HEPATOLOGY

HEPATOLOGY, VOL. 70, NO. 2, 2019

# The Role of Complement in Liver Injury, Regeneration, and Transplantation

Ebbe Billmann Thorgersen,[1,2] Andreas Barratt-Due,[1,3] Håkon Haugaa,[3,4] Morten Harboe,[1] Søren Erik Pischke,[1,3] Per H. Nilsson,[1,5] and Tom Eirik Mollnes[1,6,7]

**The liver is both an immunologically complex and a privileged organ. The innate immune system is a central player, in which the complement system emerges as a pivotal part of liver homeostasis, immune responses, and crosstalk with other effector systems in both innate and adaptive immunity. The liver produces the majority of the complement proteins and is the home of important immune cells such as Kupffer cells. Liver immune responses are delicately tuned between tolerance to many antigens flowing in from the alimentary tract, a tolerance that likely makes the liver less prone to rejection than other solid organ transplants, and reaction to local injury, systemic inflammation, and regeneration. Notably, complement is a double-edged sword as activation is detrimental by inducing inflammatory tissue damage in, for example, ischemia–reperfusion injury and transplant rejection yet is beneficial for liver tissue regeneration. Therapeutic complement inhibition is rapidly developing for routine clinical treatment of several diseases. In the liver, targeted inhibition of damaged tissue may be a rational and promising approach to avoid further tissue destruction and simultaneously preserve beneficial effects of complement in areas of proliferation. Here, we argue that complement is a key system to manipulate in the liver in several clinical settings, including liver injury and regeneration after major surgery and preservation of the organ during transplantation. (Hepatology 2019;70:725-736).**

## Overview of the Complement System

The complement system (Fig. 1) is an evolutionarily ancient part of the immune system, traditionally respected for its antimicrobial effects but today appreciated for homeostatic functions that extend far beyond microbial clearance.[1] More than fifty soluble and membrane-bound complement components have been characterized (Table 1). Hepatocytes synthesize most of the components, and the liver accounts for up to 90% of the fluid-phase complement proteins.[2] It is hypothesized that the first complement component (i.e., C3) early in evolution was expressed intracellularly and that when organisms evolved into more complex bodies, complement proteins began to be secreted into the intercellular space and were later allocated for hepatic synthesis for intravascular release.[3]

The complement system serves host tissue surveillance by reacting to the presence of danger. Activation of the complement system is typically initiated by binding of complement pattern recognition receptors within the classical and lectin pathways to patho-

*Abbreviations: ACR, acute, mostly cellular, rejection; ALT, alanine aminotransferase; AMR, antibody-mediated rejection; CD, cluster of differentiation; CR, complement receptor; DAMP, damage-associated molecular pattern; DSA, donor-specific antibody; HCC, hepatocellular carcinoma; HLA, human leukocyte antigen; Ig, immunoglobulin; IL, interleukin; IRI, ischemia–reperfusion injury; LPS, lipopolysaccharide; MAC, membrane attack complex; NASH, nonalcoholic steatohepatitis; PAMP, pathogen-associated molecular pattern; TCC, terminal C5b-9 complex; TLR, Toll-like receptor.*

*Received June 26, 2018; accepted January 10, 2019.*



*View this article online at wileyonlinelibrary.com.*

*DOI 10.1002/hep.30508*

*Potential conflict of interest: Nothing to report.*

gen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) (Fig. 1). Activation proceeds to the formation of an enzyme complex, a convertase, for cleavage of the central complement component, C3. C3 cleavage generates a small C3a anaphylatoxin and surface deposition of the larger C3b fragment. C3a promotes cell signaling by binding to its receptor, C3aR. C3b initiates the alternative pathway in which C3 activation is amplified by formation of a C3 convertase.

C3b and its degradation products are also ligands for various complement receptors. Along with increased surface deposition of C3b, the substrate specificity shifts from C3 to C5. The initiation of C5 cleavage into C5a promotes cell activation and chemotaxis through the proinflammatory receptor C5aR1, whereas C5b initiates formation of the terminal C5b-9 complex (TCC), which can be inserted into a membrane as the membrane attack complex (MAC) or released to the fluid phase as sC5b-9.

# Complement in Disease

The net complement response is the result of a delicate balance between activation and regulation. Insufficient regulation or excessive activation results in inflammation. Thus, essential regulators must be present to prevent or modulate activation. Mutational loss of regulatory function can be the primary cause of disease, like paroxysmal nocturnal hemoglobinuria and atypical hemolytic syndrome. Surfaces that lack or have lost the ability to regulate complement activation, for example, implanted biomaterials or ischemic tissue, will be targets for complement activation. Because complement can be activated on all damaged tissues, it will also be a contributor to disease in a large variety of disorders, if not all diseases. An example from hepatology, with complement in a central position, is nonalcoholic steatohepatitis (NASH) and associated hepatocellular carcinoma (HCC). The other side of the coin of complement disease is the inability to respond to PAMPs or DAMPs, typically by mutational loss of complement function. Primarily, this is associated with infection and insufficient handling of cell debris. Infections by encapsulated bacteria, typically *Neisseria meningitidis*, are more common in individuals with primary or acquired immunodeficiency of complement proteins.[4]

# Complement and Crosstalk

Almost all immune cells express receptors for complement activation fragments, which influence the innate and adaptive immune responses. C3b and the anaphylatoxins are involved in T-cell activation, and the threshold for B-cell receptor activation is lowered by simultaneous linkage of C3d fragments on the immunogenic surface to B cells.[5,6] Other important innate effector systems crosstalk with the complement system, for example, the Toll-like receptor system (Fig. 2), and act in synergy with complement activation in the synthesis and release of cytokines.[7] Inflammasome activation for the release of mature interleukin 1 beta (IL-1β) is influenced by simultaneous stimulation of complement activation products, including C5a and sublytic MAC.[8]

**ARTICLE INFORMATION:**

From the [1]Department of Immunology, Oslo University Hospital Rikshospitalet and University of Oslo; [2]Department of Gastroenterological Surgery, The Norwegian Radium Hospital, Oslo University Hospital; [3]Division of Emergencies and Critical Care, Oslo University Hospital Rikshospitalet; [4]Lovisenberg Diaconal University College, Oslo, Norway; [5]Linnaeus Centre for Biomaterials Chemistry, Linnaeus University, Kalmar, Sweden; [6]Reserach Laboratory, Nordland Hospital, Bodø, and Faculty of Health Sciences, K.G. Jebsen TREC, University of Tromsø, Tromsø, Norway; [7]Centre of Molecular Inflammation Research, Norwegian University of Science and Technology, Trondheim, Norway.

