Review

The Complement System in Liver Diseases

Xuebin Qin[1, 3] and Bin Gao[2]

The complement system plays an important role in mediating both acquired and innate responses to defend against microbial infection, and in disposing immunoglobins and apoptotic cells. The liver (mainly hepatocytes) is responsible for biosynthesis of about 80-90% of plasma complement components and expresses a variety of complement receptors. Recent evidence from several studies suggests that the complement system is also involved in the pathogenesis of a variety of liver disorders including liver injury and repair, fibrosis, viral hepatitis, alcoholic liver disease, and liver ischemia/reperfusion injury. In this review, we will discuss the potential role of the complement system in the pathogenesis of liver diseases. *Cellular & Molecular Immunology*. 2006;3(5):333-340.

Key Words: immunity, complement, liver disease

Introduction

The complement (C) system is a set of biochemical pathways that removes pathogen components from an organism as part of the innate and acquired immunity programs. Activation of the complement system triggers a wide range of cellular responses ranging from apoptosis (cell death) to opsonization (antigen/antibody binding) (1). It has been widely recognized that the complement system plays a critical role in the pathogenesis of a variety of chronic human diseases, including autoimmune diseases, atherosclerosis, the vascular complications of diabetes, complement-mediated hemolytic anemia, and infertility in both males and females (2-6). Recent evidence suggests that the complement system is also involved in the pathogenesis of a variety of liver disorders, which will be discussed in this review.

Complement system

Complement activation

The complement system consists of about 30 soluble and membrane bound proteins, and is activated by 3 distinct pathways either on pathogen surface or in plasma (Figure 1). Activation of these pathways depends on different molecules for their initiation. The classical pathway is triggered by antigen-bound antibody molecules and is initiated by the binding of a specific part of the antibody molecule (Fc) to the C1 component (7). The alternative pathway is a humoral component of the immune system's natural defense against infections and is activated by cleavage of C3 and then C5 (Figure 1). The mannose-binding lectin (MBL) pathway is initiated when the plasma MBL protein forms a complex with the MBL-associated proteases 1 and 2 (MASP1 and MASP2). MASP1 and MASP2 then bind to arrays of mannose groups on the surface of a bacterial cell (8) (Figure 1).

All three activation pathways converge at the level of C3 to form the C5 convertase such as the C4bC2aC3b from classical and MBL pathways and $(C3b)_2FBb$ from alternative pathway (Figure 1). The C5 convertase then cleaves C5 to form C5b and C5a. Importantly, thrombin has been recently identified to act as a C5 convertase in C3 deficient mice, which can cleave C5 to form C5b and C5a. Thus, thrombin generated from clotting pathway is an additional complement activation pathway, which provides a molecular basis linking clotting pathway to complement activation pathway (9). Terminal complement activation is induced initially by C5b, and followed by the sequential condensation of C6 to form C5b6, and then C7, C8, and C9. Polymerization of C9 bound to the C5b-8 complex forms the membrane attack complex (MAC), an end product of complement activation pathway. The MAC forms a lytic pore in the lipid bilayer membrane that allows the free passage of solutes and water across the membrane and destroys membrane integrity, followed by killing the foreign pathogens and cells (10) (Figure 1).

By-products of complement activation that bridge innate and adaptive immunity are critical mediators of the host

[1]Laboratory for Translational Research, Harvard Medical School, Cambridge, MA 02139, USA;

[2]Section on Liver Biology, Laboratory of Physiologic Studies, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, MD 20892, USA;

[3]Corresponding to: Dr. Xuebin Qin, Laboratory for Translational Research, Harvard Medical School, One Kendall Square, Building 600, 3rd Floor, Cambridge, MA 02139, USA. E-mail: xuebin_qin@hms.harvard.edu.

Received Sep 21, 2006. Accepted Oct 8, 2006.

