The Complement System and C1q in Chronic Hepatitis C Virus Infection and Mixed Cryoglobulinemia

Ahmed El-Shamy1,2*, Andrea D. Branch1, Thomas D. Schiano1 and Peter D. Gorevic3*

1 Division of Liver Disease, Department of Medicine, Icahn School of Medicine at Mount Sinai, New York, NY, United States,
2 Department of Pharmaceutical and Biological Sciences, California Northstate University, Elk Grove, CA, United States,
3 Division of Rheumatology, Department of Medicine, Icahn School of Medicine at Mount Sinai, New York, NY, United States

The complement system bridges innate and adaptive immunity against microbial infections, with viral infection being a major trigger. Activation of the classical, alternative, and lectin pathways have been reported in chronic hepatitis C virus (HCV) infection and/or cryoglobulinemia. HCV infection leads to dysregulation of complement-mediated immune responses. Clinical and experimental evidence support involvement of complement in intra- and extrahepatic manifestations of HCV infection, such as liver fibrosis and type II cryoglobulinemia. In this review, we summarize studies that have investigated the interplay between HCV and the complement system to establish chronic infection and autoimmunity, as well as the association between HCV pathogenesis and abnormal complement profiles. Several unanswered questions are highlighted which suggest additional informative lines of investigation.

Keywords: liver, hepatitis C virus, complement, C1q, gC1qR, mixed cryoglobulinemia

INTRODUCTION

The complement system includes major host defense mechanisms that bridge innate and adaptive immunity against microbial infections. It is also a critical mediator of the clearance of immune complexes and injured cells (1–3). Dysregulation may be associated with chronic autoimmune inflammatory conditions, such as systemic lupus erythematosus, cryoglobulinemia, and rheumatoid arthritis. Viral infections may trigger dysregulation by direct effects on complement components for the purpose of immune evasion, by effects on specific receptors used for viral entrance into cells or by promoting a pathogenic antiglobulin [i.e., rheumatoid factor (RF)] response as part of chronic immune stimulation. In particular, hepatitis C virus (HCV) infection has been associated with a number of extrahepatic disorders, such as type II mixed cryoglobulinemia (MC) and B cell lymphoma that may be accompanied by complement dysregulation. The recent introduction of direct-acting antiviral (DAA) therapy for HCV allows most patients to achieve a sustained virological response (SVR)/cure. Treatment reduces liver inflammation and improves extrahepatic disease manifestations, but cryoglobulinemia and liver dysfunction may persist (4–14).

The majority of HCV-infected patients with evidence of B cell clonality have abnormal complement profiles; a low serum level of C4 is a “signature” for type II MC patients (15, 16). However, only limited information is available regarding the mechanisms by which the complement system is involved in HCV-induced intra- and extrahepatic disease. Therefore, in this review, we aim to highlight the interplay between HCV and the complement system that become apparent with chronic infection and lymphoproliferation.
PRODUCTION SITES OF COMPLEMENT PROTEINS AND RECEPTORS

More than 40 complement-related proteins have been identified in the plasma and on cell surfaces, constituting more than 15% of the of plasma globulins (17, 18). The liver makes ~90% of the plasma components of classical, alternative, and lectin pathways. By contrast, whereas hepatocytes are the predominant source of C1r and C1s in blood, activated monocytes/macrophages and immature dendritic cells are the primary source of C1q, a recognition molecule for classical pathway activation that also has significant non-complement functions (19). Although the liver produces the majority of C4, multiple tissues may also produce this protein for local consumption, particularly in response to interferon-gamma (20). Several complement receptors, such as complement receptor type 1 (CR1), the complement receptor of the immunoglobulin superfamily (CR1g), the integrin complement receptors 3 (CR3) and 4 (CR4), and the complement component 5a receptor 1 (C5aR), are expressed on liver cells (hepatocytes, endothelial cells, Kupffer cells, and stellate cells), and contribute to a variety of functions, such as induction of gluconeogenesis, synthesis of acute-phase proteins, hepatocyte proliferation, and phagocytosis (21).

FIBROSIS AND REGENERATION

Increasing evidence implicates activation of the complement system in the pathogenesis and response to acute and chronic liver injury (22). In a clinical study investigating the relationship between complement activation and development of liver fibrosis, blood samples from 50 chronically infected HCV patients were compared to 35 patients with other various liver diseases and 50 healthy controls (23). Complement system activation, as indicated by a significant decrease in total plasma complement activity (CH50) and increase in SC5b-9 [a marker for generation of the membrane-attack complex (MAC)] was associated with high necroinflammatory activity in the HCV patients and patients with other liver diseases compared to controls. The level of SC5b-9 significantly correlated with liver fibrosis stage in HCV-infected patients but not in patients with other liver injuries. In a proteomic survey of serum samples from HCV-infected patients, liver fibrosis stage was associated with a decrease in C3, C4, and Factor H-related protein-1, a regulatory C3b-binding protein (24). C4a, a cleavage product of C4 that contrasts with C3a and C5a with regard to biologic function, was reported to be negatively correlated with the stage of liver fibrosis in children with chronic HCV infection, serum levels being significantly lower in HCV children with advanced fibrosis than those with no/mild fibrosis (25); the significance of this study, however, was limited by the low numbers of HCV-infected children, and the fact that C4a may increase as a function of classical pathway activation and C4 consumption. In a murine genomic study, the gene encoding C5 was identified as a quantitative trait locus associated with the development of liver fibrosis (26). Expression of C5aR1 was significantly upregulated on hepatic stellate cells during trans-differentiation to myofibroblasts in culture. Since myofibroblasts synthesize collagens and other extracellular matrix proteins, elevated C5aR1 expression is consistent with the concept that C5 is a modifier of liver fibrogenesis. Indeed, blockade of C5aR1 reduces hepatic fibrosis in mice (26). Critical roles for C3a and C5a for liver regeneration following carbon tetrachloride injury have been shown in C3, C5, and C3aR knock-out and double knock-out mice, reversible by restoration of C3a, C5, or C5a (27). Interestingly, in 277 patients with chronic HCV infection, two C5 single-nucleotide polymorphisms (SNPs), rs17611 and rs2300929, were associated with advanced fibrosis (26). However, in a study of 1,435 HCV-infected patients and 1,003 patients with other liver diseases, there was no significant association between these C5 SNPs and fibrosis in either patient group (28).