**ADDRESS CORRESPONDENCE AND REPRINT REQUESTS TO:**

Ebbe Billmann Thorgersen, M.D., Ph.D.
Department of Immunology and Department of Gastroenterological Surgery
Oslo University Hospital and University of Oslo
P.O. Box 4950 Nydalen
N-0424 Oslo, Norway
E-mail: ebbtho@rr-research.no
Tel: +47-48144121

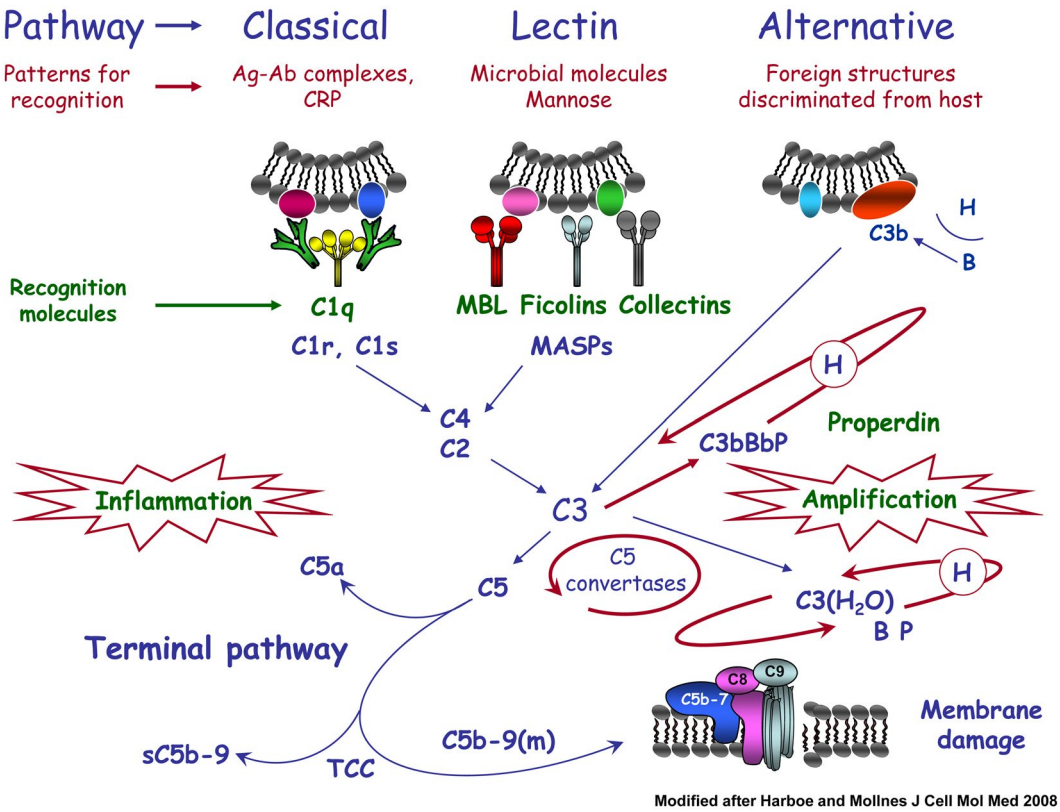


**FIG. 1.** The complement system. The complement system can be activated through three pathways (top), which converge on C3 to activate the common terminal pathway. Several pattern recognition receptors, like C1q, ficolins, mannose binding lectin, and collectins, activate the system after binding to exogenous PAMPs and DAMPs. The alternative pathway has another important function in the complement system, providing an amplification loop that enhances C3 activation independently of which pathway is initially activated. Activation of C3 leads to formation of a C5 convertase, which cleaves C5 into C5a and C5b. The anaphylatoxins C3a and C5a bind to their receptors, initiating downstream production of mediators, leading to inflammation. C5b initiates the formation of C5b-9, often called the TCC, which forms the MAC if inserted into a membrane or sC5b-9 is released to the fluid phase. The MAC may lead to lysis of bacteria and cells or, if sublytic, to activation of cells, whereas sC5b-9 is a useful plasma marker of complement activation. The complement system is tightly regulated by soluble inhibitors including the important factor H controlling the alternative pathway. Abbreviations: AB, antibody; Ag, antigen; B, factor B; CRP, C-reactive protein; H, factor H; MASP, mannose-associated serine protease; MBL, mannose binding lectin; P, factor P.

# Complement Protein Synthesis in the Normal and Diseased Liver

The level of complement proteins in plasma is determined by the balance between synthesis and consumption. Both the native components and their activation markers must be quantified in order to determine the causality. Cirrhosis, liver failure, and starvation will normally result in decreased levels of most soluble complement proteins. Decreased levels of C3 and C4 can also be found in chronic active hepatitis of any origin without liver failure, where synthesis is maintained but the levels are depressed due to simultaneous activation and thus consumption.[9] This is accompanied by increased levels of activation markers. Additionally, several complement proteins, including C3 and C4, are acute-phase reactants that will increase by up to 50% in response to systemic inflammation.[10] Thus, the level of a component can be within the normal range despite pathophysiological changes, such as increased production and ongoing consumption.

**TABLE 1. A Selection of Central Complement Proteins**

| Name | Function |
|---|---|
| C1q/r/s | The CP C1 complex containing pattern recognition molecule (C1q) and the proteases cleaving C1s (C1r) and C2/C4 (C1s) |
| MBL and ficolin-1, -2, -3 | Pattern recognition molecule of the LP |
| MASP-1, -2 | Proteases in the LP, cleaves C2 and C4 (only MASP-2) |
| C2 | Protease of the CP and LP C3/C5 convertase (C2aC4b) |
| C3 | Part of the AP C3 convertase (C3bBb) and all C5 convertases. Progenitor to cleavage fragments C3a/C3b/iC3b/C3dg/C3d |
| C4 | Part of the CP/LP C3 convertase (C2aC4b) |
| C5 | Progenitor for the anaphylatoxin C5a and the terminal C5b-9 complement complex |
| C6, 7, 8, 9 | Forms with C5b the terminal C5b-9 complement complex |
| Factor B | Protease of the AP C3/C5 convertase (C3bBb) |
| Properdin | Stabilizes the AP C3/C5 convertases (C3bBbP) |
| C3a and C3aR | Anaphylatoxin (C3a) and its associated receptor |
| CR1-4 | CR1 binds C3b and regulates C3 activation, CR2 on lymphocytes binds C3d, CR3 and CR4 phagocytose by binding iC3b opsonin |
| C5a and C5aR1, C5aR2 | Anaphylatoxin (C5a) and its associated receptors |
| C1-INH | Fluid phase regulator, inhibits CP and LP activation by inactivation of C1r/s and MASPs |
| Factor I | Fluid phase regulator, degrades C3b and C4b together with cofactors |
| C4BP and factor H | Fluid phase regulators of the CP/LP and AP, respectively; cofactor to factor I for the degradation of C4b and C3b, respectively |
| DAF and MCP | Membrane-bound regulators. DAF serves convertase decay acceleration and MCP is a cofactor for factor I degradation of C4b and C3b |
| CD59 | Regulator of the terminal complement complex, prevents assembly of C5b-9 |

Abbreviations: AP, alternative pathway; CP, classical pathway; LP, lectin pathway.