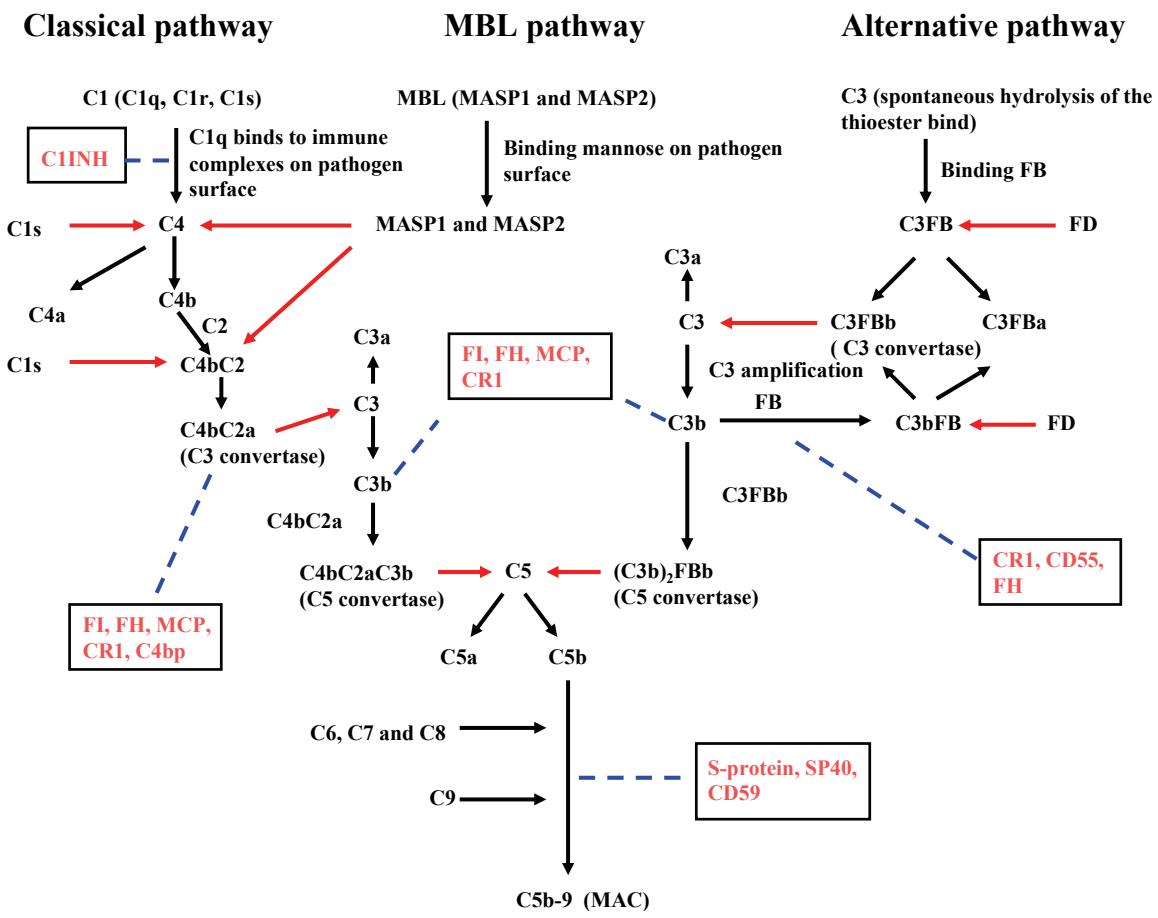

Figure 1. Complement activation and regulation. There are three complement activation pathways (classical, alternative, and MBL pathways). Black arrows indicate the direction of the activation pathway or cleavage products of complements. Red arrows demonstrate the cleavage reaction *via* enzymatic factors or proteases. Boxed words in red are the complement regulatory proteins that act on the specific stages of activation pathways and inhibit the complement activation. C1INH, C1 inhibitor; MCP, membrane cofactor protein; CR, complement receptor; C4bp, C4 binding protein; FB (D, H, I), Factor B (D, H, I).

defense against infection, and of the disposal of immune complexes and products of inflammatory injury (8). For example, fragments of C3 and C4 opsonize old red blood cells, bacteria and immunoglobulin aggregates, which are then recognized by phagocytic cells for digestion and clearance (11). The role of complement in the clearance of immune-complexes is highlighted by the strong association between immune complex disease and inherited deficiencies of certain classical pathway components (C1q, C1r, C1s, C4, C2 and C3) (12). Also, C3b and C4b bound to immune complexes potentiate antibody response and enhance immunologic memory (11). Small complement fragments C3a, C4a and C5a act on specific receptors to produce local inflammatory responses. These fragments are termed as anaphylatoxins because they can cause mast cell degranulation with the release of histamine and other mediators that increase vascular permeability and induce smooth muscle contraction (13). Of the three, C5a is the most stable and has the highest specific biological activity, which is gradually being appreciated in different diseases including liver diseases. The role of complement components and their by-products in the pathogenesis of liver diseases will be discussed below.

Complement regulation

All complement components are activated spontaneously at a low rate in plasma, also called as tick over complement activation. This tick over activation of complement occurring *in vivo* presents a serious threat to host cells. However, host cells express several plasma and membrane proteins that can inhibit self complement activation thereby preventing self damage (14). So far at least 10 plasma or membrane-bound proteins have been identified to restrict activation of complement and subsequently prevent the catastrophic effect of complement activation on "self" cells. This complement regulatory system generally acts on the inherent instability of activation pathway enzymes and reduces the production of activated complement. The soluble plasma complement regulators include: 1) C1 inhibitor that regulates C1; 2) factors H and I that regulate the alternative pathway; 3) C4 binding protein

Table 1. Expression of complements and regulators in hepatocytes

Complements regulators	Mainly synthesized or expressed in hepatocytes	References	Mainly synthesized or expressed in other cells	References
Classical	C1r/s, C2, C4, C4bp	20, 81	C1q	20-22
Alternative	C3, B	20, 81, 82	D, P	20, 23-25
Lectin	MBL, MASP1, 2, 3, Map19	80, 83		
Terminal	C5, C6, C8, C9	20, 81	C7	20, 26
Regulators (in plasma)	I, H, C1INH	20		
Regulators (membrane bound)			CD59, CD46, CD55, CD35	27-29

(C4bp) that catalyses the cleavage of C4b by factor I; 4) S-protein, clusterin, and serum lipids that compete with membrane lipids for reacting with nascent C5b67 (7). Moreover, 3 membrane proteins that are expressed on the surface of almost all cell types have been shown to inhibit autolgous complement activation, thereby protecting self cells from consequent complement-mediated injury (7). These regulators include decay accelerating factor (DAF or CD55), membrane cofactor protein (MCP or CD46), and the membrane inhibitor of reactive lysis (MIRL or CD59). DAF inactivates the C3 (C4b2a and C3bBb) and C5 (C4b2a3b and C3bBb3b) convertases by accelerating the decay of these enzymes (15-17). MCP acts as a cofactor for the cleavage of (non-convertase-associated) self cell-bound C4b and C3b by the serum protease factor I (18). CD59 restricts MAC formation by preventing C9 incorporation and polymerization (19) (Figure 1).

