

Emerging recognition of the complement system in hepatic ischemia/reperfusion injury, liver regeneration and recovery (Review)

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Abstract. Hepatic ischemia/reperfusion injury (IRI) is a result of the ischemic cascade and may occur in the settings of liver trauma, resection and transplantation. Components of the complement system have been indicated to be mediators of hepatic IRI and regulators of liver regeneration. As such, their potential to mediate both beneficial and harmful effects render them key targets for therapy. In the present study, the mechanisms of complement mediating hepatic IRI were discussed with a focus on the different functions of complement in hepatic injury and liver recovery, and an explanation for this apparent paradox is provided, i.e. that the complement products C3a and C5a have an important role in liver damage; however, C3a and C5a are also necessary for liver regeneration. Furthermore, situated at the end of the complement activation cascade, the membrane attack complex is crucial in hepatic IRI and inhibiting the complex with a site-targeted murine complement inhibitor, complement receptor 2-CD59, may improve liver regeneration after partial hepatectomy, even when hepatectomy is combined with ischemia and reperfusion.

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1. Introduction

Ischemia/reperfusion injury (IRI) of the liver occurs when there is a transient blockage of blood supply to the organ, followed by re-establishment of the blood supply, which results in severe injury to the liver. Hepatic IRI is frequently associated with severe liver trauma and operations on the liver, such as hepatic lobe resection and liver transplantation (1,2). IRI causes an acute inflammatory response, which may lead to local and remote liver cell damage and dysfunction (3,4). Numerous crucial elements of the pathogenesis of hepatic IRI have been identified. These include activated polymorpho-nuclear leukocytes (PMNs) and Kupffer cells (KCs), formation of oxygen radicals and activation of complement (5-7). Complement activation is critical during hepatic IRI, which may rapidly promote complement activation through activating PMNs and KCs by the release of cellular proteins, thereby leading to the formation of reactive oxygen species (ROS) and the promotion of inflammatory response (8).

Studies in animal models of hepatic IRI have increased the understanding of the pathogenesis of the complement system in IRI and guided clinical investigations aimed at preventing or minimizing this condition. Blockade of complement activation has been determined to reduce hepatic IRI (9). Of note, in experimental models of IRI, targeted complement inhibitors complement receptor 2 (CR2)-complement receptor1-related protein (Crry) and CR2-CD59 were demonstrated to improve the ability of the liver to recover after injury (10,11).

In the present review, the current knowledge of complement activation in the pathophysiology of hepatic IRI is summarized with a focus on the importance of targeted complement inhibitors in regulating both the injury and the recovery from

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IRI, highlighting relevant novel approaches and experimental discoveries that may improve the clinical management of liver transplantation and resection surgery.

2. Complement-mediated injury cascades in hepatic IRI

Although the exact mechanisms of hepatic IRI remain to be fully elucidated, factors involved in liver injury include sinusoidal congestion and microcirculatory dysfunction, adhesion-molecule expression, aggregation and activation of leukocyte and platelets, free radicals, pro-inflammatory cytokines and complement activation products (12,13).

In general, there are two phases of hepatic injury follow IRI (14). The first phase (<2 h after reperfusion) is considered oxidative stress, which is induced by KCs. The KCs are able to produce and release a large quantity of ROS in this phase. Accumulation of ROS may induce mitochondria-dependent apoptosis and has a key role in hepatic injury (15), which was confirmed by providing partial protection against hepatic IRI with free radical scavengers, chemical antioxidants and blockage of KC activity with gadolinium chloride (16-18). The second phase is characterized by the recruitment of activated neutrophils into the liver parenchyma (19-21). The recruitment is initiated during the first phase of hepatic IRI and may activate a series of inflammatory pathways. During the second phase of hepatic IRI, KCs and infiltrating neutrophils further increase the ROS and pro-inflammatory cytokines, leading to irreversible tissue damage in the form of sinusoidal congestion, cytoplasmic vacuolation of hepatocytes, necrosis and apoptosis (21,22). Most importantly, complement products are also critically involved in KC activation and neutrophil infiltration (8).