# Liver Injury

## ISCHEMIA–REPERFUSION INJURY

Warm hepatic ischemia–reperfusion injury (IRI) regularly occurs during liver resection as several procedures require intermittent total vascular occlusion (the "Pringle maneuver") in order to reduce blood loss and facilitate surgery (Fig. 3). While the ischemia time is relatively short during the Pringle maneuver, it is substantially longer in the course of liver transplantation, with both cold ischemia up to 12 hours during preservation and warm ischemia during implantation, leading to increased reperfusion injury.

Several experimental models targeting different complement effectors have demonstrated an ameliorating effect on hepatic IRI-induced injury in rats. Soluble complement receptor 1 (CR1) treatment, which efficiently reduces C3 activation, improved microvascular circulation and reduced adherent leukocytes.[11] C1 inhibitor, although not a specific complement inhibitor, attenuated plasma alanine aminotransferase (ALT) levels.[12] Treatment with the C5aR1 antagonist PMX53 has reduced mortality after total hepatic IRI (i.e., occlusion of both the portal vein and the common hepatic artery) and attenuated increases in liver enzyme levels and neutrophil infiltration after partial hepatic IRI (i.e., occlusion of the left branches of the portal vein and common hepatic artery).[13] Finally, a study using C6-deficient rats clearly demonstrated that formation of the TCC led to increased inflammation and cell injury in hepatic IRI.[14]

Targeted complement inhibition using the fusion protein CR2–cluster of differentiation 59 (CD59) has revealed promising effects in a mouse model of hepatic IRI.[15] CR2 binds to cell-bound C3d at sites with ongoing complement activation, whereas CD59 binds to C8 and C9, thereby preventing C5b-9 formation. This complement inhibitory strategy might be particularly beneficial to prevent liver injury due to increased bioavailability and efficacy while preserving host immunity compared to systemic complement inhibition. Furthermore, CR2–CD59 inhibition does not impact C3 and C5, allowing the prosurvival properties of C5a and C3a to operate locally in hepatic IRI. Marshall et al. identified membrane TCC as the principal mediator of hepatic injury, demonstrating that CR2–CD59 increased local liver concentrations

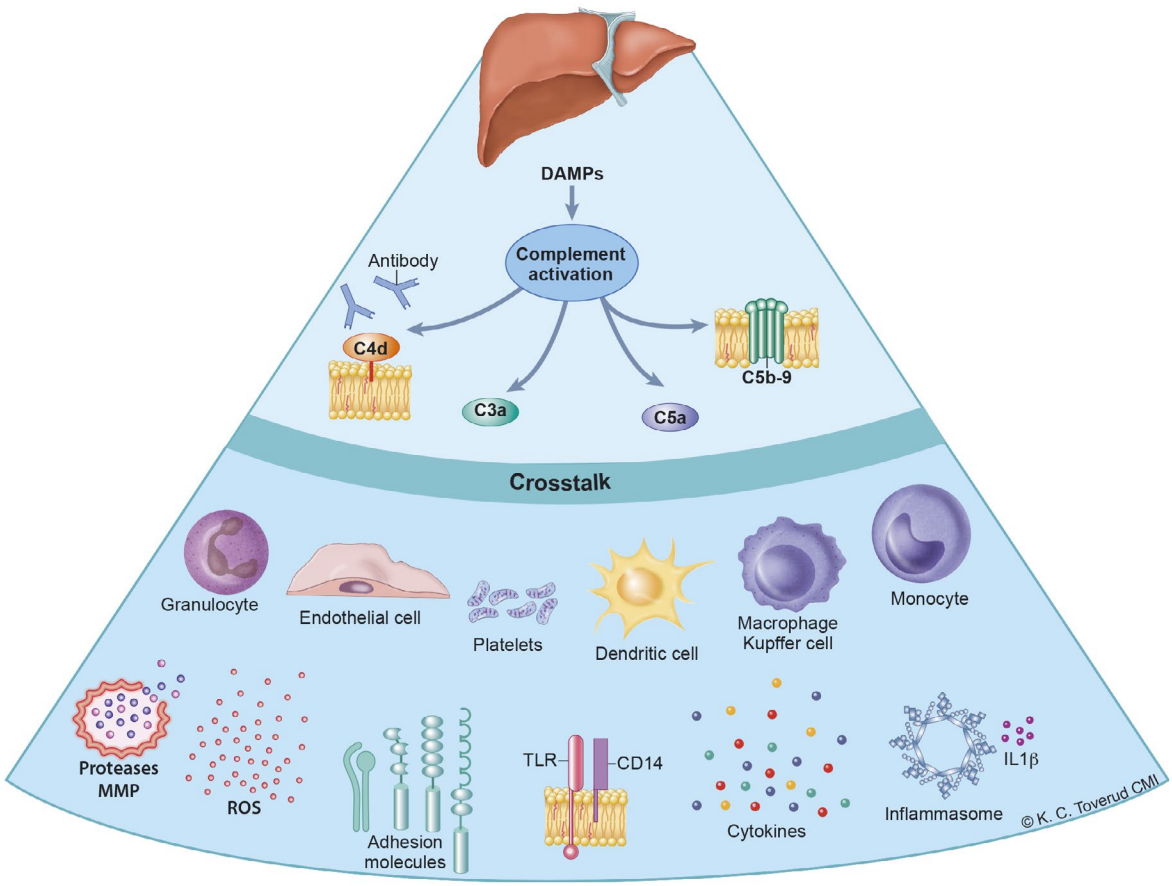


**FIG. 2.** The complement system and crosstalk with other innate immune effector systems in liver IRI, regeneration, and transplantation. The complement system is activated by endogenously derived DAMPs in IRI, regeneration, and transplantation. The activation products from the complement cascade activate cells by binding to cell receptors, which frequently crosstalk with receptors and mediators of a number of effector molecules and mechanisms downstream in the innate and adaptive immune system. The most important complement effector molecules are C3a, C5a, and C5b-9. C4d is a split product of the classical and lectin pathway activation and is covalently bound to cell membranes. If immunohistopathological staining in liver biopsies from liver grafts shows binding of C4d, AMR is suspected. Complement activates other effector systems, which frequently crosstalk and activate complement, consistent with a widespread bilateral and multilateral crosstalk between the complement system and these other effector systems. In particular, abundant crosstalk occurs with the hemostatic system ("thromboinflammation") including the other plasma cascades and the platelets. Complement activation promotes hemostasis by several routes; C5a activation of C5aR1 on monocytes and endothelial cells induces up-regulation of tissue factor (not shown) and thereby promotes coagulation through the extrinsic pathway. C3a binding to C3aR on platelets primes the cell for activation and insertion of MAC through the platelet membrane, promoting release of prothrombotic platelet microvesicles. Furthermore, there is an extensive crosstalk between complement and granulocytes, releasing enzymes like matrix metalloproteinases, and reactive oxygen species. Finally, there are advanced crosslinks between complement and the TLRs, producing a range of proinflammatory and anti-inflammatory cytokines, and with the inflammasome. This crosstalk may lead to a synergistic effect on the effector mechanisms involved and thereby produce a stronger inflammatory response. The main cellular players in these mechanisms, except for hepatocytes, are granulocytes, endothelial cells, platelets, dendritic cells, monocytes, and macrophages/Kupffer cells. Although there is much overlap with respect to the complement-induced activation of the different cells, granulocytes typically produce and release proteases and ROS; endothelial cells express adhesion molecules; and macrophages/Kupffer cells, monocytes, and dendritic cells typically up-regulate TLRs and their coreceptor CD14, produce and release cytokines, and assemble and activates the inflammasome. Platelets are increasingly recognized as important immune cells and can display or release all of the effector molecules shown, in particular dependent on the C5b-9 insertion. Abbreviations: MMP, matrix metalloproteinase; ROS, reactive oxygen species. Printed with permission from Kari C. Toverud.

