INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a growing public health threat and becoming the leading cause of liver transplantation. Nevertheless, no approved specific treatment is currently available for NAFLD. The pathogenesis of NAFLD is multifaceted and not yet fully understood. Accumulating evidence suggests a significant role of the complement system in the development and progression of NAFLD. Here, we provide an overview of the complement system, incorporating the novel concept of complosome, and summarise the up-to-date evidence elucidating the association between complement dysregulation and the pathogenesis of NAFLD. In this process, the extracellular complement system is activated through various pathways, thereby directly contributing to, or working together with other immune cells in the disease development and progression. We also introduce the complosome and assess the evidence that implicates its potential influence in NAFLD through its direct impact on hepatocytes or non-parenchymal liver cells. Additionally, we expound upon how complement system and the complosome may exert their effects in relation with hepatic zonation in NAFLD. Furthermore, we discuss the potential therapeutic implications of targeting the complement system, extracellularly and intracellularly, for NAFLD treatment. Finally, we present future perspectives towards a better understanding of the complement system’s contribution to NAFLD.

KEYWORDS
complement, complosome, immunometabolism, NAFLD, spatial localisation
to 38.2% in 2016–2020, as demonstrated in a recent meta-analysis.\textsuperscript{7} The prevalence dramatically increases up to 70% in high-risk populations such as individuals with diabetes and obesity.\textsuperscript{3} Nevertheless, NAFLD has also been reported to present in 9.2% of lean individuals.\textsuperscript{4} Concurrently, NASH, the more severe form of NAFLD, has become the leading indication for liver transplantation and emerged as one of the most common causes of cirrhosis, cirrhotic complications, hepatocellular carcinoma and liver-related death.\textsuperscript{5,6} Remarkably, despite ostensibly being a hepatic condition, NAFLD, in its all histological stages, is inextricably linked to an increased risk for all-cause mortality, which increases progressively with worsening NAFLD histology.\textsuperscript{7} Unfortunately, currently, there are no approved specific therapeutic treatments for the disease. Consequently, it leads to extremely high costs in association with the disease and its comorbidities.\textsuperscript{8} Thus, there is an urgent need for collective efforts in mechanistic research and the development of innovative therapeutics to tackle the disease.

Mechanistically, NAFLD is heterogeneous and multifactorial, with a complex interplay between metabolic, genetic, epigenetic, environmental factors, gut microbiota and host immune response. In this context, various immune processes, both innate and adaptive immunity, have been implicated in the development and progression of NAFLD.\textsuperscript{9,10} Recently, there has been growing attention to the role of the complement system in the pathogenesis of NAFLD.\textsuperscript{11–13} Classically, the complement system is a pivotal component of the innate immune system, playing an essential part in protecting the host from infection and promoting tissue homeostasis and repair. However, once dysregulated in its activity, the complement system has been demonstrated to be involved in a variety of both infectious and non-infectious diseases.\textsuperscript{14} Intriguingly, mounting evidence suggests that complement components could play a role as an immunometabolic factor in driving various pathological conditions, including NAFLD. Furthermore, the unveiling of novel facets of complement biology, including the complosome, has paved the way for broadening our insights into the canonical and non-canonical roles of the complement system in physiology as well as pathobiology.\textsuperscript{15–17} In this review, we highlight the current knowledge on the emerging roles of the classical complement systems and the complosome in NAFLD. Furthermore, we discuss the potential implications of complement-targeted therapeutic strategies in NAFLD management.

In order to retrieve relevant evidence, a literature review was conducted in PubMed, Medline, and Embase to identify relevant published articles. The search terms for NAFLD included "nonalcoholic fatty liver disease", "non-alcoholic fatty liver disease", NAFLD, "nonalcoholic fatty liver", "non-alcoholic fatty liver", NAFL, "non-alcoholic steatohepatitis", "non-alcoholic steatohepatitis", NASH, "metabolic dysfunction associated fatty liver disease", NAFLD, "metabolic associated steatohepatitis", and MASH. The search terms for the complement system were "complement system", "complement activation", "complement protein\textsuperscript{*}\", "complement pathway\textsuperscript{**,} "complement receptor\textsuperscript{**}\", "complement inhibitor\textsuperscript{**}\", complosome, and "intracellular complement".

Key points

As the front line of defence in our human bodies, the complement system, along with a new concept called the complosome, play a big role in the beginning and growth of non-alcoholic fatty liver disease (NAFLD), a main reason people need liver transplants. In this review, we discuss how this system directly impacts different types of liver cells, influencing how NAFLD starts and worsens. Targeting this system, both inside and outside cells, has a high potential for treating NAFLD.