Biosynthesis of plasma complements and expression of complement receptors in the liver

Synthesis of plasma complements in the liver

The liver (mainly hepatocytes) is responsible for biosynthesis of 90% of plasma complement components and their soluble regulators, including the classical (C1r/s, C2, C4, C4bp), alternative (C3, factor B), lectin (MBL, MASP1-3, Map19), terminal (C5, C6, C8, C9) pathways of the complement system, and soluble regulators (factors I, H, and C1 inhibitor) (Table 1) (20). Many other types of cells including immune cells and endothelial cells also synthesize these components, but their contributions to plasma levels appear to be minor compared to hepatocytes.

In contrast, several other plasma components including C1, factor D, properdin, and C7 are mainly produced outside of the liver. C1 includes three subcomponents including C1q, C1r, and C1s. Many cell types including epithelial cells, fibroblasts, and cells of the monocyte/macrophage lineage can produce C1q (21, 22). Among these cells, monocytes can produce all three C1 subcomponents to form intact C1, and are likely the major source for plasma C1. Hepatocytes only produce C1r and C1s but not C1q, therefore, intact C1 can not assemble in hepatocytes. The biological significance of C1r and C1s synthesized in hepatocytes is not clear. Plasma factor D is mainly produced by adipocyte (23, 24) while macrophages and monocytes are primarily responsible for production of plasma properdin (25) and C7 (26). Finally, soluble complement regulatory proteins are produced by hepatocytes (20) while membrane bound complement components including CD59, CD35, CD46 and CD55 are expressed ubiquitously in all tissues (27-29).

Analyses of promoter regions of the complement components revealed that many of them are controlled by several liver specific transcription factors (such as hepatocyte nuclear factors [HNFs] and C/EBP) (30, 31), which may explain why hepatocytes are the major source to synthesize these proteins. For example, disruption of the HNF-1α gene abolished or significantly decreased expression of C5, C8, and C9 mRNA in the liver (30). Transcriptional regulation of complement factor B is controlled by HNF4 (31) while C4BP is regulated by HNF3 and nuclear factor-1 (32). In addition, expression of many complement components in the liver is significantly elevated during acute phase response and induced by proinflammatory cytokines such as IL-6, IL-1, TNF-α and IFN-γ (33-35).

Complement receptors in the liver

Several complement receptors have been detected in liver cells and contribute to a variety of functions in the liver (Table 2). The liver is composed of hepatocytes, Kupffer cells, stellate cells, and sinusoidal endothelial cells. In normal liver, expression of C5aR was not detected on the surface of hepatocytes, but can be induced after lipopolysaccharide challenge or after partial hepatectomy *via* an IL-6-dependent mechanism (36-38). The functions of *de novo* expression of C5aR on hepatocytes through interaction with C5a include: 1) stimulation of glucose release; 2) induction of acute phase protein synthesis; 3) stimulation of hepatocyte proliferation (36-40).

In contrast to hepatocytes, normal Kupffer cells express complement receptors for both C3 and C5a at high levels (40, 41). Three types of C3-receptors have been detected on the surface of Kupffer cells, including CR1 (C3b-receptor; CD35), CR3 (iC3b- and β-glucan-receptor), and CR4 (iC3b-receptor; CD11c/CD18). Through interaction with these receptors, Kuppfer cells contribute to the blood clearance of C3-opsonized immune complexes, therapeutic β-glucan polysaccharides, IgM-opsonized E and β-glucans (42, 43). Recently, Helmy et al. identified a novel complement receptor

Table 2. Expression of complement receptors in the liver

Liver cell types	Complement receptors	Functions	References
Hepatocytes	C5aR	Induction of acute phase response, glucose release, hepatocyte proliferation	36-40
Kupffer cells	C5aR	Prostanoid release, synthesis of proinflammatory cytokines, clearance of red cells	36, 40, 44, 45
	CR1, CR3, and CR4, CRIg	Clearance of C3-opsonized immune complexes, β-glucan polysaccharides, IgM-opsonized E and β-glucans	41, 43, 44
Stellate cells	C5aR	Upregulation of fibronectin expression, induction of prostanoid release and liver fibrosis	40, 46, 49
Endothelial cells	C5aR (weak expression)	Minimal effect	7, 20, 26, 40, 48

of the immunoglobulin superfamily CRIg on Kupffer cells and demonstrated that CRIg is required for efficient binding and phagocytosis of complement C3-opsonized particles through binding to complement fragments C3b and iC3b (44). Activation of C5aR by C5a is able to stimulate Kupffer cells to produce prostanoid and synthesize proinflammatory cytokines (36) and participates in the clearance of red blood cells (45). High levels of C5aR were also detected on the surface of stellate cells (46) that are known to play a key role in the induction of liver fibrosis. Activation of C5aR by C5a has been shown to upregulate fibronectin expression and induce prostanoid release in hepatic stellate cells (46, 47), which may contribute to the involvement of C5a in liver fibrosis (see below). Finally, sinusoidal endothelial cells also express C5aR at low levels, and the function of C5aR on these cells appears to be minor (47, 48).