of IL-6 and tumor necrosis factor (TNF) accompanied by increased signal transducer and activator of transcription 3 and Akt activation, reduced hepatic neutrophil infiltration, inhibition of mitochondrial depolarization, and recovery of adenosine triphosphate stores.[15]

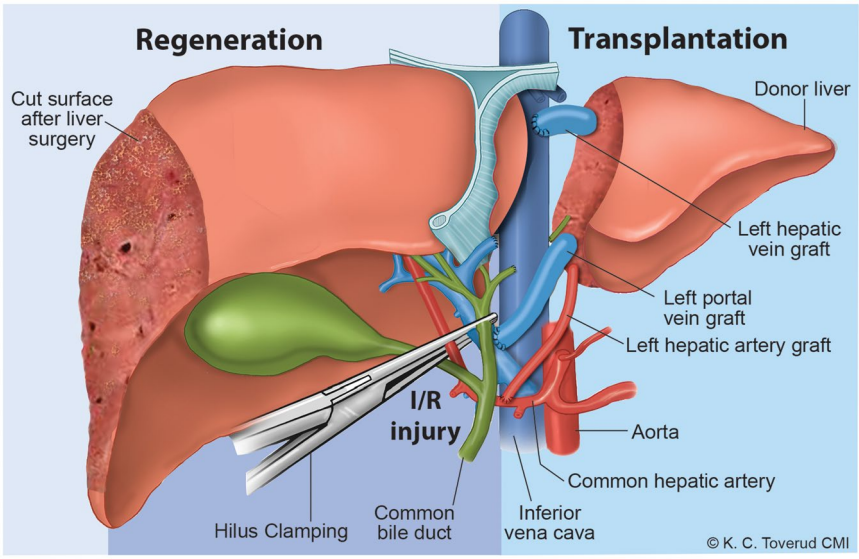


**FIG. 3.** Liver IRI, regeneration, and transplantation. The figure represents an overview of the text's three main topics. Liver IRI exemplified by hilar clamping ("Pringle maneuver"), regeneration exemplified after liver surgery, and transplantation exemplified by a segment 2 to 3 allograft (the "Resection And Partial Liver Segment 2/3 Transplantation With Delayed Total Hepatectomy [RAPID] concept"). Printed with permission from Kari C. Toverud.

Furthermore, the inhibitory strategy exerted protective effects and enhanced liver regeneration after hepatic IRI, also after 90% hepatectomy.[15] The protective effects associated with reduced levels of membrane TCC may be obtained through reduced NLR family pyrin domain containing 3 (NLRP3) inflammasome activation and subsequently reduced levels of IL-1β and IL-18.[16] In the future, a therapeutic preferably site-targeting, complement-modulating pharmaceutical[15] might be useful after large liver surgery to prevent complement-mediated liver injury and simultaneously promote liver regeneration. Complement activation appears to play an essential role in liver regeneration and is required for liver cell survival and proliferation. Thus, in hepatic IRI, complement activation balances its fine-tuned effects between injury and protection, an interplay that needs to be further elucidated in humans. We propose that C5 with C5a generation should be prevented at a very early stage to avoid tissue damage but compensated by complement being free to act in repair and regeneration.

## SYSTEMIC INFLAMMATORY RESPONSE

The liver comprises the highest content of tissue-resident immune cells in the body, including Kupffer cells, dendritic cells, and natural killer cells.[17] These immune cells function together with infiltrating myeloid and lymphoid cells to respond to PAMPs and DAMPs. Thus, it is not surprising that the liver reacts vividly to systemic inflammation. Under normal physiological conditions, complement activation is tightly regulated and maintains host homeostasis by acting only locally. However, improper or excessive complement activation may cause detrimental tissue and organ damage and may amplify a systemic inflammatory and counterproductive reaction as seen in septic shock.

Noninfectious systemic inflammatory response with activation of the complement system occurs in the course of trauma, intoxication, response to extracorporeal membrane oxygenation, whole-body ischemia (e.g., after cardiac arrest), burns, and local nonhepatic ischemia (e.g., bowel ischemia). Circulating complement products like C5a induce hepatic endothelial cell activation with up-regulation of cell adhesion molecules, including intercellular cell adhesion molecule 1 and vascular cell adhesion molecule 1 (VCAM-1), and subsequent recruitment of neutrophils to the liver sinusoids.[18]

Experimental C1 inhibitor treatment has been shown to reduce hepatic VCAM-1 expression,[19] while C3 knockout mice, C3aR/C5aR antagonists,

and CR2-fH protect against fulminant hepatic failure due to systemic inflammation.[20] In a primate model of *Escherichia coli* sepsis, single inhibition of C3 or C5 both attenuated *E. coli*–induced liver injury by reducing ALT and alanine aminotransferase (AST) levels and reduced congestion, leukocyte infiltration, and hepatocellular vacuolization,[21] confirming the detrimental effects of uncontrolled complement activation.

The reaction of hepatic cells to systemic complement activation should be highlighted. The liver is constantly exposed to activated complement, immune complexes, and intestinal bacterial products like lipopolysaccharide (LPS) from gram-negative bacteria. Large amounts of LPS enter the portal vein from the intestines but do not reach the systemic circulation. Usually, LPS binding to its receptor, Toll-like receptor 4 (TLR4)–CD14, induces a strong proinflammatory signal. However, in the liver the response is different. For example, in experimental studies, resident liver Kupffer cells stimulated by LPS binding to TLR4–CD14 produce the anti-inflammatory cytokine IL-10.[22] Hepatocytes are more resistant to activated complement products than endothelial cells, due to intracellular signaling through the phosphoinositide 3-kinase (PI3K)/Akt pathway.[23] Specifically, phosphorylation of Akt, a prerequisite for increased and thus protective PI3K activity, is complement-dependent in hepatocytes; and much higher concentrations of phosphorylated Akt are found in hepatocytes compared to heart and kidney. Concordantly, only when the system is exhausted by overwhelming systemic stimuli will the liver be injured. The mechanism of increased resistance of hepatocytes to complement injury should thus be further investigated in order to develop therapeutic approaches.