In IRI, complement activation results in the rapid induction of multiple parallel downstream pathways of hepatic injury. Studies in animal models have indicated that IRI in several organ systems results in activation of complement components through either the antibody-dependent classical pathway (23), the alternative pathway (24) or the mannose-binding lectin-mannan-binding lectin serine protease pathway (25). Activation of complement by any of these pathways results in the formation of biologically active potent inflammatory complement substances, including the anaphylatoxins (C3a and C5a) and the cytolytic membrane attack complex (MAC) (26). Diffusible anaphylatoxin-mediated responses and MAC-mediated lysis of hepatocytes may have parallel roles in exacerbating hepatic IRI.

The anaphylatoxins C3a and C5a are both crucial chemoattractant factors for leukocytes. They also contribute to the accumulation and infiltration of PMNs, induce smooth muscle contraction, increase vascular permeability, cause the release of histamine and activate KCs (27-29). Activated KCs and PMNs secrete inflammatory cytokines, such as TNF- α and IL-1 β (30-32), which amplify the inflammatory response and cause hepatic injury. Furthermore, inflammatory cells may also release myeloperoxidase and toxic products when activated, resulting in the production of ROS, which may directly injure hepatocytes. In addition, endothelium activated by complement results in the production of numerous factors, such as adhesion molecules, cytokines, platelet-activating factors, leukotrienes, P-selectin and endothelin (33); activity of these

factors may result in fibrin deposition, platelet aggregation and adhesion of neutrophils to the vascularendothelium (34). This complement-associated mechanism contributes to impaired hepatic microcirculatory blood flow in the reperfusion period, which has an important role in hepatic IRI (12). The anaphylatoxins C3a and C5a also have a significant role in renal and cerebral IRI (35,36).

In the terminal complement pathway, the MAC is formed, which directly causes cell lysis and stimulates cells to release pro-inflammatory molecules, which may amplify the inflammatory response by promoting the expression of pro-inflammatory mediators (37). Furthermore, the MAC may influence the recruitment of inflammatory cells, adhesion of leukocytes to endothelium and enhancement of the pro-coagulant properties of the endothelium (38). While the MAC promotes hepatocyte lysis, it may also be a parallel injury pathway during hepatic IRI. Direct cellular injury via this mechanism also triggers inflammation, thus amplifying the hepatic injury response. A previous study has proved that livers of C6-deficient rats (blocked in only the terminal pathway) are protected from the deleterious effects of IRI (39). It has provided direct evidence for an important role of the MAC in hepatic IRI. Therefore, inhibition of the complement cascade may be a novel approach for minimizing liver damage and preventing fatal organ failure after hepatic ischemia. Furthermore, a study by our group has indicated that steatotic livers are more sensitive to IRI and complement C3 has a critical role during IRI (40). On this basis, it may be suggested that complement inhibition with CR2-Crry maybe a strategy for reducing liver-donor shortage by allowing greater use of marginal steatotic donor livers (hepatic steatosis typically renders the donor organ unusable, as donor organs with >30% steatosis are more likely to develop graft failure).