Role of complements in liver diseases

Liver fibrosis

C5 and C5aR were recently demonstrated to exhibit a critical role in the pathogenesis of liver fibrosis by Hillebrandt et al (49). In this study, through genetic mapping in experimental mouse intercrosses between fibrosis-susceptible BALB/cJ and fibrosis-resistant A/J inbred strain, the 44.7 Mb region on mouse chromosome 2 was found to contain a gene that is responsible for liver fibrosis (49). The fibrosis-resistant A/J inbred strain carries a 2-bp deletion in the C5 gene in exon 6 within the 44.7 Mb region of mouse chromosome 2, which is responsible for the C5 deficiency in this strain. Therefore, C5 was considered as a strong candidate gene for promoting liver fibrosis. The several other inbred mouse strains that are C5 deficient with a 2-bp deletion in the C5 gene have significantly lower stages of fibrosis after CCl_4 treatment than did mouse strains with C5 sufficiency. The prediction that C5 is a strong candidate gene was further supported by several lines of evidence (49). Firstly, introduction of C5 deficiency to the C5 sufficient strain by multiple backcrosses resulted in the fibrosis-resistant phenotype. Secondly, transgenic introduction of C5 gene into C5 deficient inbred strain resulted in the fibrosis-susceptible phenotype. Finally, blockade of C5 receptor 1 (C5aR1) attenuates liver fibrosis in mice. Consistently, genetic analyses suggest that human C5 gene variants are also associated with liver fibrosis in patients with chronic HCV infection (49). However, the molecular mechanisms responsible for the involvement of C5aR1 in liver fibrosis remain obscure.

Immunocytochemistry and confocal laser scanning microscopy analyses showed that expression of C5aR1 was detected on the hepatic stellate cells at high levels and increased significantly in these cells during trans-differentiation to myofibroblasts in culture (49). Stimulation of C5aR1 by C5a can upregulate fibronectin expression and induce prostanoid release in hepatic stellate cells (40, 46), which may contribute to the involvement of C5aR1 in liver fibrosis. Further studies are required to investigate the molecular mechanisms underlying the C5aR1 contribution to liver fibrosis.

Liver regeneration

Complement has also been involved in liver regeneration after partial hepatectomy or after toxic injury (50-52). By using a murine model of partial hepatectomy, Strey et al. demonstrated that the anaphylatoxins C3a and C5a are essential for liver regeneration (51). C3 or C5 deficiency results in diminished liver regeneration, accompanied by transient or fatal liver failure after partial hepatectomy (51). Liver regeneration was severely impaired in C3 and C5 double knockout mice, which was restored after simultaneous reconstitution with C3a and C5a (51). Furthermore, they demonstrated that 1) C5aR blockage disrupted liver regeneration; 2) C5aR stimulation was required for intra-hepatic TNF-α and IL-6 release; 3) C5aR engagement recruited NF-κB and STAT-3 dependent pathways in the regenerating liver; 4) C3 was required for normal STAT-3/NF-κB activation in liver regeneration (51). A series of similar experiments were also performed to address the role of C3 and C5 in liver regeneration after CCl_4-induced liver damage (50, 52). C5-deficient mice developed severely defective liver regeneration and persistent parenchymal necrosis after exposure to CCl_4 and also showed a marked delay in the re-entry of hepatocytes into the cell cycle (S

phase) and diminished mitotic activity (50). Administration of murine C5 or C5a restored hepatocyte regeneration in C5 deficient mice while blockage of the C5aR abrogated the ability of hepatocytes to proliferate in response to liver injury (50). Disruption of the C3 gene delayed liver regeneration post CCl_4 injection, which can be restored by C3a reconstitution (52). C3a receptor-deficient mice also showed impaired liver regeneration (52). Taken together, the results from these studies suggest that both C3 and C5 participate in liver regeneration after liver injury or loss of tissue (50-52) *via* enhancing the priming signals STAT-3 and NF-κB, the two important signals for the initiation of the regenerating response (53).

Viral hepatitis

More than half a billion people worldwide are chronically infected with the hepatitis B virus (HBV) or hepatitis C virus (HCV), which is a leading cause of liver injury, fibrosis, and cirrhosis. The mechanisms responsible for HBV and HCV persistence and disease pathogenesis remain poorly understood, and the interaction of hepatitis viruses and the host immune system is likely involved (54). Although the complement system has been shown to contribute to the protection of host from virus infection (55, 56), the involvement of complement in viral hepatitis has not been well documented. It was reported that cold activation of complement (loss of hemolytic activity in sera during storage at low temperature) was observed in the patients with chronic HCV infection and contributed to HCV-associated liver damage and was useful for monitoring response to interferon therapy in these patients (57, 58). However, the reason why HCV infection results in the high titer of cold complement activation and its role in pathogensis of HCV infection still remains to be addressed. A recent study demonstrated that an increased prevalence of anti-C1q antibodies was present in HCV-infected patients and anti-C1q antibodies were associated with low complement levels (59). The anti-C1q antibodies have been shown to be associated strongly with immune complex diseases, most prominently with hypo-complementaemic urticarial vasculitis syndrome, systemic lupus erythmatosus, diffuse proliferative lupus nephritis, and severe rheumatoid arthritis (60). Thus, the high levels of anti-C1q antibodies in HCV patients suggest that immune complex may also contribute to the pathogenesis of HCV-associated liver injury. Another autoantibody against asialo-glycoprotein receptor that is expressed on hepatocytes and mediates clearance of desialylated serum proteins is also involved in the pathogenesis of hepatitis virus-induced liver disease through disrupting clearance of desialylated proteins and activation of the complement-mediated cytolysis (61, 62). Immunohistochemistry analyses showed that MAC was detected in hepatocytes surrounding necrotic areas in the patients with fulminant and acute hepatitis (63), further supporting that the complement system is activated and is involved in the pathogenesis of HCV-associated liver disease.