Importantly, complement-dependent IRI has been found in experimental studies to be delicately balanced with complement regulation of liver regeneration, suggesting that complement is a double-edged sword in these processes.[24]

## EMERGING CHALLENGES IN LIVER INJURY: NASH

The incidence of nonalcoholic fatty liver disease progressing to NASH is increasing worldwide in parallel to the obesity epidemic, leading to fibrosis, cirrhosis, and associated HCC.[25] The innate immune system plays a major role in the pathogenesis, including a significantly increased synthesis of C3.[26] Additionally, widespread complement activation with deposition of iC3b, C3d, C4d, and C5b-9 around steatotic hepatocytes is associated with increased numbers of invading neutrophils, proinflammatory cytokine expression, and disease severity.[27] C1q has been associated with HCC development through activation of the collagen receptor discoidin domain receptor 1.[28] Thus, NASH and related HCC could be decreased by inhibition of a constantly activated complement system, for instance, by targeting the alternative pathway.[29]

# Complement and Liver Regeneration

Liver regeneration has been evolutionarily preserved in mammals. Animal models of both toxic and surgical liver injury have shown the importance of complement in the process. Knockout animals paved the way for these discoveries. In a model of toxic liver injury, using carbon tetrachloride ($CCl_4$) injected intraperitoneally, C5-deficient ($C5^{-/-}$) mice showed severe toxic damage to the liver with defective liver regeneration and persistent parenchymal necrosis compared to wild-type mice.[30] After the toxic injury, hepatocytes, which constitute 90% of all liver cells, showed a marked delay of reentry into the cell cycle (S phase) and diminished mitotic activity in $C5^{-/-}$ mice. Reconstitution of C5-deficient mice with murine C5 or C5a significantly restored hepatocyte regeneration. Furthermore, blockade of C5aR1 in wild-type mice abrogated the ability of hepatocytes to proliferate in response to liver injury, providing a mechanism by which C5 exerts its function.[30]

Furthermore, C5aR1 was up-regulated on hepatocytes, which normally do not express C5aR1 constitutionally, during liver regeneration in a model of surgical liver injury.[31] C5a binding to C5aR1 induced a growth response in hepatocytes, and C5aR1 was involved in a cell cycle signaling pathway. The findings led to the hypothesis that C5a amplifies the proliferative response through C5aR1.

As with C5, the other central component of the complement cascade, C3 also impacts on liver regeneration. C3-deficient mice ($C3^{-/-}$) were shown to have impaired liver regeneration after $CCl_4$ injury, which,

as shown with C5a reconstitution in $C5^{-/-}$ mice, was restored by C3a reconstitution.[32] The authors confirmed their findings using C3aR-deficient mice ($C3aR^{-/-}$), indicating that the effect was through C3a–C3aR signaling. Increased C3b in serum and C3b deposition in the liver found early after injury in wild-type mice would also be important as an inducer of C5 convertase, producing C5a. In addition to C3b, iC3b deposition found in damaged liver parenchyma in wild-type, but not in C3-deficient, mice coincided with delayed removal of damaged tissue in $C3^{-/-}$ mice. Indeed, both C3a and C5a, as well as C3b/iC3b, may contribute in the regenerative process after liver injury.[32]

Experimental two-thirds partial hepatectomy in rodents was pioneered in the early 1930s by Higgins et al., and although later refined, the 70%-80% hepatectomy in rodents is still an excellent and widely used model system for studies of liver regeneration. Using a hepatectomy model in mice, the importance of both C3 and C5 for liver regeneration was studied.[33] Both $C3^{-/-}$ and $C5^{-/-}$ mice showed increased apoptosis, parenchymal damage, liver failure, mortality, and impaired liver regeneration, with more severe pathological changes in the C3-deficient mice. Crossbred mice with dual deficiency ($C3^{-/-}$ and $C5^{-/-}$) had a more exacerbated phenotype, with signs of liver failure in all animals. Reconstitution with C3a, C5a, or both, prior to and directly after the surgical insult, partly reversed the changes in the deficient mice, with an additive effect with C3a/C5a combined reconstitution.[33]

Nontraditional activation of the complement system (i.e., activation not dependent on the classical, lectin, or alternative pathway) is frequently reported, including direct activation of C5 by proteases of the coagulation system, like thrombin. In liver regeneration, a similar activation of complement is suggested.[34] Using the hepatectomy model, knockout mice with defects in essential components of the three initiating pathways ($C4^{-/-}$ mice for the classical and lectin pathways, factor $B^{-/-}$ mice for the alternative pathway) were studied, as well as $C3^{-/-}$ mice for the initial common pathway in the activation cascade. The authors found that $C4^{-/-}$, factor $B^{-/-}$, and $C4^{-/-}$ mice challenged with an anti–factor B antibody, effectively preventing C3/C5 convertase formation by all three pathways, had normal regeneration, while regeneration in $C3^{-/-}$ mice was delayed. Plasmin-mediated C3 activation was demonstrated *in vitro* in plasma from all mouse strains used in the study. Thus, the authors proposed that plasmin, a known regulator of liver regeneration,[35] may contribute in nontraditional complement activation in liver regeneration.[34]

In a study of the role of complement in both IRI and liver regeneration following 70% hepatectomy, C3 was confirmed to play a central role in complement-dependent liver regeneration.[24] The authors suggested that involvement of complement C3 in the proliferative response could be independent of C3a–C3aR interaction and that the signaling could involve desarginated C3a, also termed acylation-stimulating protein, interacting with the enigmatic complement receptor C5aR2, previously called C5L2.

Interestingly, inhibition of the TCC without inhibiting upstream generation of C3a and C5a enhanced liver regeneration after 70% hepatectomy and enhanced survival in an extreme 90% hepatectomy model.[15] Proliferative effects and reduced injury as outlined above (see Liver Injury) are thought to contribute to these findings. Thus, the prosurvival properties of C3a and C5a seem to be exerted through crosstalk with the cytokine network, in particular local formation of IL-6 and TNF.[36] The versatile cytokine IL-4, produced locally by natural killer T cells, may contribute to liver regeneration, possibly through a regulatory positive feedback loop with C3a and C5a also involving induction of macrophage-produced IL-6.[37]

In liver surgery, the concept of liver regeneration is widely used.[38] Interestingly, in a principle of partial liver transplantation, necessary regeneration has been demonstrated despite a standard immunosuppressive regimen (Fig. 3).[39] The complete mechanisms behind liver regeneration in humans are unknown, but experimental models reveal that complement seems to have an important role in these complex cellular processes.

# Liver Transplantation

## LIVER TRANSPLANTATION, IMMUNOTOLERANCE, AND REJECTION

The liver has unique characteristics when it comes to immunotolerance, and the field is still incompletely understood. The tolerability has been found

in several species including rodents, pigs, dogs, and humans. Approximately 20%-25% of liver-transplanted patients could be weaned off immunosuppression without experiencing rejection.[40] Another interesting feature of liver transplantation is that the need for human leukocyte antigen (HLA) cross-matching has been seen as debatable with respect to transplant outcome, in sharp contrast to kidney and heart transplantation where HLA cross-matching is critical for outcome.