2 | THE ‘CLASSICAL’ COMPLEMENT SYSTEM AND NAFLD

2.1 | Overview of the ‘classical’ complement system

The complement system, an intricate network comprising over 50 plasma proteins and membrane receptors, assumes a pivotal role as a frontline defence mechanism against microbial pathogens. It operates in a highly coordinated manner, bridging the innate and adaptive arms of the immune system by regulating inflammatory responses, leukocyte trafficking, and antigen processing, presentation, and clearance. In doing so, it safeguards homeostasis and combats infections.\textsuperscript{18,19}

Activation of the complement system can occur via three distinct pathways—namely, the classical pathway (CP), lectin pathway (LP), and alternative pathway (AP)—triggered by pathogen- or damage-associated molecular patterns, each characterised by unique contextual cues.\textsuperscript{18,19} The CP is initiated by the attachment of the C1q subcomponent to the Fc portion of antibodies, leading to a cascade of conformational changes and subsequent activation of C1r. Thereafter, the activated C1r cleaves and activates the C1s. C1s, in turn, splits C4 and also C2, producing C4a, C4b, C2a, and C2b. Next, C4b and C2a assemble on the target, forming the CP C3 convertase (C4b2a complex). On the other hand, the LP is instigated by the binding of mannose-binding lectin (MBL), ficolins or collectins to mannose residues on the pathogen surface. Following the formation of lectin-sugar complexes, the MBL-associated serine proteases (MASP), MASP-1, and MASP-2, are activated and cleave C4 and C2, which then generate the C3 convertase as in the classical pathway.\textsuperscript{18–20} As for the AP, it is a continuous, low-level activation and does not require antibody or prior exposure to a microbe to function. The pathway commences with the spontaneous hydrolysis of C3 (also referred as “C3 tickover”), which then binds to factor B (FB). This is followed by a proteolytic cleavage mediated by another serine protease, factor D (FD), leading to the production of Bb and Ba fragments. Subsequently, the alternative pathway C3 convertase, C3bBb, is formed, and then stabilised by properdin, creating the stabilised C3 convertase (C3bBb complex). Regardless of its origin,
all forms of C3 convertases then promote the cleavage of C3 into C3a and C3b. After that, C3b joins with C4bC2a to generate the C5 convertases (C4b2a3b or C3bBbC3b complexes). These complexes then cleave C5 into C5a and C5b, resulting in the assembly of the membrane attack complex (MAC) through the serial addition of C6–C9. C3 convertases can also amplify branch of AP by activating C3, thereby causing the deposition of large amounts of C3b on the target, and establishing an efficient cycle of C3 cleavage and convertase assembly (see Figure 1A). 19

While MAC creates a hole or pore in the membrane that can kill or damage the pathogen or noxious target cells, resultant anaphylatoxins (C3a, C5a) trigger local and systemic inflammatory responses, increasing vascular permeability and attracting neutrophils through their chemotactic properties. Additionally, generated opsonins (such as C3b, iC3b, C4b, etc.) enhance the phagocytic capability of complement receptors-expressed macrophages and neutrophils.18,19

In physiological conditions, the complement system is persistently activated at a low grade, serving to eliminate dying cells without engaging other elements of the innate or adaptive immune system. However, upon detection of pathogen infection or target cells, the complement system undergoes full activation, giving rise to effectors that participate in target opsonization, inflammation amplification, and target cell lysis. These vascular space-related responses are mediated by systemic complement through its canonical antimicrobial functions. Intriguingly, the complement system also regulates adaptive immune responses in a manner that is majorly governed

**FIGURE 1** Current understanding of the complement and complosome. (A) Activation of the complement system occurs when target epitopes are recognised by C1q (Classical pathway) or MBL/FCNs/CL (Lectin pathway), leading to the cleavage of C4 and the formation of C3 convertase, which cleaves C3. The alternative pathway initiates spontaneously (continuous “tick-over” process) that can be boosted under pathological conditions. Cleaved C3 triggers the formation of the C5 convertase, which cleaves C5 and ultimately generates the membrane attack complex (MAC). The MAC is capable of lysing membrane and activating cells. Activation of C4, C3, and C5 also results in the production of anaphylatoxins, which induce chemotaxis and inflammation. The complement system’s activation is tightly regulated by soluble and membrane-bound proteins (blue, in boxes), which inhibit convertase formation, accelerate decay, or prevent C5b9 complex assembly; (B) Visualisation of the current knowledge of observed complosome components, including their sources and associated binding partners; (C) The figure summarises the up-to-date list of cells that have been identified to harbour the complosome. AGT16L1, autophagy related 16-Like 1; C1INH, C1 inhibitor; C3aR, C3a receptor; C4BP, C4 binding protein; C5aR1, C5a receptor 1; CL, Collectins; CR1, complement receptor 1; CTSL, Cathepsin L; FB, factor B; FCNs, Ficolins; FD, factor D; FH, factor H; FHR-3, Factor H-Related Protein 3; FI, factor I; MASP, MBL-associated serine proteases; MBL, mannos-binding lectin; p32/gC1qr, p32/global C1q receptor; SNARE, soluble N-ethylmaleimide-sensitive factor attachment protein receptors.
by local cell-derived canonical complement. Ultimately, such orchestrated reactions are meticulously controlled to avert microbial invasion and infection, eliminate noxious target cells, and simultaneously avoid damaging inflammation.18,19