MBL has been shown to be an important component of innate immunity and has an important role in host defense against infection of bacteria, viruses, and fungi. Genetic polymorphisms are one of the most important factors to determine plasma levels of MBL. Individuals possessing variant alleles of MBL had low plasma MBL levels and showed a dose-dependent correlation with cirrhosis and hepatocellular carcinoma in progressed HBV carries (61, 64), and likely became viral persistence after HBV infection (65). Moreover, Chong et al. also demonstrated that MBL bound HBsAg *in vitro* in a dose- and calcium-dependent and mannan-inhibitable manner and this binding also enhanced C4 deposition (63). Finally, chronic HBV and HCV infection resulted in a decrease of serum MBL levels, which was likely due to impairment of MBL production (62). Together, these findings suggest that patients with variant alleles of MBL have low levels of plasma MBL and are associated with progression of liver disease and viral persistence after HBV infection.

Alcoholic liver injury

It has been known for many years that serum levels of complement components are lower in alcoholic cirrhotic patients than normal healthy individuals, which may be associated with high risk of bacterial and fungal infections in these patients (66). Recent studies in animal models suggest that complements are activated in alcoholic liver disease and contribute to its pathogenesis. Both endotoxin and oxidative stress have been implicated in complement activation and disposition in alcoholic liver disease (67). Feeding mice and rats with ethanol diet resulted in increased disposition of C1, C3, C8, and C9 but decreased expression of complement regulators Crry and CD59 in the liver (68). C3-deficient mice are resistant to ethanol-induced hepatic steatosis, elevation of liver malondialdehyde level, and serum alanine amino-transferase activity (69) while C6-deficient mice are more susceptible to ethanol-induced hepatic injury and steatosis (68). These studies suggest that C3 contributes to the pathogensis of alcoholic disease while the terminal complement component C6 provides a protective function against alcohol-induced liver injury. However, clinical data showed that activation of complement was not different in acute alcoholic hepatitis patients compared with normal healthy control groups and there was no relationship between clinical or laboratory indicators of disease severity and complement activation in acute alcoholic hepatitis, suggesting that complement activation may not contribute to the clinical and histological features of human alcoholic liver disease (70). Extensive studies are needed to define precise roles of the complement system in human alcoholic liver disease.

Liver ischemia/reperfusion injury and transplantation

Liver ischemia/reperfusion (I/R) injury occurs during liver surgery and transplantation and is triggered by a complex inflammatory response following temporary deprivation of blood supply. Two distinct phases of hepatic I/R have been identified, including the initial and later phases of injury. The initial phase involves Kupffer cell activation and production of oxidative stress. The later phase is characterized by massive neutrophil infiltration (71). The involvement of

complement activation in liver I/R injury in a rat model was first reported by Jaeschke and colleagues (72). In this study, they demonstrated that depletion of serum complement by treatment with cobra venom factor before ischemia prevented Kupffer cell-induced oxidant stress, accumulation of polymorphonuclear leukocyte (PMNs) in the liver, and hepatic injury caused by I/R (72). Furthermore, treatment with C5aR antagonist reduced total hepatic I/R-induced mortality and ameliorated the partial hepatic I/R-induced liver injury and accumulation of PMNs in the liver (73). Blocking complement activation with sCR1 or C1 inhibitor also significantly ameliorated necrosis and inflammation during hepatic I/R injury and liver transplantation in rodent models (74-76). Clinical data suggest the involvement of complements in liver I/R injury during human liver transplantation. MAC deposition was found to be elevated in the postoperative specimens of patients with liver transplantation, and correlated positively with the number of leukocytes and platelets accumulating within the graft, and with an increase in postoperative aspartate aminotransferase levels in the serum (77). Complement activation was also reported in the human livers after partial hepatectomy and was likely caused by I/R *via* the classical pathway (76). Recently, Schmeding et al. showed that elevation of C4d deposition was detected in 68% of liver allograft biopsies with acute rejection while it was detected in only 12% of liver allografts with HCV recurrence and 7% of protocol biopsies from the subjects without rejection or HCV recurrence (78). This suggests that activation of complement may contribute to rejection after liver transplantation (78). Moreover, complement activation may play an important role in the rejection of hepatic xenotransplantation (79).

In contrast to induction of the detrimental effect in liver injury, activation of complement may play a beneficial effect in preventing infection after liver transplantation. Transplantation of patients with donor livers carrying MBL variant alleles resulted in a significant decrease of serum MBL levels, and was associated with a significantly increased incidence of infections after liver transplantation, suggesting that activation of complement is an important factor in controlling infection after liver transplantation (80).

Summary

The liver is the major site for biosynthesis of 80-90% plasma complement components, and expresses a variety of complement receptors. The complement and complement receptors appear to be involved in liver injury and repair. However, the underlying mechanisms remain poorly understood, such as how C5aR contributes to liver fibrosis and how C3 and C5 contribute to liver regeneration. Moreover, although several studies have been carried out in animal models, the role of complement in human liver disease has been poorly documented and investigated. Further detailed studies about the potential contribution of complements to the pathogenesis of human liver diseases are urgently needed to answer these questions.

Acknowledgement

We thank the NIH Fellow Editorial Board for the helpful editorial assistance.