Despite the relative immune tolerance, rejection occurs. Complement is the prime immune effector system in antibody-mediated rejection (AMR) to mismatched organs as preformed donor-specific antibodies (DSAs) directed against HLA or non-HLA antigens activate complement.[41] DSA-initiated activation of complement is mediated through the classical pathway. Complement activation in AMR targets donor endothelium and causes inflammation and injury.[42] Both innate and adaptive immune responses are induced. C3b binds to the surface and acts as an opsonin, C5b-9 (MAC) is inserted in the membranes, and both ion influx and endocytosis of vesicles with the complex induce nuclear factor kappa B–mediated inflammatory responses. C3a and C5a generate a range of inflammatory responses through their receptors. T-cell alloreactivity is increased, and B-cell CR2 binding of C3d may amplify their response to target antigens (Table 1). "Sensitized" patients, who have preformed DSAs, are prone to AMR. Both preformed antibodies without any known previous immunization, so-called natural antibodies, which includes immunoglobulin M (IgM), IgA, and $IgG_3$ (the IgG subclass with the highest complement-activating ability), and antibodies produced in response to immunization contribute to the complement-mediated rejection. In a population-based study of over 1,000 kidney-transplanted patients, complement-binding anti-HLA DSAs correlated highly significantly with graft loss compared to patients with non-complement-binding anti-HLA DSAs or patients without anti-HLA DSAs.[43]

The liver is far less prone to AMR than other organ grafts, and although cross-matching seems to have overall limited value, cases have been published with AMR caused by complement-binding anti-HLA antibodies.[44] Approximately 8% of liver-transplanted patients develop *de novo* mostly anti-HLA antibodies within the first year after transplantation, resulting in lower graft and overall survival.[45] Complement fixation of these antibodies is unknown, but it is reasonable to speculate that they can activate complement. These data support the hypothesis that the liver, although to a far lesser extent than other solid organ grafts, is able to induce a humoral response. Because of this, AMR in liver transplantation has regained interest in recent years.

As is the case in kidney grafts, both acute and chronic AMR seems to exist in liver grafts. Interestingly, the DSA-positive liver transplant recipients with the highest complement-binding affinity were at greatest risk of developing AMR.[46] Despite recent evidence of AMR also in liver grafts, the liver has a unique ability to suppress immune responses. As described, the regenerative potential of the liver, where complement plays an important part, might repair damage without fibrosis formation and thereby "hide" damage. Along with a range of other proposed mechanisms for immune tolerance in the liver, these are of essential benefit to liver transplant recipients. Interestingly, the immune tolerance of the liver could be used clinically as liver grafts have been found to protect kidney grafts from rejection, including complement-mediated hyperacute AMR in simultaneous liver/kidney transplantation.[47] It is even suggested that auxiliary partial liver transplantation combined with kidney transplantation could be a treatment option in highly sensitized patients waiting for kidney transplantation. The mechanism seems to be clearance of complement-inducing DSAs from the circulation,[48] and DSAs against HLA class I seem better cleared than DSAs against HLA class II.

## MONITORING REJECTION BY COMPLEMENT DEPOSITION

Biopsy staining of the stable, inactive, covalently bound complement split product C4d on endothelial cells has been acknowledged as a valuable detection tool of AMR in kidney transplants and is included in the Banff criteria for rejection. C4d is a split product of C4b generated from C4, typically by antibody-induced (e.g., DSAs) classical pathway activation of the complement system. However, C4 activation with C4d deposition may also be achieved by antibody-independent lectin pathway complement activation. Antibodies interact noncovalently with the surface on the endothelial cells and are readily washed away by

the bloodstream; the covalently bound C4d is unaffected and serves as a "footprint" of antibody-mediated tissue damage. C4d also has value in the concept of chronic AMR. Diffuse staining of C4d has been associated with chronic changes of kidney grafts reflecting chronic DSA-mediated renal allograft rejection.

The value of C4d staining in monitoring liver graft rejection has been debated, both because AMR has not been well established in liver grafts and because it has been difficult to determine in which of the many liver vascular departments C4d deposits would be of value for diagnostics. However, positive C4d staining is now also recommended as a criterion by the Banff Working Group on Liver Allograft Pathology in both acute and chronic AMR of liver grafts (Fig. 2).[49]

## COMPLICATIONS AFTER LIVER TRANSPLANTATION AND REAL-TIME MONITORING

Standard postoperative monitoring following liver transplantation includes laboratory assessment of circulating lactate, AST, ALT, international normalized ratio (INR), and bilirubin. A sudden increase in lactate may be the first sign of graft ischemia and is usually followed up by diagnostic radiology. Elevated lactate, transaminases, INR, and bilirubin during the first week after transplantation are associated with delayed graft function.[50] Later, such as from day 7 onwards, such increases may reflect acute, mostly cellular, rejection (ACR).[51] A biopsy of the liver graft is needed to diagnose ACR.

Microdialysis catheters with a semipermeable membrane at the tip enable real-time tissue monitoring of the condition of the transplanted organ. Metabolic substances (lactate, pyruvate, glucose, glycerol, urea, and glutamate) and mediators of inflammation (cytokines, chemokines, and complement activation products) could be sampled from the intercellular space in hepatic tissue by this method. In liver transplantation, high concentrations of intrahepatic lactate before reperfusion correlated with the magnitude of reperfusion injury.[52] Poorly functioning liver grafts were associated with high lactate concentrations prior to reperfusion and complement activation as seen by C4d deposition detected by histological examination.[53] After reperfusion, simultaneous increases of lactate, the lactate-to-pyruvate ratio, and glycerol are highly indicative of an ischemic complication,[54] whereas simultaneous increases in lactate and pyruvate with a stable lactate-to-pyruvate ratio may be the first evidence of ACR.[54]

Following reperfusion of liver grafts, time-dependent decreases in C5a, IL-1 receptor antagonist, IL-6, IL-10, and macrophage inflammatory protein 1 beta were found, whereas the courses of the chemokines IL-8 (chemokine [C-X-C motif] ligand 8 [CXCL8]) and interferon gamma-induced protein 10 (IP-10; CXCL10) were more stable.[55] In the same study, C5a increased with ischemia but not with rejection, as opposed to IP-10, which increased 2-5 days before circulating ALT and bilirubin in grafts with ACR but not in ischemic grafts. There were no correlations between the concentration levels of any of the markers sampled within 24 hours after graft reperfusion and graft function during the first week. Furthermore, neither the magnitude of inflammatory mediators nor metabolic parameters following graft reperfusion could predict later occurrence of ACR. Thus, more studies exploring dynamic changes in complement activation markers and other immunologically active mediators in microdialysate from hepatic tissue during the process of organ harvest, ischemia, and reperfusion are needed.