HEPATOCELLULAR CARCINOMA (HCC)

Cancer growth is determined by intrinsic properties of malignant cells as well as several modifiers, including the complement system, which may either reduce or increase progression (29). HCC is the second leading cause of organ-specific cancer-related death worldwide (30) and is the most rapidly increasing cause of cancer-related death in the United States, Europe, and Japan (31). Of note, C3a was identified by mass spectrometry and 2-dimen-sional gel electrophoresis (2-DE) of serum samples obtained from HCV-HCC, chronic HCV, HBV-HCC, chronic HBV, and healthy subjects as a differentially expressed biomarker protein with significantly higher levels among HCV-HCC patients compared to the other groups (32).

TREATMENT

Until recently, HCV treatment has centered on interferon-alpha (IFNα), a cytokine with both antiviral and immunologic effects. However, IFNα-based treatment failed to eliminate HCV in many patients and was often poorly tolerated, particularly because of its ability to induce, uncover and/or exacerbate autoimmune/inflammatory disorders (33, 34). The CC genotype associated with the rs285009 SNP of the C4 gene closely correlated with decreased level of mRNA expression and C4 protein which was more striking at baseline in HCV patients compared to healthy controls. More importantly, the presence of this SNP was significantly associated with a poor response to IFNα-based therapy as well as the development of a high degree of liver fibrosis (35). Interestingly, a significant reduction in C4 activity was also observed in relapers after IFNα treatment compared to patients who achieved SVR (36). Polymorphism at the rs2230201 SNP of C3 was also associated with IFNα treatment outcome (37). The rs2230201 ‘C’ allele was associated with increased serum C3 levels compared to the ‘T’ allele, which conferred an advantage in attaining SVR, especially in homozygotes. Patients with serum C3 value < 53 mg/dl and non-CC genotypes may not respond to IFNα treatment (37). Recently developed DAA therapies provide an opportunity for HCV patients with autoimmune/inflammatory disorders to be cured with a low risk of side effects (38–40). In the era of DAA, how a patient’s complement profile contributes to the treatment response remains to be defined.

In summary, accumulating clinical observations support a role for the complement system in mediating liver inflammation and fibrosis in HCV infection (Table 1). However, the mechanisms...
underlying these observations are still unclear. Thus, further experimental and molecular studies are required to dissect how the complement system contributes to intrahepatic HCV pathogenesis, including roles in innate and adaptive immunity, regulation of apoptosis, fibrosis, and regeneration.

### HCV策略 TO OVERCOME ANTIVIRAL RESPONSES OF THE COMPLEMENT SYSTEM

Hepatitis C virus lacks a DNA intermediate; thus, it is incapable of integrating into host chromosomal DNA. Despite this, unlike most RNA viruses, chronic infection is established in ~80% of cases (41) through multiple strategies to evade innate and adaptive antiviral responses (42). In part, this is accomplished directly by inhibition of complement components and/or indirectly by induction of regulators of complement activation (RCA) (Table 2). Mazumdar et al. examined the relationship between HCV infection and C3 concentrations in blood. C3 has a central role in modulating all three pathways of the complement system. In matched serum and liver biopsy samples from HCV patients, both the levels of C3 in serum and the expression of mRNA in biopsies were significantly lower compared to serum and tissue obtained from healthy donors (43). Further in vitro studies showed that HCV-NS5A protein strongly downregulated C3 promoter activity at the basal level. In addition, expression of the transcription factor C/EBP-β, which induces C3 promoter activity, was reduced in immortalized human hepatocytes and human hepatoma cells (Huh7) that were either infected with cell culture-adapted HCV or transfected with HCV-NS5A (43). Moreover, HCV inhibited C3 convertase activity, which is critical in promoting the activity of classical and lectin pathways of complement system (44). Infection of a hepatoma cell line with HCV resulted in inhibition of C2 expression and hence impairment of C3 convertase function. On the other hand, C3b deposition onto bacterial membrane was reduced by sera from HCV patients as compared to healthy controls, which further indicates impaired C3 convertase (44). C4 contributes to the eradication of several viral infections by its role as opsonin and by its central role in promoting the activity of the classical and lectin pathways (3). Notably, C4 protein levels in the serum and mRNA expression levels in liver tissue were lower in HCV patients compared to patients with unrelated liver diseases (45). In vitro studies showed that the expression levels of the two C4 isoforms (C4A and C4B) were significantly reduced in hepatocytes transfected with a full-length HCV genome. In particular, among different HCV proteins, only core and NS5A contributed to HCV’s inhibitory effect on C4 as shown by in vitro transfection experiments, using the Huh7 hepatoma cell line and plasmids containing different HCV proteins (45). Consistent with these in vitro results, the expression levels of C4 mRNA in liver tissue of HCV-core or NS5A transgenic mice were also significantly reduced. Mechanistic studies showed that HCV-core downregulated the expression of upstream stimulatory factor-1, a transcription factor critical for C4 expression, while NS5A inhibited the expression of interferon regulatory factor-1, which is required for IFN-γ-induced C4 promoter activation (45).