References

1. Holt DS, Botto M, Bygrave AE, Hanna SM, Walport MJ, Morgan BP. Targeted deletion of the CD59 gene causes spontaneous intravascular hemolysis and hemoglobinuria. Blood. 2001;98:442-449.
2. Acosta J, Qin X, Halperin J. Complement and complement regulatory proteins as potential molecular targets for vascular diseases. Curr Pharm Des. 2004;10:203-211.
3. Morgan BP. Complement: clinical aspects and relevance to disease. London: Academic Press, 1990.
4. Qin X, Goldfine A, Krumrei N, et al. Glycation inactivation of the complement regulatory protein CD59: a possible role in the pathogenesis of the vascular complications of human diabetes. Diabetes. 2004;53:2653-2661.
5. Qin X, Krumrei N, Grubissich L, et al. Deficiency of the mouse complement regulatory protein mCd59b results in spontaneous hemolytic anemia with platelet activation and progressive male infertility. Immunity. 2003;18:217-227.
6. Qin X, Dobarro M, Bedford SJ, et al. Further characterization of reproductive abnormalities in mCd59b knockout mice: a potential new function of mCd59 in male reproduction. J Immunol. 2005;175:6294-6302.
7. Morgan BP. Regulation of the complement membrane attack pathway. Crit Rev Immunol. 1999;19:173-198.
8. Walport MJ. Complement. First of two parts. N Engl J Med. 2001;344:1058-1066.
9. Huber-Lang M, Sarma JV, Zetoune FS, et al. Generation of C5a in the absence of C3: a new complement activation pathway. Nat Med. 2006;12:682-687.
10. Mayer MM. Complement. Historical perspectives and some current issues. Complement. 1984;1:2-26.
11. Walport MJ. Complement. Second of two parts. N Engl J Med. 2001;344:1140-1144.
12. Whaley K. Measurement of complement activation in clinical practice. Complement Inflamm. 1989;6:96-103.
13. Steinberg JB, Kapelanski DP, Olson JD, Weiler JM. Cytokine and complement levels in patients undergoing cardiopulmonary bypass. J Thorac Cardiovasc Surg. 1993;106:1008-1016.
14. Fisicaro N, Aminian A, Hinchliffe SJ, et al. The pig analogue of CD59 protects transgenic mouse hearts from injury by human complement. Transplantation. 2000;70:963-968.
15. Medof ME, Gottlieb A, Kinoshita T, et al. Relationship between decay accelerating factor deficiency, diminished acetylcholinesterase activity, and defective terminal complement pathway restriction in paroxysmal nocturnal hemoglobinuria erythrocytes. J Clin Invest. 1987;80:165-174.
16. Davitz MA, Low MG, Nussenzweig V. Release of decay-accelerating factor (DAF) from the cell membrane by phosphatidylinositol-specific phospholipase C (PIPLC). Selective modification of a complement regulatory protein. J Exp Med. 1986;163:1150-1161.
17. Nicholson-Weller A, Spicer DB, Austen KF. Deficiency of the complement regulatory protein, "decay-accelerating factor", on membranes of granulocytes, monocytes, and platelets in paroxysmal nocturnal hemoglobinuria. N Engl J Med. 1985;312:1091-1097.