3. Complement cascades are essential in liver recovery and regeneration

Liver regeneration may occur after partial hepatectomy, liver transplantation, viral infection, IRI or toxic injury. The regeneration process is complex and well-orchestrated (41,42), and it has been widely studied in order to understand the mechanisms that regulate hepatocyte proliferation and survival (43,44). In normal adult mouse liver, mature differentiated hepatocytes are not vegetative and are quiescent. However, when the liver receives a regenerative stimulus (e.g., resection or toxic injury), ~95% of hepatocytes undergo cell division and maintain their metabolic functions (45). The process of regeneration is tightly regulated through controlled delivery of cytokines, growth factors, paracrine signals and complement activation products that control the proliferation of hepatic cells (46). The initiation step is activation by priming of the quiescent hepatocytes (G0 phase) with factors such as TNF- α , IL-6 and nitric oxide, which then provides a regenerative stimulus that promotes entry into the cell cycle (G1 phase) by complete mitogens such as hepatic growth factor (HGF), ligands for epidermal growth factor receptor (EGFR), transforming growth factor- α , EGF, heparin-binding EGF and amphiregulin (47). Furthermore, the cytokines TNF- α and IL-6 are crucial in regulating the early priming phase of liver regeneration by contributing to the mitogenic priming of hepatocytes and the early hepato-

cyte proliferative response of the liver (48). These cytokines are components of the TNF- α /TNFR1/NF- κ B/IL-6/STAT3 pathway that drives hepatocyte gene expression, although the inhibitors and stop signals of hepatic regeneration remain to be elucidated (49).

Recent evidence indicates that complement activation products serve important physiological roles in liver regeneration and recovery, after either resection or toxic injury (50-53). Mice deficient in C3 and/or C5 after partial hepatectomy and toxic injury with carbon tetrachloride have an impaired regenerative response, which may be reversed by reconstitution of C3a and C5a. Furthermore, interception of C5a receptor (C5aR) signaling may suppress IL-6/TNF- α induction, and lack of C3aR and C5aR stimulation attenuates NF- κ B-STAT3 activation after hepatectomy, which abrogates the proliferative response of hepatocytes to liver injury (50,52). According to one study, restoration of hepatocyte proliferative capabilities in C3aR^{-/-} mice by systemic C3a reconstitution was not beneficial (50). Another similar study indicated that treatment with C5a agonist in rats after partial hepatectomy facilitated the synthesis of the growth factor/receptor pair HGF/c-Met to upregulate the expression of HGF and c-Met mRNA, which significantly increased the expression of cyclin E and D1mRNA levels and incorporation of 5-bromodeoxuridin (54). Taken together, these data indicate that: i) C3a and C5a signaling are critical for the early priming phase of liver regeneration; ii) C3a exerts its regenerative effects via the C3aR; and iii) C5a is upregulated during liver regeneration. Binding of C5a to the C5aR promotes a growth response by inducing the expression of HGF mRNA and its corresponding c-MET receptor. Furthermore, C5aR is also involved in a cell-cycle signaling pathway (54,55).

With regard to complement activation pathways, a study reported that animals in which all of the traditional upstream C3 activation pathways were disrupted, i.e. C4-null mice treated with complement factor B neutralizing antibody (monoclonal antibody1379), exhibited normal C3 activation and hepatocellular proliferative response after partial hepatectomy. The study concluded that a non-traditional mechanism of complement activation was involved in the regenerative process (53).

4. Complement regulation between hepatic IRI and liver regeneration

After hepatic resection, the liver undergoes both IRI injury and regeneration. As outlined above, complement activation is necessary for liver regeneration and is extensively involved in hepatic IRI. Thus, it was postulated that a balance exists, in which excessive complement activation (which may occur in liver resection) or excessive inhibition or inadequate blockade of complement activity may be deterrent to regeneration. This hypothesis is supported in part by the results of a study by our group (56), in which C3 deficiency or inhibition of complement was achieved by using an inhibitor of C3 activation and CR2-Crry was indicated to protect against hepatic IRI; CR2-mediated targeting of complement inhibition has the potential to be of therapeutic benefit for numerous complement-associated diseases or disease states (57), such as traumatic brain injury, age-related macular degeneration and