Likewise, Kim et al., showed that liver biopsies from HCV patients had lower expression of C9 mRNA compared to samples from unrelated diseases or healthy controls. This indicates that HCV regulates the MAC via C9. C9 mRNA was significantly downregulated in cultured hepatocytes infected with HCV (46). In particular, HCV-core protein had a critical role in regulating C9 promoter activity. Furthermore, in a subsequent in vitro study, HCV NS2 and NS5B proteins were found to be responsible for

---

| Complement system-related factor | HCV-induced immune evasion | Reference |
|----------------------------------|---------------------------|-----------|
| C3                              | Downregulation of C3 promoter activity by HCV-NS5A via inhibition of C/EBP-β | (43) |
| C2                              | Impairment of C3 convertase function via inhibition of C2 | (44) |
| C4                              | Inhibition of C4 activity through HCV core-induced inhibition of upstream stimulatory factor-1 and HCV-NS5A-induced inhibition of interferon regulatory factor-1 | (45) |
| C9                              | Impairment of membrane-attack complex (MAC) formation through inhibition of C9 promoter activity by HCV-core | (46) |
| C3 and C4                       | Downregulation of C3 and C4 hepatocyte synthesis through the inhibition of the hepatocyte MICA/B | (47) |
| MAC                             | Impairment of MAC formation through incorporation of CD59 in HCV envelope | (55) |
| C3 convertase                   | Upregulation of CD55 expression which accelerates the decay of C3 convertase | (56) |
| gC1qR                           | Impairment of T-cell immunity through the interaction of HCV core to gC1qR on T-cells and monocyte-derived dendritic cells | (65–67) |
HCV-associated inhibition of the hepatocyte protein major histocompatibility complex class I-related chains A and B (MICA/B) which functions as a key receptor ligand for NKG2D on NK cells resulting in downregulation of C3 and C4 hepatocyte synthesis (47).

A general role for lectins and pattern recognition of viral glycoproteins has been identified for HIV and HCV (48). In vitro studies showed that mannan-binding lectin (MBL) bound to the HCV E2 ectodomain and E1/E2 heterodimers through its lectin domain, as well as activate complement through MBL-associated serine protease 2 (49). Ficolin-2, a known lectin pathway activator was found to inhibit attachment of HCV envelope E1 and E2 N-glycans to their low-density lipoprotein (LDL) and scavenger B1 receptors (50), with elevated blood levels of L-ficolin or MBL being found in the serum of some patients, possibly correlating with MBL2 genetic variants and response to IFNα (51).

Host expression of RCAs, such as CD35, CD46, CD55, and CD59, serves to protect the cells from MAC lysis (52, 53). HCV has developed strategies to attenuate complement activation by regulating RCAs. Amet et al. first showed that CD59, a key member of RCA, associated with the external membrane of HCV particles obtained from infected patients and Huh7.5.1 cells and had a direct role in abrogating antibody-dependent complement-mediated lysis (54). In vitro studies by Eiaz et al. indicated that HCV selectively incorporates CD59 in its envelope, which inhibits the formation of the MAC complex (55). Also, it was found that HCV infection upregulates the expression of CD55, which accelerates the decay of C3 convertase (56). Taken together, HCV has the capability to attenuate the complement system at multiple steps to weaken the innate immune response.

**ROLE OF gC1q RECEPTOR (gC1qR) IN HCV PATHOGENESIS**

gC1q receptor is an acidic multifunctional cellular protein ubiquitously expressed on somatic cells (57). It binds to the globular heads of C1q and modifies complement activation (58). Apart from its interaction with C1q, gC1qR binds to several host cell-surface ligands, such as vitronectin and high molecular weight kininogen (59). Interaction of these ligands with gC1qR leads to classical complement pathway activation with generation of inflammatory cytokines, cell adhesion, and activation of the intracellular coagulation cascade leading to the production of bradykinin, increased vascular permeability, and infiltration of vascular tissue with proinflammatory cells (60). In addition to cellular proteins, gC1qR interacts with several microbial proteins, such as adenovirus core protein (61), HIV rev (62), and protein A of *Staphylococcus aureus* (63), suggesting its role in the pathogenesis of these infections. Interestingly, by using HCV-core protein as bait in yeast two-hybrid assay, Kittlesen et al. was the first to report the interaction between gC1qR and HCV-core (64). The interaction of HCV-core to gC1qR on T-lymphocytes resulted in inhibition of T-cell proliferation and function through impairment of ERK/MEK phosphorylation (65) and Lck/Akt activation (66). Also, engagement of gC1qR on monocyte-derived dendritic cells with HCV-core resulted in an impaired capacity to generate type 1 CD4+ T cell immunity via inhibition of TLR-induced IL-12 production (67). Therefore, HCV might utilize the direct interaction of its core protein with gC1qR on T cells as a tool to suppress cellular immunity which might imply an important role in persistent infection, an observation that might extend to minicore isoforms of this protein, which lack the RNA binding domain of the p21 core (68).

By contrast to the inhibitory influence of HCV-core protein and gC1qR interaction on T cell responses, this interaction on B cells resulted in hyper-activation and proliferation indicated by upregulation of CD69, overexpression of costimulatory and chemokine receptors, and increased production of IgM and IgG (69). This might partially explain the link between chronic HCV infection, B-cell lymphoproliferative disorders, and several autoimmune-related diseases (70–72). In support of this, the level of circulating gC1qR and gC1qR mRNA of PBMC in HCV patients with MC, one of the major B-cell disorders associated HCV infection, is significantly increased compared to HCV patients without MC or healthy controls (73). Interestingly, there was also a positive correlation between circulating gC1qR with RF activity and C1q concentrations in HCV patients with MC (73). Taken together, these observations suggest the involvement of gC1qR in the pathogenesis of HCV-induced autoimmunity.

**ROLE OF C1q IN HCV-INDUCED MC**

Mixed cryoglobulins are cold-precipitable complexes of monoclonal or polyclonal IgM RF with polyclonal or oligoclonal IgG (15). Type II MCs, which are composed of monoclonal IgMκ RF and polyclonal IgG, are heavily represented among cryoglobulins associated with chronic HCV infection, and those found in patients with primary Sjögren’s syndrome, both of which may be complicated by clonal B-cell proliferations and specific types of non-Hodgkin’s lymphoma (15). HCV patients with symptomatic type II MC suffer from various extrahepatic manifestations, including vascular, renal, and neurological lesions (74), i.e., cryoglobulinemic vasculitis (CryoVas) (75).

