., 2011). Further investigation with pexelizumab in CABG or MI is not currently planned as of this writing.

Additional inhibitors of the MBL complex have been recently developed and used in I/R models. MBL/ficolin-associated protein-1 (MAP-1) (Skjoedt et al., 2010) also named MAp44 (Degn et al., 2009) is an endogenous and natural complement inhibitor. MAP-1, at pharmacologic concentrations, displaces MASP-1, -2 and -3 from the MBL complex and significantly inhibits inflammation, complement activation, myocardial dysfunction and coagulation in mice following MI/R (Skjoedt et al., 2012). Polyman2 is a dendrimeric molecule comprised of multiple copies of synthetic mannoside, which is equi-effective as anti-MBL mAb in rat or mouse stroke models. Further, Polyman2 or anti-MBL mAb demonstrated significant reductions in neurological deficits and infarct volumes when therapy was given up to 18 hours after cerebral injury (Orsini et al., 2012). These novel complement inhibitors extend our knowledge about the importance of complement and support/extend the importance of the MBL complex and its associated MASPs in the sequelae of I/R injury.

No matter the method taken, the goal of complement inhibition to prevent IR injuries will in the future have to focus on whether to inhibit all or specific components of complement activation. Whereas certain disease processes may require varying specific inhibition as others may not, it is not yet clear what the overall ramification will be in either component inhibition or complete cascade inhibition. Some components may surely need to remain active as in the recognition of pathogens to prevent bacterial sequela or for low level expression for which certain protection during IR may be required.

Complement I/R Injuries in the era of –omics research

The in vitro and in vivo molecular techniques that have been the mainstay of complement I/R research are now being aided by a new era of –omics based research. Traditional bioinformatics and chemoinformatics have already been used widely in drug-target elucidation. It is however clear that a better understanding of the genetic expression and transcripts involved in complement induced I/R injury is needed in order to develop new classes of therapeutics to either alter the expression levels of complement genes or to inhibit specific transcripts. It is also of vital importance to understand the genetic polymorphisms that are involved or influenced by I/R.

The general genome underlying complement and the I/R transcriptome of such processes must simultaneously be understood. Patients undergoing cardiopulmonary bypass had RNA isolated and globin mRNA depleted from whole blood postoperatively which revealed a gene regulatory network of 50 genes. The pro-inflammatory and protective pathways associated with I/R injury initiated by CPB included upregulation of TLR-4 and -3, IL1R2/IL1RAP, IL6, IL18RAP, MMP-9, HGF/HGFR, Calgranulin-A/B, and coagulation factors V

and XII (Liangos et al., 2010). It is currently not known if these gene transcripts are a “result of” or “assist in” initiation of complement I/R injury. When these clinical studies are more clearly defined, then specifically directed therapies can be developed.

Gene microarrays for knowledge discovery and drug-target elucidation in I/R allow for direct gene expression profiling for not only identification purposes, but for gene function, protein network and pathway analysis. Use of such methods were employed in a hyperglycemia model to study acute vasculopathy, cardiomyopathy, the specific role of MBL (Zou et al., 2012) and other complement components (Stahl laboratory; unpublished data) compared to WT. Knowledge derived from these data sets will allow us to formulate gene interaction sets for therapeutic targeting and hypothesis testing (Zou et al., 2012).

Complement component specific siRNA for C5aR inhibits C5aR gene expression in vivo and in vitro. Treated mice were protected from renal I/R injury, neutrophil influx and cell necrosis in renal tissue by an observed decreased expression of TNF-alpha and chemokines MIP-2 and KC (Zheng et al., 2008). C3 siRNA also reduced complement mediated I/R renal injury and decreased expression of TNF-alpha (Zheng et al., 2006). These studies reveal the usefulness of gene expression analysis when using specific target therapeutics and the resultant insight into interacting network pathways in I/R injuries.

Approximately 5–10% of the Caucasian population display MBL deficiencies, which are due to homozygosity in one of three SNPs in the *MBL2* coding region. The occurrence of MASP-2 deficiency is about 6 in 10,000 (Degn et al., 2011). Deficiencies also exist in the classical pathway, alternative pathway, C3, terminal complement components, and regulators. Approximately 75% of classical pathway deficiencies are due to C4 or C1 complex which can result in systemic lupus erythema (SLE). C3 deficiency is rare but associated with severe life threatening bacterial infections (Botto et al., 2009). These studies not only reveal the importance of understanding polymorphisms and deficiencies in complement, but are also applicable in the development of study designs and focus for drug target elucidation in I/R injuries.

The current era of –omics generated technologies is rapidly changing the face of knowledge discovery in terms of complement I/R injuries. The difficulty however has still remained in advancing these new therapeutics from the bench to bedside. It becomes also important to realize that as more knowledge is obtained, so is its utilization for interpretation and application. With the high rate of –omics driven information gained from highly trained personnel to perform and interpret the results, future studies will need to have the ability to evaluate human genes within an animal vector/model system(s). Only then can true drug target elucidation, including off target binding complications, be assessed for more rapid bench to bedside approval of safe complement I/R therapeutics.

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Figure 1.
Brief graphical representation of the complement system.