# Summary

Knowledge of the role of complement in homeostasis and disease is rapidly evolving. The liver is an organ where most complement characteristics come to play. Not only is the liver the main producer of complement proteins, but it is also an immunoprivileged organ with fascinating tolerance against complement attack, clinically important in liver transplantation. The liver is the human organ with the highest regenerative ability. Intriguingly, complement appears to be a central player as experimental studies indicate that liver regeneration is largely complement-dependent. Complement-induced liver injury, as seen after ischemia–reperfusion, is delicately balanced with complement-dependent regulation of regeneration, suggesting that complement is a double-edged sword in these processes. Complement inhibition evolves as a therapeutic acute strategy in IRI and acute liver failure, while long-term treatment might be beneficial in NASH. In these scenarios targeted complement inhibition preventing or reducing cleavage of

C5 with specific C5 inhibitors would reduce detrimental hepatic C5a actions and deposition of C5b-9 on hepatocytes. This approach appears promising as it entails several advantages: C5 inhibition is already used clinically (i.e., eculizumab) for other indications and thus has been evaluated as a safe therapy even in critically ill patients, in which host immunity is preserved, and beneficial C3a hepatocyte-proliferative effects are conserved. Still, while global C5 or even upstream C3 inhibition might be beneficial in some situations, could a more direct targeting of parts of the system be beneficial in others? Inhibitors targeting C1s (classical pathway), factor D (alternative pathway), or specifically the C5aR1 could be alternative approaches when retention of parts of the systems is desirable. Tissue targeting is another promising complement therapeutic principle where a conjugate of CR2, binding to C3d deposited in the tissue, and a complement inhibitor like factor H will go directly to the complement-attacked tissue after systemic infusion and inhibit complement locally but not compromise the systemic complement function. More research is needed, but as mediator-directed therapy rapidly develops, complement could be a key system to manipulate and optimize the unique physiological characteristics of the liver in various clinical settings, including hepatic protection during major surgery, transplantation, and acute liver failure.

## REFERENCES


1) Ricklin D, Hajishengallis G, Yang K, Lambris JD. Complement: a key system for immune surveillance and homeostasis. Nat Immunol 2010;11:785-797.
2) Qin X, Gao B. The complement system in liver diseases. Cell Mol Immunol 2006;3:333-340.
3) Elvington M, Liszewski MK, Atkinson JP. Evolution of the complement system: from defense of the single cell to guardian of the intravascular space. Immunol Rev 2016;274:9-15.
4) Mahmoudi M, Nilsson PH, Mollnes TE, Roos D, Sullivan KE. Complement deficiencies. In: Rezaei N, Aghamohammadi A, Notarangelo L, eds. Primary Immunodeficiency Diseases: Definition, Diagnosis, and Management. 2nd edn. Heidelberg: Springer; 2017:437-460.
5) Strainic MG, Liu J, Huang D, An F, Lalli PN, Muqim N, et al. Locally produced complement fragments C5a and C3a provide both costimulatory and survival signals to naive $CD4^+$ T cells. Immunity 2008;28:425-435.
6) Dempsey PW, Allison ME, Akkaraju S, Goodnow CC, Fearon DT. C3d of complement as a molecular adjuvant: bridging innate and acquired immunity. Science 1996;271:348-350.
7) Barratt-Due A, Pischke SE, Nilsson PH, Espevik T, Mollnes TE. Dual inhibition of complement and Toll-like receptors as a novel approach to treat inflammatory diseases-C3 or C5 emerge together with CD14 as promising targets. J Leukoc Biol 2017;101:193-204.
8) **Samstad EO**, **Niyonzima N**, Nymo S, Aune MH, Ryan L, Bakke SS, et al. Cholesterol crystals induce complement-dependent inflammasome activation and cytokine release. J Immunol 2014;192:2837-2845.
9) Inai S, Kitamura H, Fujita T, Kojima J, Nagaki K. Differences between plasma and serum complement in patients with chronic liver disease. Clin Exp Immunol 1976;25:403-409.
10) Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. N Engl J Med 1999;340:448-454.
11) Lehmann TG, Koeppel TA, Munch S, Heger M, Kirschfink M, Klar E, et al. Impact of inhibition of complement by sCR11 on hepatic microcirculation after warm ischemia. Microvasc Res 2001;62:284-292.
12) 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.
13) Arumugam TV, Woodruff TM, Stocks SZ, Proctor LM, Pollitt S, Shiels IA, et al. Protective effect of a human C5a receptor antagonist against hepatic ischaemia–reperfusion injury in rats. J Hepatol 2004;40:934-941.
14) Fondevila C, Shen XD, Tsuchihashi S, Uchida Y, Freitas MC, Ke B, et al. The membrane attack complex (C5b–9) in liver cold ischemia and reperfusion injury. Liver Transpl 2008;14:1133-1141.
15) **Marshall KM**, **He S**, Zhong Z, Atkinson C, Tomlinson S. Dissecting the complement pathway in hepatic injury and regeneration with a novel protective strategy. J Exp Med 2014; 211:1793-1805.
16) Triantafilou K, Hughes TR, Triantafilou M, Morgan BP. The complement membrane attack complex triggers intracellular $Ca^{2+}$ fluxes leading to NLRP3 inflammasome activation. J Cell Sci 2013;126:2903-2913.
17) Lu L, Zhou H, Ni M, Wang X, Busuttil R, Kupiec-Weglinski J, et al. Innate immune regulations and liver ischemia-reperfusion injury. Transplantation 2016;100:2601-2610.
18) Rittirsch D, Redl H, Huber-Lang M. Role of complement in multiorgan failure. Clin Dev Immunol 2012;2012:962927.
19) Croner RS, Lehmann TG, Fallsehr C, Herfarth C, Klar E, Kirschfink M. C1-inhibitor reduces hepatic leukocyte-endothelial interaction and the expression of VCAM-1 in LPS-induced sepsis in the rat. Microvasc Res 2004;67:182-191.
20) **Sun S**, **Guo Y**, Zhao G, Zhou X, Li J, Hu J, et al. Complement and the alternative pathway play an important role in LPS/D-GalN-induced fulminant hepatic failure. PLoS One 2011;6:e26838.
21) **Keshari RS**, **Silasi R**, Popescu NI, Patel MM, Chaaban H, Lupu C, et al. Inhibition of complement C5 protects against organ failure and reduces mortality in a baboon model of *Escherichia coli* sepsis. Proc Natl Acad Sci USA 2017;114:E6390-E6399.
22) Knolle P, Schlaak J, Uhrig A, Kempf P, Meyer zum Buschenfelde KH, Gerken G. Human Kupffer cells secrete IL-10 in response to lipopolysaccharide (LPS) challenge. J Hepatol 1995;22:226-229.
23) **Koch CA**, **Kanazawa A**, Nishitai R, Knudsen BE, Ogata K, Plummer TB, et al. Intrinsic resistance of hepatocytes to complement-mediated injury. J Immunol 2005;174:7302-7309.
24) **He S**, **Atkinson C**, Qiao F, Cianflone K, Chen X, Tomlinson S. A complement-dependent balance between hepatic ischemia/reperfusion injury and liver regeneration in mice. J Clin Invest 2009;119:2304-2316.
25) **Said A**, **Ghufran A**. Epidemic of non-alcoholic fatty liver disease and hepatocellular carcinoma. World J Clin Oncol 2017;8:429-436.