In type III MCs, both the IgM and IgG components appear to be polyclonal; extrahepatic disease manifestations may occur, but cryoglobulin levels are lower than type II, and an association with asymptomatic disease is more frequent. In addition, intermediate types characterized by oligoclonality or mixed IgM clonality with polyclonal IgM (type IIa) have also been described (76). Type III MCs may be found in HCV infection, as well as associated with rheumatic diseases [e.g., systemic lupus erythematosus (SLE)] in which complement activation may occur (16). The significance of Type IIa and related intermediate forms with regard to the progression to clonality that may occur in cirrhosis associated with HCV, and in primary Sjögren’s syndrome, remains to be fully defined (77, 78).

As noted, a low serum level of C4 is a significant “signature” of type II MC patients (15, 16). This selective depression of C4 strongly implicates classical pathway activation of the complement in cryoglobulin formation. However, the level and function of C4 may be significantly influenced by inter-individual copy-number variation of C4A or C4B genes, charge variation, or isotype deficiency of these genes (79). Incorporation of C4 into isolated cryoprecipitates was first demonstrated as an 11S peak on density gradient ultracentrifugation in patients with lupus nephritis (80).
More recent studies have confirmed that cryoprecipitates from patients with HCV-associated CryoVas are enriched in C1q (81), antibodies to HCV antigens (82), and may contain HCV-core protein as indicated by results obtained using an enhanced highly sensitive chemiluminescent microparticle immunoassay (81). Based on evidence that C1q and HCV-core bind to gC1qR, gC1qR/HCV-core complexes might provide a platform for complement activation and deposition of C4D at sites of vasculopathy (83). Additional factors that might be reflected in depletion of C4/ C1q and localization to cryocomplexes include the ability of C1q to bind promiscuously to > 100 known ligands, including both IgG- and IgM-containing immune complexes, surface-bound C-reactive protein, and molecules exposed at the surface of apoptotic cells, with binding through charged residues on the apex of the gC1q heterotrimer (19, 84), acquired C1-inhibitor deficiency (85), regulation of activation by C4-binding protein (86, 87), and antibodies to C1q and/or potentially to other components of the C1 complex (88).

ROLE OF RF IN MC PATHOGENESIS

Although MC may be associated with a rheumatoid-like arthritis, it is distinct from RA in that anticollagen activity is restricted to the IgM isotype; although it is presumed that the antigen specificity is directed primarily to determinants in the Fc portion of IgG uncovered by aggregation or complexing to antigen, there have been some suggestions of F(ab)2 anti-hinge or anti-idiotypic activity is directed primarily to determinants in the Fc portion of IgG (80). Although MC may be associated with a rheumatoid-like arthritis, it is distinct from RA in that antiglobulin activity is restricted to the IgM isotype; although it is presumed that the antigen specificity is directed primarily to determinants in the Fc portion of IgG uncovered by aggregation or complexing to antigen, there have been some suggestions of F(ab)2 anti-hinge or anti-idiotypic activity is directed primarily to determinants in the Fc portion of IgG (80).

APOPTOTIC ROLE OF C1q IN SLE

Activation of complement is a central feature of SLE, intimately related to the pathogenesis of lupus nephritis, and a marker for disease activity and relapse; deficiencies or polymorphisms of molecules central to the classical, alternative, and lectin pathways have been linked to disease susceptibility, immune-complex nephritis, and severity (94). In particular, a central role for C1q and C1q receptors, both with regard to deficiency and as molecular cell-surface sensors for innate and acquired immune responses, has been reviewed (95). The observation that SLE develops in ~90% patients genetically deficient in C1q highlighted a function of C1q as “protector” against autoimmunity that may be independent of its classical role ion complement activation (96). In SLE, C1q deficiency may result either from genetic disorders or anti-C1q autoantibodies (97). The contributions of C1q deficiency in the development of SLE can be related to abrogation of binding to molecules (phosphatidylerine, double-stranded DNA, glycerylaldehyde-3-phosphate dehydrogenase, annexins 2 and 5, calreticulin) expressed on the surface of dying cells (17) and resultant lack of generation of activated C1s to cleave these apoptotic autoantigens (19). In addition, all three pathways of complement can be activated on the surface of apoptotic cells without further activation of innate or adaptive immune components (17); impaired clearance of dying cells and immune complexes in absolute or functional C1q deficiency is linked to the development of self-reactive B cells with affinity toward multiple autoantigens, and effects on monocyte and dendritic cell differentiation (98, 99).

Anti-C1q autoantibodies have been reported in 30–50% of SLE patients, most commonly correlating with antibodies to double-stranded DNA, nephritis, and low levels of C3 and C4 (100, 101). While antibodies with unique specificities for the globular head and collagen tail of C1q have been identified, the impact of blocking C1q domains on biological activity remains uncertain compared to a number of other (e.g., calreticulin) known inhibitors (17). Although the C1q is a major component of HCV-induced cryoprecipitate, to the best of our knowledge there are no published studies addressing this issue. Low levels of C1q and a significant prevalence of anti-C1q autoantibodies are shared features of SLE and HCV-induced cryoglobulinemia (36, 73, 102, 103).

Interferon-alpha is well-known to have both antiviral and inflammatory effects (104). Plasmacytoid dendritic cells (pDC) are the major producers of IFNα (105). Interestingly, C1q collagen tail interacts with LAIR-1 (CD305), an inhibitory receptor for C1q (106), on pDCs and restricts the production of IFNα (107). Therefore, anti-C1q autoantibodies might contribute to HCV-induced cryoglobulinemia by blocking the interaction between the C1q tail and its inhibitory receptor, LAIR-1, on pDCs resulting in uncontrolled overproduction of IFNα, which may in turn drive the inflammation associated with progression of MC in HCV patients. Alternatively, elevated levels of IFNα produced by uncontrolled pDCs might promote differentiation of B cells into plasma cells resulting in production of pathogenic autoantibodies reported in SLE (108).