18. Brodbeck WG, Mold C, Atkinson JP, Medof ME. Cooperation between decay-accelerating factor and membrane cofactor protein in protecting cells from autologous complement attack. J Immunol. 2000;165:3999-4006.
19. Sugita Y, Tobe T, Oda E, et al. Molecular cloning and characterization of MACIF, an inhibitor of membrane channel formation of complement. J Biochem (Tokyo). 1989;106:555-557.
20. Morgan BP, Gasque P. Extrahepatic complement biosynthesis: where, when and why? Clin Exp Immunol. 1997;107:1-7.
21. Tenner AJ, Volkin DB. Complement subcomponent C1q secreted by cultured human monocytes has subunit structure identical with that of serum C1q. Biochem J. 1986;233:451-458.
22. Gulati P, Lemercier C, Guc D, Lappin D, Whaley K. Regulation of the synthesis of C1 subcomponents and C1-inhibitor. Behring Inst Mitt. 1993;196-203.
23. Choy LN, Rosen BS, Spiegelman BM. Adipsin and an endogenous pathway of complement from adipose cells. J Biol Chem. 1992;267:12736-12741.
24. White RT, Damm D, Hancock N, et al. Human adipsin is identical to complement factor D and is expressed at high levels in adipose tissue. J Biol Chem. 1992;267:9210-9213.
25. Maves KK, Weiler JM. Properdin: approaching four decades of research. Immunol Res. 1993;12:233-243.
26. Wurzner R, Joysey VC, Lachmann PJ. Complement component C7. Assessment of *in vivo* synthesis after liver transplantation reveals that hepatocytes do not synthesize the majority of human C7. J Immunol. 1994;152:4624-4629.
27. Qin X, Miwa T, Aktas H, et al. Genomic structure, functional comparison, and tissue distribution of mouse Cd59a and Cd59b. Mamm Genome. 2001;12:582-589.
28. Mead R, Hinchliffe SJ, Morgan BP. Molecular cloning, expression and characterization of the rat analogue of human membrane cofactor protein (MCP/CD46). Immunology. 1999; 98:137-143.
29. Harris CL, Rushmere NK, Morgan BP. Molecular and functional analysis of mouse decay accelerating factor (CD55). Biochem J. 1999;341 (Pt 3):821-829.
30. Pontoglio M, Pausa M, Doyen A, Viollet B, Yaniv M, Tedesco F. Hepatocyte nuclear factor 1α controls the expression of terminal complement genes. J Exp Med. 2001;194:1683-1689.
31. Garnier G, Circolo A, Colten HR. Constitutive expression of murine complement factor B gene is regulated by the interaction of its upstream promoter with hepatocyte nuclear factor 4. J Biol Chem. 1996;271:30205-30211.
32. Arenzana N, Rodriguez de Cordoba S. Promoter region of the human gene coding for β-chain of C4b binding protein. Hepatocyte nuclear factor-3 and nuclear factor-I/CTF transcription factors are required for efficient expression of C4BPB in HepG2 cells. J Immunol. 1996;156:168-175.
33. Stapp JM, Sjoelund V, Lassiter HA, Feldhoff RC, Feldhoff PW. Recombinant rat IL-1β and IL-6 synergistically enhance C3 mRNA levels and complement component C3 secretion by H-35 rat hepatoma cells. Cytokine. 2005;30:78-85.
34. Prada AE, Zahedi K, Davis AE, 3rd. Regulation of C1 inhibitor synthesis. Immunobiology. 1998;199:377-388.
35. Phillips DJ, Novinger MS, Evatt BL, Hooper WC. TNF-α suppresses IL-1α and IL-6 upregulation of C4b-binding protein in HepG-2 hepatoma cells. Thromb Res. 1996;81: 307-314.
36. Schieferdecker HL, Schlaf G, Jungermann K, Gotze O. Functions of anaphylatoxin C5a in rat liver: direct and indirect actions on nonparenchymal and parenchymal cells. Int Immunopharmacol. 2001;1:469-481.
37. Koleva M, Schlaf G, Landmann R, Gotze O, Jungermann K, Schieferdecker HL. Induction of anaphylatoxin C5a receptors in rat hepatocytes by lipopolysaccharide *in vivo*: mediation by interleukin-6 from Kupffer cells. Gastroenterology. 2002;122: 697-708.
38. Daveau M, Benard M, Scotte M, et al. Expression of a functional C5a receptor in regenerating hepatocytes and its involvement in a proliferative signaling pathway in rat. J Immunol. 2004;173:3418-3424.
39. Buchner RR, Hugli TE, Ember JA, Morgan EL. Expression of functional receptors for human C5a anaphylatoxin (CD88) on the human hepatocellular carcinoma cell line HepG2. Stimulation of acute-phase protein-specific mRNA and protein synthesis by human C5a anaphylatoxin. J Immunol. 1995;155: 308-315.
40. Schieferdecker HL, Rothermel E, Timmermann A, Gotze O, Jungermann K. Anaphylatoxin C5a receptor mRNA is strongly expressed in Kupffer and stellate cells and weakly in sinusoidal endothelial cells but not in hepatocytes of normal rat liver. FEBS Lett. 1997;406:305-309.
41. Hinglais N, Kazatchkine MD, Mandet C, Appay MD, Bariety J. Human liver Kupffer cells express CR1, CR3, and CR4 complement receptor antigens. An immunohistochemical study. Lab Invest. 1989;61:509-514.
42. Benacerraf B, Sebestyen MM, Schlossman S. A quantitative study of the kinetics of blood clearance of P32-labelled *Escherichia coli* and *Staphylococci* by the reticuloendothelial system. J Exp Med. 1959;110:27-48.
43. Yan J, Vetvicka V, Xia Y, Hanikyrova M, Mayadas TN, Ross GD. Critical role of Kupffer cell CR3 (CD11b/CD18) in the clearance of IgM-opsonized erythrocytes or soluble β-glucan. Immunopharmacology. 2000;46:39-54.
44. Helmy KY, Katschke KJ, Jr., Gorgani NN, et al. CRIg: a macrophage complement receptor required for phagocytosis of circulating pathogens. Cell. 2006;124:915-927.
45. Kumar V, Ali SR, Konrad S, et al. Cell-derived anaphylatoxins as key mediators of antibody-dependent type II autoimmunity in mice. J Clin Invest. 2006;116:512-520.
46. Schlaf G, Schmitz M, Heine I, Demberg T, Schieferdecker HL, Gotze O. Upregulation of fibronectin but not of entactin, collagen IV and smooth muscle actin by anaphylatoxin C5a in rat hepatic stellate cells. Histol Histopathol. 2004;19:1165-1174.
47. Beekman B, van El B, Drijfhout JW, Ronday HK, TeKoppele JM. Highly increased levels of active stromelysin in rheumatoid synovial fluid determined by a selective fluorogenic assay. FEBS Lett. 1997;418:305-309.
48. Schieferdecker HL, Pestel S, Rothermel E, Puschel GP, Gotze O, Jungermann K. Stimulation by anaphylatoxin C5a of glycogen phosphorylase in rat hepatocytes *via* prostanoid release from hepatic stellate cells but not sinusoidal endothelial cells. FEBS Lett. 1998;434:245-250.
49. Hillebrandt S, Wasmuth HE, Weiskirchen R, et al. Complement factor 5 is a quantitative trait gene that modifies liver fibrogenesis in mice and humans. Nat Genet. 2005;37:835-843.
50. Mastellos D, Papadimitriou JC, Franchini S, Tsonis PA, Lambris JD. A novel role of complement: mice deficient in the fifth component of complement (C5) exhibit impaired liver regeneration. J Immunol. 2001;166:2479-2486.
51. Strey CW, Markiewski M, Mastellos D, et al. The proinflammatory mediators C3a and C5a are essential for liver regeneration. J Exp Med. 2003;198:913-923.
52. Markiewski MM, Mastellos D, Tudoran R, et al. C3a and C3b activation products of the third component of complement (C3) are critical for normal liver recovery after toxic injury. J Immunol. 2004;173:747-754.