collagen antibody-induced arthritis (58-61). Although either C3 deficiency or high-dose CR2-Crry protected against IRI in mice, they significantly increased injury and impaired regeneration in the partial hepatectomy model (56). However, a low dose of CR2-Crry actually enhanced regeneration after partial hepatectomy (56). Furthermore, this dose was protective in a model of IRI, albeit somewhat less protective than the higher dose (56). Given these results, the effect of C3 deficiency and dose of complement inhibitor were examined in a model incorporating both IRI and 70% partial hepatectomy (which mimics the procedure used for massive liver resection under the Pringle maneuver) (56). In the combined model, either C3 deficiency or high-dose CR2-Crry resulted in steatosis, severe hepatic injury and high mortality compared to the wild-type controls. However, mice treated with low-dose CR2-Crry had increased hepatic proliferative responses and significantly decreased damage compared with these responses in wild-type controls. A large quantity of C3 deposition was observed in wild-type mice at 48 h after partial hepatectomy, whilst C3 immunohistochemical tissue staining was absent in C3^{-/-} mice and mice treated with high-dose CR2-Crry. Furthermore, a low level of C3 immunohistochemical staining was present in mice treated with low-dose CR2-Crry. In reconstitution experiments, it was indicated that the C3a degradation product C3adesArg (also known as acylation-stimulating protein) is important in the balance between inflammation/injury and regeneration. These results suggest a balance between complement-dependent injury and regeneration (56).

The aforementioned study also indicated that either C3 deficiency or treatment with CR2-Crry after IRI resulted in improved hepatocyte proliferation and accelerated recovery from injury as compared with the corresponding values in the wild-type controls, a result which demonstrated that complement is not critical to the recovery of hepatic IRI. Of note, recovery in the one-dose CR2-Crry treatment group is better than that in the C3 deficiency group; these results suggested that a low level of complement rather than complement deficiency is beneficial for the recovery of hepatic IRI injury. Although the precise mechanisms of the divergent effects of complement on liver regeneration between IRI and partial hepatectomy models remain elusive, preliminary data suggest that the differences are related to the amount of complement produced during these insults. Levels of C3a have been moderately increased (3- to 5-fold) after 70% partial hepatectomy but sharply increased (30- to 50-fold) after IRI. It was postulated that moderate increases in complement, which occur after 70% partial hepatectomy, promote liver regeneration via activation of regenerative signaling pathways, whereas much higher increases in the expression of complement components, as occurs after IRI injury, may be hepatotoxic and/or impair hepatocyte proliferation and regeneration. This concept was supported by experiments by our group in which animals were treated with various concentrations of CR2-Crry after 70% partial hepatectomy; low concentrations of CR2-Crry had hepatoprotective effects, whereas high concentrations induced significant cytotoxicity. Administration of low concentrations of CR2-Crry immediately after the onset of reperfusion downregulated inflammatory cytokines, decreased hepatic neutrophil infiltration, inhibited liver cell apoptosis and necrosis, and reduced liver injury and mortality in a mouse

model of hepatic IRI. These data further support the concept of a balance between the extent of IRI and an ability to regenerate that is determined by the level of complement activation (56).

Several agents that inhibit all or part of the complement system have demonstrated a protective effect against liver IRI: i) Complement depletion with cobra venom factor (8,62); ii) treatment with C5aR antagonist in rats after hepatic IRI (9); iii) intravenous soluble complement receptor 1 given prior to reperfusion after hepatic ischemia (63); and iv) inhibition of the classical pathway by pre-ischemic administration of C1 inhibitor (64). Therefore, there appear to be multiple potential pharmacologic strategies to protect against hepatic IRI by targeting the complement cascade. However, from the evidence of experimental models, the strategies of targeting C3 may provide the most effective complement inhibition during hepatic IRI, which does not only inhibit the formation of both C3a and C5a anaphylatoxins, but also inhibits the formation of the MAC, which may directly result in hepatocyte lysis. However, given the beneficial role for complement, particularly its putative role in liver recovery and regeneration and the potential danger of systemic complement inhibition, therapeutic inhibition of complement must be performed to protect from hepatic IRI as much as possible while increasing liver regeneration.