CONCLUSION

The complement system plays a central role in rheumatic and autoimmune diseases, several of which are associated with the presence in blood of cold-perceptible immune complexes enriched in IgM RF, specific antibody activities, putative antigens and C1q as part of a cascade capable of activating the classical pathway, leading
in turn to the generation of anaphylatoxins, chemotactic factors, and inflammatory mediators. Both C1q and its globular receptor are promiscuous with regard to ligand specificity, allowing for alternative functions that include binding to specific intracellular antigens expressed on the surface of apoptotic cells, as well as to specific domains of HCV. A research agenda includes the mapping of C1q epitopes responsible for binding to diverse ligands, anti-C1q antibodies, heterotrimeric formation, and C4/C2 serine protease generation that might in turn be targets for therapy. Similar therapeutic strategies might be targeted to C1qR binding to C1q and High Molecular Weight Kininogen in plasma, on the surface of endothelial cells as a mechanism for vasculopathy, and the regulation of danger sensors on mononuclear cells and immature dendritic cells. A second line of investigation is the delineation of factors responsible for the strikingly low C4 levels in sera of patients with Type II MC and some patients with SLE with regard to mechanisms such as copy-number variation, polymorphisms, cleavage and deposition in tissue, and specific inhibitors. With regard to HCV, a focus on liver pathology would provide an arena to identify complement-defined mechanisms of disease, including immune activation in lymphoid follicles, steatosis, fibrosis, and regeneration.

**AUTHOR CONTRIBUTIONS**

AE and PG wrote the review. AB and TS revised the manuscript.

**ACKNOWLEDGMENTS**

This work was supported by the Seaver Foundation and an Investigator-initiated grant from Gilead Sciences.

**REFERENCES**

1. Walport MJ. Complement. First of two parts. *N Engl J Med* (2001) 344: 1058–66. doi:10.1056/NEJM200104053441406
2. Sarma JV, Ward PA. The complement system. *Cell Tissue Res* (2011) 343: 227–35. doi:10.1007/s00441-010-1034-0
3. Hölters VM. Complement and its receptors: new insights into human disease. *Annu Rev Immunol* (2014) 32:433–59. doi:10.1146/annurev-immunol-032113-120154
4. D'Ambrosio R, Aghemo A, Rumi MG, Ronchi G, Donato MF, Paradis V, et al. A morphometric and immunohistochemical study to assess the benefit of a sustained virological response in hepatitis C virus patients with cirrhosis. *Hepatology* (2012) 56:532–43. doi:10.1002/hep.25606
5. Chekuri S, Nickerson J, Bichoupan K, Sefcik R, Doobay K, Chang S, et al. Liver stiffness decreases rapidly in response to successful hepatitis C treatment and then plateaus. *PLoS One* (2016) 11:e0159413. doi:10.1371/journal.pone.0159413
6. Gragnani L, Visentini M, Fognani E, Urraro T, De Santis A, Petraccia L, et al. Prospective study of guideline-tailored therapy with direct-acting antivirals for hepatitis C virus-associated mixed cryoglobulinaemia. *Hepatology* (2016) 64:1473–82. doi:10.1002/hep.28753
7. Mandorfer M, Kozbail K, Schwabl P, Freismuth C, Schwarzer R, Stern R, et al. Sustained virologic response to interferon-free therapies ameliorates HCV-induced portal hypertension. *J Hepatol* (2016) 65:692–9. doi:10.1016/j.jhep.2016.05.027
8. Sise ME, Bloom AK, Wisocky J, Lin MV, Gustafson JL, Lundquist AL, et al. Ongoing liver inflammation in patients with chronic hepatitis C and C4 by stabilization of mRNA. *J Immunol* (2016) 15:575–83.e1. doi:10.1016/j.jimmunol.2016.09.158
9. Comarmond C, Garrido M, Pol S, Desbois AC, Costopoulos M, Le Garff-Le Normand and then plateaus. *PLoS One* (2012) 11:e0159413. doi:10.1371/journal.pone.0159413
10. Comarmond C, Garrido M, Pol S, Desbois AC, Costopoulos M, Le Garff-Le Normand and then plateaus. *PLoS One* (2012) 11:e0159413. doi:10.1371/journal.pone.0159413
11. Emery JS, Kuczynski M, La D, Almarzooqi S, Kowgier M, Shah H, et al. Novel serum bio-markers for liver fibrosis in children with chronic hepatitis C. *Clin Gastroenterol Hepatol* (2016) 6:2318–28. doi:10.1016/j.cgh.2016.05.027
12. Lautella G, Russi S, Pavone F, Vaccara A, Dammacco F. Direct-acting antiviral agents in the therapy of hepatitis C virus-related mixed cryoglobulinaemia: a single-centre experience. *Arthritis Res Ther* (2017) 19:74. doi:10.1186/s13075-017-1280-6
13. Saudou D, Pol S, Ferfar Y, Alric L, Hezode C, Si Ahmed SN, et al. Efficacy and safety of sofosbuvir plus dasabuvir for treatment of HCV-associated cryoglobulinaemia vasculitis. *Gastroenterology* (2017) 153:49–52.e5. doi:10.1016/j.gastro.2017.03.006
14. Welsch C, Efinger M, Von Wagner M, Herrmann E, Zeuzem S, Welzl TM, et al. Inflammatory mediator levels in patients with chronic hepatitis C and sustained virological response. *PLoS One* (2017) 12:e0171755. doi:10.1371/journal.pone.0171755
15. Gorevic PD. Rheumatoid factor, complement, and mixed cryoglobulinemia. *Clin Dev Immunol* (2012) 2012:439018. doi:10.1155/2012/439018
16. Gorevic PD, Galanakis DC. Cryoglobulins, cryofibrinogenemia, and pyroglobulins. 8th ed. In: Detrick B, Schmitz J, editors. *Manual of Molecular and Clinical Laboratory Immunology*. Washington, DC: ASM Press (2016). p. 101–11.
17. Merle NS, Church SE, Fremeaux-Bacchi V, Roumenina LT. Complement system part I – molecular mechanisms of activation and regulation. *Front Immunol* (2015) 6:262. doi:10.3389/fimmu.2015.00262
18. Reddy YN, Siedlecki AM, Francis JM. Breaking down the complement system: a review and update on novel therapies. *Curr Opin Nephrol Hypertens* (2017) 26:123–8. doi:10.1097/HNE.0000000000000305
19. Lu J, Kishore U. C1 complex: an adaptable proteolytic module for complement and non-complement functions. *Front Immunol* (2017) 8:592. doi:10.3389/fimmu.2017.00592
20. Mitchell TJ, Naughton M, Norsworthy P, Davies KA, Walport MJ, Morley BJ. IFN-gamma up-regulates expression of the complement components C3 and C4 by stabilization of mRNA. *J Immunol* (1996) 156:4429–34.
21. Qin X, Gao B. The complement system in liver diseases. *Cell Mol Immunol* (2006) 3(5):333–40.
22. Ricklin D, Reis ES, Lambris JD. Complement in disease: a defence system turned against itself. *Nat Rev Immunol* (2017) 17:646–57. doi:10.1038/nri.2016.104
23. Emery JS, Kuczynski M, La D, Almarzooqi S, Kowgier M, Shah H, et al. Novel serum biomarkers for liver fibrosis in patients with chronic hepatitis C. *Clin Chem* (2017) 63:301–7. doi:10.1373/clinchem.2017.283504
24. Ganghadharan B, Antrobus R, Dwek RA, Zitzmann N. Novel serum bio-markers for liver fibrosis in patients with chronic hepatitis C. *Clin Chem* (2018) 64:496–507. doi:10.1373/clinchem.2017.283504
25. Behairy BE, El-Mashad GM, Abd-Elghany RS, Ghoneim EM, Sira MM. Serum complement C4a and its relation to liver fibrosis in patients with chronic hepatitis C. *World J Hepatol* (2013) 5:445–51. doi:10.4254/wjh.v5.i8.445
26. Hillebrandt S, Wasmuth HE, Weiskirchen R, Hellerbrand C, Kepperl H, Werth A, et al. Complement factor 3 is a quantitative trait gene that modulates liver fibrogenesis in mice and humans. *Nat Genet* (2005) 37:835–43. doi:10.1038/ng1399
27. Rutkowska MJ, Sughrue ME, Kane AJ, Ahn BJ, Fang S, Parsa AT. The complement cascade as a mediator of tissue growth and regeneration. *Inflamm Res* (2010) 59:897–905. doi:10.1007/s00011-010-0220-6
28. Halangk J, Sarrazin C, Neumann K, Puhl G, Mueller T, Teuber G, et al. Evaluation of complement factor 5 variants as genetic risk factors for the development of advanced fibrosis in chronic hepatitis C infection. *J Hepatol* (2008) 49:339–45. doi:10.1016/j.jhep.2008.05.021
29. Markiewski MM, Deangelis RA, Benenica F, Ricklin-Lichtsteiner SK, Koutoudaki A, Gerard C, et al. Modulation of the antitumor immune response by complement. *Nat Immunol* (2008) 9:1225–35. doi:10.1038/nature06155
30. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods, and major patterns in GLOBOCAN 2012. Int J Cancer (2015) 136:E359–86. doi:10.1002/ijc.29210