53. Fausto N, Campbell JS, Riehle KJ. Liver regeneration. Hepatology. 2006;43:S45-53.
54. Rehermann B, Nascimbeni M. Immunology of hepatitis B virus and hepatitis C virus infection. Nat Rev Immunol. 2005;5:215-229.
55. Speth C, Stoiber H, Dierich MP. Complement in different stages of HIV infection and pathogenesis. Int Arch Allergy Immunol. 2003;130:247-257.
56. Mehlhop E, Diamond MS. Protective immune responses against West Nile virus are primed by distinct complement activation pathways. J Exp Med. 2006;203:1371-1381.
57. Nomura H, Ogo T, Rikimaru N, et al. Hepatitis C virus-related liver damage is related to cold activation of complement. J Clin Gastroenterol. 1997;25:529-534.
58. Akahane Y, Miyazaki Y, Naitoh S, et al. Cold activation of complement for monitoring the response to interferon in patients with chronic hepatitis C. Am J Gastroenterol. 1996;91:319-327.
59. Saadoun D, Sadallah S, Trendelenburg M, et al. Anti-C1q antibodies in hepatitis C virus infection. Clin Exp Immunol. 2006;145:308-312.
60. Seelen MA, Trouw LA, Daha MR. Diagnostic and prognostic significance of anti-C1q antibodies in systemic lupus erythematosus. Curr Opin Nephrol Hypertens. 2003;12:619-624.
61. Diao J, Slaney DM, Michalak TI. Modulation of the outcome and severity of hepadnaviral hepatitis in woodchucks by antibodies to hepatic asialoglycoprotein receptor. Hepatology. 2003;38:629-638.
62. Diao J, Churchill ND, Michalak TI. Complement-mediated cytotoxicity and inhibition of ligand binding to hepatocytes by woodchuck hepatitis virus-induced autoantibodies to asialoglycoprotein receptor. Hepatology. 1998;27:1623-1631.
63. Pham BN, Mosnier JF, Durand F, et al. Immunostaining for membrane attack complex of complement is related to cell necrosis in fulminant and acute hepatitis. Gastroenterology. 1995;108:495-504.
64. Chong WP, To YF, Ip WK, et al. Mannose-binding lectin in chronic hepatitis B virus infection. Hepatology. 2005;42: 1037-1045.
65. Thio CL, Mosbruger T, Astemborski J, et al. Mannose binding lectin genotypes influence recovery from hepatitis B virus infection. J Virol. 2005;79:9192-9196.
66. Homann C, Varming K, Hogasen K, et al. Acquired C3 deficiency in patients with alcoholic cirrhosis predisposes to infection and increased mortality. Gut. 1997;40:544-549.
67. Collard CD, Lekowski R, Jordan JE, Agah A, Stahl GL. Complement activation following oxidative stress. Mol Immunol. 1999;36:941-948.
68. Bykov IL, Vakeva A, Jarvelainen HA, Meri S, Lindros KO. Protective function of complement against alcohol-induced rat liver damage. Int Immunopharmacol. 2004;4:1445-1454.
69. Bykov I, Junnikkala S, Pekna M, Lindros KO, Meri S. Complement C3 contributes to ethanol-induced liver steatosis in mice. Ann Med. 2006;38:280-286.
70. Bird GL, Lau JY, Davies ET, Williams RS. Lack of plasma complement activation in severe acute alcoholic hepatitis. Alcohol Clin Exp Res. 1995;19:1537-1540.
71. Jaeschke H. Mechanisms of liver injury. II. Mechanisms of neutrophil-induced liver cell injury during hepatic ischemia-reperfusion and other acute inflammatory conditions. Am J Physiol Gastrointest Liver Physiol. 2006;290:G1083-1088.
72. Jaeschke H, Farhood A, Bautista AP, Spolarics Z, Spitzer JJ. Complement activates Kupffer cells and neutrophils during reperfusion after hepatic ischemia. Am J Physiol. 1993;264: G801-809.
73. Arumugam TV, Shiels IA, Woodruff TM, Granger DN, Taylor SM. The role of the complement system in ischemia-reperfusion injury. Shock. 2004;21:401-409.
74. Chavez-Cartaya RE, DeSola GP, Wright L, Jamieson NV, White DJ. Regulation of the complement cascade by soluble complement receptor type 1. Protective effect in experimental liver ischemia and reperfusion. Transplantation. 1995;59:1047-1052.
75. Lehmann TG, Koeppel TA, Munch S, et al. Impact of inhibition of complement by sCR1 on hepatic microcirculation after warm ischemia. Microvasc Res. 2001;62:284-292.
76. Heijnen BH, Straatsburg IH, Padilla ND, Van Mierlo GJ, Hack CE, Van Gulik TM. Inhibition of classical complement activation attenuates liver ischaemia and reperfusion injury in a rat model. Clin Exp Immunol. 2006;143:15-23.
77. Scoazec JY, Borghi-Scoazec G, Durand F, et al. Complement activation after ischemia-reperfusion in human liver allografts: incidence and pathophysiological relevance. Gastroenterology. 1997;112:908-918.
78. Schmeding M, Dankof A, Krenn V, et al. C4d in acute rejection after liver transplantation--a valuable tool in differential diagnosis to hepatitis C recurrence. Am J Transplant. 2006;6: 523-530.
79. Tector AJ, Berho M, Fridell JA, et al. Rejection of pig liver xenografts in patients with liver failure: implications for xenotransplantation. Liver Transpl. 2001;7:82-89.
80. Bouwman LH, Roos A, Terpstra OT, et al. Mannose binding lectin gene polymorphisms confer a major risk for severe infections after liver transplantation. Gastroenterology. 2005; 129:408-414.
81. Morris KM, Aden DP, Knowles BB, Colten HR. Complement biosynthesis by the human hepatoma-derived cell line HepG2. J Clin Invest. 1982;70:906-913.
82. Alper CA, Johnson AM, Birtch AG, Moore FD. Human C'3: evidence for the liver as the primary site of synthesis. Science. 1969;163:286-288.
83. Schwaeble W, Dahl MR, Thiel S, Stover C, Jensenius JC. The mannan-binding lectin-associated serine proteases (MASPs) and MAp19: four components of the lectin pathway activation complex encoded by two genes. Immunobiology. 2002;205:455-466.