26) **Jia Q**, **Li C**, **Xia Y**, Zhang Q, Wu H, Du H, et al. Association between complement C3 and prevalence of fatty liver disease in an adult population: a cross-sectional study from the Tianjin Chronic Low-Grade Systemic Inflammation and Health (TCLSIHealth) cohort study. PLoS One 2015;10:e0122026.
27) Rensen SS, Slaats Y, Driessen A, Peutz-Kootstra CJ, Nijhuis J, Steffensen R, et al. Activation of the complement system in human nonalcoholic fatty liver disease. Hepatology 2009;50:1809-1817.
28) **Lee JH**, **Poudel B**, Ki HH, Nepali S, Lee YM, Shin JS, et al. Complement C1q stimulates the progression of hepatocellular tumor through the activation of discoidin domain receptor 1. Sci Rep 2018;8:4908.
29) Segers FM, Verdam FJ, de Jonge C, Boonen B, Driessen A, Shiri-Sverdlov R, et al. Complement alternative pathway activation in human nonalcoholic steatohepatitis. PLoS One 2014;9:e110053.
30) 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.
31) Daveau M, Benard M, Scotte M, Schouft MT, Hiron M, Francois A, 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.
32) Markiewski MM, Mastellos D, Tudoran R, DeAngelis RA, Strey CW, Franchini S, 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.
33) **Strey CW**, **Markiewski M**, **Mastellos D**, Tudoran R, Spruce LA, Greenbaum LE, et al. The proinflammatory mediators C3a and C5a are essential for liver regeneration. J Exp Med 2003;198:913-923.
34) Clark A, Weymann A, Hartman E, Turmelle Y, Carroll M, Thurman JM, et al. Evidence for non-traditional activation of complement factor C3 during murine liver regeneration. Mol Immunol 2008;45:3125-3132.
35) Shanmukhappa K, Matte U, Degen JL, Bezerra JA. Plasmin-mediated proteolysis is required for hepatocyte growth factor activation during liver repair. J Biol Chem 2009;284:12917-12923.
36) Schmidt-Arras D, Rose-John S. IL-6 pathway in the liver: from physiopathology to therapy. J Hepatol 2016;64:1403-1415.
37) **DeAngelis RA**, **Markiewski MM**, **Kourtzelis I**, **Rafail S**, Syriga M, Sandor A, et al. A complement-IL-4 regulatory circuit controls liver regeneration. J Immunol 2012;188:641-648.
38) **Schnitzbauer AA**, **Lang SA**, Goessmann H, Nadalin S, Baumgart J, Farkas SA, et al. Right portal vein ligation combined with in situ splitting induces rapid left lateral liver lobe hypertrophy enabling 2-staged extended right hepatic resection in small-for-size settings. Ann Surg 2012;255:405-414.
39) Line PD, Hagness M, Berstad AE, Foss A, Dueland S. A novel concept for partial liver transplantation in nonresectable colorectal liver metastases: the RAPID concept. Ann Surg 2015;262:e5-e9.
40) Orlando G, Soker S, Wood K. Operational tolerance after liver transplantation. J Hepatol 2009;50:1247-1257.
41) Biglarnia AR, Ekdahl KN, Nilsson B. Complement interception across humoral incompatibility in solid organ transplantation: a clinical perspective. Adv Exp Med Biol 2015;865:211-233.
42) Stites E, Le Quintrec M, Thurman JM. The complement system and antibody-mediated transplant rejection. J Immunol 2015;195:5525-5531.
43) **Loupy A**, **Lefaucheur C**, Vernerey D, Prugger C, Duong van Huyen JP, Mooney N, et al. Complement-binding anti-HLA antibodies and kidney-allograft survival. N Engl J Med 2013;369:1215-1226.
44) Ducreux S, Guillaud O, Bosch A, Thaunat O, Morelon E, Hervieu V, et al. Monitoring efficiency of humoral rejection episode therapy in liver transplantation: any role for complement binding Luminex Single Antigen assays? Transpl Immunol 2016;35:23-28.
45) Kaneku H, O'Leary JG, Banuelos N, Jennings LW, Susskind BM, Klintmalm GB, et al. *De novo* donor-specific HLA antibodies decrease patient and graft survival in liver transplant recipients. Am J Transplant 2013;13:1541-1548.
46) O'Leary JG, Kaneku H, Banuelos N, Jennings LW, Klintmalm GB, Terasaki PI. Impact of IgG3 subclass and C1q-fixing donor-specific HLA alloantibodies on rejection and survival in liver transplantation. Am J Transplant 2015;15:1003-1013.
47) Gutierrez A, Crespo M, Mila J, Torregrosa JV, Martorell J, Oppenheimer F. Outcome of simultaneous liver-kidney transplantation in highly sensitized, crossmatch-positive patients. Transplant Proc 2003;35:1861-1862.
48) Olausson M, Mjornstedt L, Norden G, Rydberg L, Molne J, Backman L, et al. Successful combined partial auxiliary liver and kidney transplantation in highly sensitized cross-match positive recipients. Am J Transplant 2007;7:130-136.
49) Demetris AJ, Bellamy C, Hubscher SG, O'Leary J, Randhawa PS, Feng S, et al. 2016 Comprehensive update of the Banff Working Group on Liver Allograft Pathology: introduction of antibody-mediated rejection. Am J Transplant 2016;16:2816-2835.
50) Olthoff KM, Kulik L, Samstein B, Kaminski M, Abecassis M, Emond J, et al. Validation of a current definition of early allograft dysfunction in liver transplant recipients and analysis of risk factors. Liver Transpl 2010;16:943-949.
51) Germani G, Rodriguez-Castro K, Russo FP, Senzolo M, Zanetto A, Ferrarese A, et al. Markers of acute rejection and graft acceptance in liver transplantation. World J Gastroenterol 2015;21:1061-1068.
52) Silva MA, Murphy N, Richards DA, Wigmore SJ, Bramhall SR, Buckels JA, et al. Interstitial lactic acidosis in the graft during organ harvest, cold storage, and reperfusion of human liver allografts predicts subsequent ischemia reperfusion injury. Transplantation 2006;82:227-233.
53) Silva MA, Mirza DF, Murphy N, Richards DA, Reynolds GM, Wigmore SJ, et al. Intrahepatic complement activation, sinusoidal endothelial injury, and lactic acidosis are associated with initial poor function of the liver after transplantation. Transplantation 2008;85:718-725.
54) Haugaa H, Thorgersen EB, Pharo A, Boberg KM, Foss A, Line PD, et al. Early bedside detection of ischemia and rejection in liver transplants by microdialysis. Liver Transpl 2012;18:839-849.
55) Haugaa H, Thorgersen EB, Pharo A, Boberg KM, Foss A, Line PD, et al. Inflammatory markers sampled by microdialysis catheters distinguish rejection from ischemia in liver grafts. Liver Transpl 2012;18:1421-1429.


Author names in bold designate shared co-first authorship.