31. Hoşienda Y, Fuchs BC, Bardeesy N, Baumert TF, Chung RT. Pathogenesis and prevention of hepatitis C virus-induced hepatocellular carcinoma. J Hepatol (2014) 61:579–90. doi:10.1016/j.jhep.2014.07.010

32. Lee IN, Chen CH, Sheu JC, Lee HS, Huang GT, Chen DS, et al. Identification of complement C3a as a candidate biomarker in human chronic hepatitis C and HCV-related hepatocellular carcinoma using a proteomics approach. Proteomics (2006) 6:2685–73. doi:10.1002/pmc.20500488

33. McHutchison JG, Gordon SC, Schiff ER, Shiffman ML, Lee WM, Rustgi VK, et al. Complement C3 expression in chronic hepatitis C patients. Hepatology (2003) 38:1343–52. doi:10.1053/jhep.2003.08.030

34. Liang TJ, Ghany MG. Therapy of hepatitis C—back to the future. J Clin Transl Hepatol (2013) 1:77–90. doi:10.4145/JCTH.2014.8302

35. Zhao Y, Ren Y, Zhang X, Zhao P, Tao W, Zhong J, et al. Ficolin-2 inhibits hepatitis C virus infection, whereas apolipoprotein E3 mediates viral immune escape. J Immunol (2014) 193:783–96. doi:10.4049/jimmunol.1302563

36. Mason CP, Tarri AW. Human lectins and their roles in viral infections. Molecules (2015) 20:2229–71. doi:10.3390/molecules20022229

37. Brown KS, Keogh MJ, Owaisian AM, Adair R, Patel AH, Arnold IN, et al. Specific interaction of hepatitis C virus glycoproteins with mannann binding lectin inhibits virus entry. Protein Cell (2013) 4:8558–67. doi:10.1007/s13238-010-0088-9

38. Zhao Y, Ren Y, Zhang X, Zhao P, Tao W, Zhong J, et al. Ficolin-2 inhibits hepatitis C virus infection, whereas apolipoprotein E3 mediates viral immune escape. J Immunol (2014) 193:783–96. doi:10.4049/jimmunol.1302563