Of note, when liver regeneration is the major concern as compared with hepatic IRI injury (such as in massive liver resection and small-for-size liver transplantation), selective inhibition at certain points in the complement pathway, rather than total complement inhibition (at the C3 point), may be more appropriate. Complement inhibition is considered a potential strategy for hepatic IRI, but upon complete inhibition at the C3 point, liver regeneration is decreased or may even stop completely. Zhou *et al* (33) demonstrated that the MAC is a potential target for the prevention of renal IRI. Overexpression of human CD55 and CD59 or treatment with human CD55 protects against renal IRI in mice (65). Zhang *et al* (66) reported that CD59-deficient mice had more severe liver dysfunction, as evidenced by increased aspartate aminotransferase levels and increased injury of liver parenchymal and nonparenchymal cells compared to CD59-sufficient mice during warm hepatic IR. Collectively, it may be hypothesized that the MAC has a predominant role in IRI but has a minor effect on liver regeneration, whereas more proximal activation products (C3a/C5a) are more important for liver regeneration. In a study by our group (11), CR2-CD59, a special complement inhibitor which may inhibit the formation of the MAC, was used to investigate how the complement works in the balance between liver injury and regeneration in a clinical setting of pharmacological inhibition. The results indicated that CR2-CD59 does not impair the generation of C3 and C5 activation products, but it may also ameliorate hepatic IRI, which is as effective as the C3 activation inhibitor CR2-Crry as reported previously (56). This result indicates that the MAC is crucial to hepatic IRI. Furthermore, unlike inhibition of C3 or C5, CR2-CD59 does not only protect against hepatic IRI but also significantly improves liver regeneration after partial hepatectomy, even when the hepatectomy is combined with IRI. Of note, CR2-CD59 may also improve long-term survival from 0 to 70% after 90% hepatectomy (11). This result demonstrates that specific inhibition of the MAC is an optimal

potential therapeutic strategy to improve liver regeneration and reduce injury. In addition, inhibiting complement late in the pathway is less likely to disrupt normal immune homeostatic functions and host defense than earlier inhibition, which is an important consideration in the transplant recipient (11). These results suggest that the extent of complement activation may tip the balance between injury and the ability of the liver to regenerate (56).

5. Future perspective

Hepatic IRI is unavoidable in the process of liver operations. Data from animal studies indicate that complement is important in both hepatic IRI and recovery. At present, there is no effective pharmacological therapy for hepatic IRI in humans. Balanced approaches of complement inhibition or selective inhibition at certain points in the complement pathway (which may be obtained by temporal treatment of selective targeted inhibitors) are attractive strategies for reducing inflammation, minimizing hepatic IRI and enhancing liver recovery after hepatic IRI-inducing procedures, such as liver resection and transplantation. The present review shed new light on the role of the complement system in hepatic IRI and the failure of liver regeneration. Evidence from preclinical studies has revealed that regulation of the complement system represents a potential promising intervention strategy for patients undergoing massive liver resection or liver transplantation.

To date, studies on the complement system in hepatic IRI and liver regeneration have mainly focused on animal models of various liver diseases. A long road lies ahead before their clinical application. However, complement inhibitors such as eculizumab, Amy-101 and ravulizumab have entered or completed clinical trials (67-69). They have demonstrated good results in the treatment of diabetes mellitus, atypical hematuric urinary syndrome, paroxysmal nocturnal hemoglobinuria and Covid-19 (67-69). In conclusion, complement regulation strategies may be a promising application in the field of hepatic IRI and liver regeneration.

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Availability of data and materials

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Authors' contributions

SQH, GDY, ZGH, YZ and CJL designed the study, wrote the manuscript, performed the literature research and selected the

included studies. SQH and GDY provided critical intellectual revision. All authors read and approved the final manuscript.

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Not applicable.

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Not applicable.

Competing interests

The authors declare that they have no competing interests.

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