39. Zupin L, Polesello V, Alberi G, Morattelli G, Croce SL, Masutti F, et al. MLB2 genetic variants in HCV infection susceptibility, spontaneous viral clearance and pegylated interferon plus ribavirin treatment response. Scand J Immunol (2016) 84:61–9. doi:10.1111/sji.12444

40. Stover KA, Morrison TE. Complement and viral pathogenesis. Virology (2011) 411:362–73. doi:10.1016/j.virology.2010.12.045

41. Agrawal P, Nawadkar R, Ojha H, Kumar J, Sahu A. Complement evasion strategies of viruses: an overview. Front Microbiol (2017) 8:1117. doi:10.3389/fmicb.2017.01117

42. Amet T, Ghabril M, Chalasani N, Byrd D, Hu N, Grantham A, et al. CD59 incorporation protects hepatitis C virus against complement-mediated destruction. Hepatology (2012) 55:354–63. doi:10.1002/hep.24686

43. Eizay, A.; Steinmann, E.; Banki, Z.; Anggakusuma, K.; Ségayer, L.; Langer, S.; et al. Specific acquisition of functional CD59 by hepatitis C virus. PLoS One (2012) 7:e45770. doi:10.1371/journal.pone.0045770

44. Zhao Y, Chen X, Zhao P, Tao W, Zhong J, et al. Ficolin-2 inhibits hepatitis C virus infection, whereas apolipoprotein E3 mediates viral immune escape. J Immunol (2014) 193:783–96. doi:10.4049/jimmunol.1302563

45. Liang TJ, Ghany MG. Therapy of hepatitis C—back to the future. J Clin Transl Hepatol (2013) 1:77–90. doi:10.4145/JCTH.2014.8302

46. Dembitter FR, Kinosita Y, Burstein D, Phelps MB, Asghar MB, et al. gC1q-R/p33, a member of a new class of multifunctional and multicompartamental cellular proteins, is involved in inflammation and infection. Immuno Rev (2001) 189:65–77. doi:10.1034/j.1600-065X.2001.180008.x

47. Joseph K, Ghebrehiwet B, Peerschke EL, Reid KB, Kaplan AP. Identification of the zinc-dependent endothelial cell binding protein for high molecular weight kinogen and factor XII: identity with the receptor that binds to the globular “heads” of C1q (gC1q-R). Proc Natl Acad Sci U S A (1996) 93:8552–7. doi:10.1073/pnas.93.16.8552

48. Joseph K, Ghebrehiwet B, Kaplan AP. Activation of the kinin-forming cascade on the surface of endothelial cells. Biol Chem (2001) 382:71–5. doi:10.1515/BC.2001.012

49. Matthews DA, Russell WC. Adenovirus core protein V interacts with p32—a protein which is associated with both the mitochondria and the nucleus. J Gen Virol (1997) 79(Pt 7):1677–85. doi:10.1099/00221281-77-1677-7

50. Luo Y, Yu H, Peterlin BM. Cellular protein modulates effects of human immunodeficiency virus type 1 Rev. J Virol (1994) 68:3850–6.

51. Nguyen T, Ghebrehiwet B, Peerschke EL. Staphylococcus aureus protein A recognizes platelet gC1qR/p33: a novel mechanism for staphylococcal interactions with platelets. Infect Immun (2000) 68:2061–8. doi:10.1128/IAI.68.4.2061-2068.2000

52. Kittleson DJ, Chisena-Bullock KA, Yaq QZ, Braciale TJ, Hahn YS. Interaction between complement receptor gC1qR and hepatitis C virus core protein inhibits T lymphocyte proliferation. J Clin Invest (2000) 106:1239–49. doi:10.1172/JCI10332

53. Yao ZQ, Nguyen DT, Hiotellis AI, Hahn YS. Hepatitis C virus core protein inhibits its human T lymphocyte responses by a complement-dependent regulatory pathway. J Immunol (2001) 167:5264–72. doi:10.4049/jimmunol.167.9.5264

54. Yao ZQ, Eisen-Vandervelde A, Waggoner SN, Cale EM, Hahn YS. Direct binding of hepatitis C virus core to gC1qR on CD4+ and CD8+ T cells leads to impaired activation of Lck and Akt. J Virol (2004) 78:6409–6419.2004

55. Waggoner SN, Hall CH, Hahn YS. HCV core protein interaction with gC1q receptor inhibits Th1 differentiation of CD4+ T cells via suppression of dendritic cell IL-12 production. J Leukoc Biol (2007) 82:1407–19. doi:10.1189/jlb.0705268

56. Eng FJ, EI-Shamy A, Doyle EH, Kleeper A, Muerhoff AS, Branch AD. Newly discovered hepatitis C virus minicore circulate in human blood. Hepatol Commun (2018) 2:21–8. doi:10.1002/hep4.1125

57. Yao ZQ, Prayther D, Trubace C, Dong ZP, Moorman J. Differential regulation of SOCS-1 signalling in B and T lymphocytes by hepatitis C virus core protein. Immunology (2008) 125:197–207. doi:10.1111/j.1365-2678.2007.02829.x

58. McMurray RW, Elbourne K. Hepatitis C virus infection and autoimmunity. Semin Arthritis Rheum (2007) 36:191–201. doi:10.1016/j.semarthrit.2006.09.007

59. Strassburg CP, Vogel A, Manns MP. Autoimmunity and hepatitis C. Autoimmun Rev (2003) 2:232–31. doi:10.1016/S1531-6914(03)00036-3

60. Paroli M, Iannucci G, Accpetizzato D. Hepatitis C virus infection and autoimmune diseases. Int J Gen Med (2012) 5:903–7. doi:10.2147/IJGM.S37580
73. Sansonno D, Tucci FA, Ghebrehiwet B, Lauletta G, Peerschke EI, Conteduca V, et al. Role of the receptor for the globular domain of C1q protein in the pathogenesis of hepatitis C virus-related cryoglobulin vascular damage. *J Immunol* (2009) 183:6013–20. doi:10.4049/jimmunol.0902038

74. Cacoub P, Gragnani L, Comarmond C, Zignego AL. Extrahepatic manifestations of chronic hepatitis C virus infection. *Dig Liver Dis* (2014) 46(Suppl 5): S165–73. doi:10.1016/j.dld.2014.10.005

75. Quartuccio L, Isola M, Corazza L, Maset M, Monti G, Gabrielli A, et al. Performance of the preliminary classification criteria for cryoglobulinemia vasculitis and clinical manifestations in hepatitis C virus-unrelated cryoglobulinemia vasculitis. *Clin Exp Rheumatol* (2012) 30(Suppl7):548–52.

76. Tissot JD, Invernizzi F, Schifferli JA, Spertini F, Schneider P. Two-dimensional electrophoretic analysis of cryoproteins: a report of 335 samples. *Electrophoresis* (1999) 20:606–13. doi:10.1002/(SICI)1522-2683(19990301)20:3<606::AID-ELPS606>3.0.CO;2-N

77. Sene D, Ghillani-Dalbin P, Thibault V, Guis L, Musset L, Duhaut P, et al. Lymphoid system in hepatitis C patients infected with hepatitis C virus. *J Rheumatol* (2004) 31(11):2199–206.

78. De Rosa FG, Abel G, Agnello V. Observations on cryoglobulin testing II. The association of oligoclonal mixed cryoglobulinemia with cirrhosis in patients infected with hepatitis C virus. *J Rheumatol* (2009) 36:1956–7. doi:10.3899/jrheum.090189

79. Menegatti E, Messina M, Oddone V, Bubini E, Sciacsia S, Naretto C, et al. Immunogenetics of complement in mixed cryoglobulinaemia. *Clin Exp Immunol* (2016) 134(Suppl 97):S12–5.

80. Hanauer LB, Christian CL. Studies of cryoproteins in systemic lupus erythematosus. *J Clin Invest* (1967) 46:400–8. doi:10.1172/JCI110541

81. Sansonno D, Lauletta G, Nisi L, Gatti P, Pesola F, Pansini N, et al. Non-protective association of low-density lipoprotein receptor genotypes with hepatitis C viral load. *Genes Immun* (2014) 15:16–24. doi:10.1038/gene.2013.56

82. Sansonno D, Piccoli C, Racanelli V, D’Amore FP, Lauletta G. The lymphoid system in hepatitis C virus infection: autoimmunity, mixed cryoglobulinemia, and overt B-cell malignancy. *Semin Liver Dis* (2000) 20:143–57. doi:10.1055/s-2000-9613

83. Bryan AR, Wu EY. Complement deficiencies in systemic lupus erythematosus. *Curr Allergy Asthma Rep* (2014) 14:448. doi:10.1007/s11882-014-0448-2

84. Ghebrehiwet B, Peerschke EI. Role of C1q and C1q receptors in the pathogenesis of systemic lupus erythematosus. *Curr Dir Autoimmun* (2004) 7:87–97. doi:10.1159/000075688

85. Macedo AC, Isaac L. Systemic lupus erythematosus and deficiencies of early components of the complement classical pathway. *Front Immunol* (2016) 7:55. doi:10.3389/fimmu.2016.00055

86. Son M, Diamond B, Santiago-Schwarz F. Fundamental role of C1q in autoimmunity and inflammation. *Immunol Res* (2015) 63:101–6. doi:10.1007/s10204-015-8705-6

87. Botto M, Walport MJ. C1q autoimmunity and apoptosis. *Immunobiology* (2002) 205:395–406. doi:10.1007/0-7817-2985-0141

88. Lintner KE, Wu YL, Yang Y, Spencer CH, Hauptmann G, Hebert LA, et al. C4b-binding protein in primary Sjogren’s syndrome and its association with type II cryoglobulinemia. *N Engl J Med* (1992) 327:1490–5. doi:10.1056/nejm199211193272104

89. Bryan AR, Wu EY. Complement deficiencies in systemic lupus erythematosus. *J Exp Med* (1997) 185:1721–3. doi:10.1084/jem.185.10.1721

90. De Rosa FG, Agnello V. Observations on cryoglobulin testing: I. The association of C-reactive protein in cryoglobulins. *Clin Exp Immunol* (2001) 125:316–22. doi:10.1046/j.1365-2249.2001.01606.x

91. Casali P, Borzini P, Piottelli P, Invernizzi F, Zanussi C. Acquired C1-inhibitor deficiency in essential cryoglobulinemia and macroglobulinemia. *Acta Haematol* (1978) 59:277–84. doi:10.1111/j.1365-2249.1978.tb00773.x

92. Hayday AC, Patarroyo De Rosas M, Giigi L. A newly described control mechanism of complement activation in patients with mixed cryoglobulinemia (cryoglobulin and complement). *J Invest Dermatol* (1980) 74:328–32. doi:10.1111/1523-1747.ep12543575

93. Zadura AE, Theander E, Blom AM, Trouw LA. Complement inhibitor C4b-binding protein in primary Sjogren’s syndrome and its association with other disease markers. *Scand J Immunol* (2009) 69:374–80. doi:10.1111/j.1365-3083.2009.02229.x

94. Lintner KE, Wu YL, Yang Y, Spencer CH, Hauptmann G, Hebert LA, et al. Early components of the complement classical activation pathway in human systemic autoimmune diseases. *Front Immunol* (2016) 7:36. doi:10.3389/fimmu.2016.00036

95. Posnett DN, Edinger J. When do microbes stimulate rheumatoid factor? *J Exp Med* (1997) 185:1721–3. doi:10.1084/jem.185.10.1721

96. Lintner KE, Wu YL, Yang Y, Spencer CH, Hauptmann G, Hebert LA, et al. Complement system in HCV infection. *Complement System in HCV Infection*.