Complement components manifest their effector functions by targeting “danger” signals exhibited by pathogens, while ensuring the preservation of healthy self-tissue from undesired complement activation through the presence of complement inhibitors in both fluid-phase and membrane surfaces.21 Figure 1A illustrates the pivotal role of key complement regulators. Nevertheless, unrestrained complement overactivation, in turn, can become destructive and act as a pathogenic effector. In these scenarios, any abnormalities in complement activity are associated with a larger number of disorders including inflammatory, autoimmune, thrombotic, and age-related diseases.14,22,23

2.2  Evidence linking ‘classical’ complement dysregulation to metabolic syndrome, obesity, lipid metabolism disorders, insulin resistance, and diabetes

NAFLD is intricately linked to metabolic syndrome, obesity, and lipid metabolism disorders, insulin resistance, and diabetes. Predictably, the complement system’s role in these disorders has been extensively investigated. Elevated C3 levels have been consistently linked to metabolic syndrome, independent of factors like ethnicities, lifestyle, cholesterol, insulin resistance, and inflammation.24–29 C3, along with C4 and acylation stimulating protein (ASP), was also positively correlated with metabolic syndrome in the work of Nilsson et al.30 Meanwhile, obesity is characterised by increased levels of complement components such as AP components (FB, FD), factor H (FH), C3 and ASP whereas a decrease in these components was noted following weight loss.30–34 Serum C3 levels correlate with obesity,30,35–43 body fat content,38 and insulin resistance,35 serving as a predictor of substantial weight gain in men.39 It warrants mention that adipose tissues are the main producing source for a crucial AP component (FD), and can also produce FB and C3.38,44 Indeed, C3 levels exhibited a proportional increase in relation to the total volume of adipose tissues.30,31 These abovementioned lines of evidence imply the activation of AP contributing to obesity and insulin resistance. Furthermore, with insulin-like properties, ASP, can promote the synthesis of triacylglycerols through the transportation of free fatty acids (FFAs) into adipocytes.31,45

Complement components can also play a role in lipid metabolism, with C3 levels positively correlating with cholesterol and triglyceride levels.46,47 Hyperlipidemia patients exhibited elevated levels of C3 and ASP compared to healthy individuals. In healthy subjects, an oral fat loading test resulted in a notable rise in plasma C3, whereas hyperlipidemia patients displayed a delayed C3 response. A negative correlation was also noted between the increase in postprandial C3 and postprandial FFAs.48 Additionally, deficiencies in complement FB and C3, which lead to a lack of ASP, result in delayed lipid clearance.49 It should also be noted that chylomicron, a form in which dietary fat is carried, can activate the AP, inducing overproduction of ASP.50,51 Therefore, it is reasonable to speculate that impaired C3/ASP response may be associated with impaired fatty acid uptake, leading to increased plasma fatty acid concentration and enhanced flux to the liver.

In type 2 diabetes, higher serum C3 levels have been identified as a disease predictor.36,52,53 Elevated serum ficolin 3 levels were associated with reduced risk of type 2 diabetes,54 while decreased MBL levels increased diabetes risk after renal transplantation.55 In contrast to these genes, there are several complement components may be protective against type 2 diabetes. Specifically, C4b binding protein can preserve β-cells function by inhibiting the aggregation into protofibrils of islet amyloid polypeptide, an important influencer in type 2 diabetes pathogenesis.36 CD59, on the other hand, was shown to maintain glucose-stimulated insulin secretion by β-cells.57,58

Collectively, the aforesaid data suggest that the complement system plays a crucial role in lipid metabolism. Dysregulated complement activity is implicated in metabolic syndrome, obesity, lipid metabolism disorders, insulin resistance, and diabetes, ultimately contributing to the pathogenesis of NAFLD.

2.3  Evidence linking ‘classical’ complement dysregulation to NAFLD pathogenesis

Hepatocytes, apart from functioning as a colossal metabolic machine, have been long established as the primary source of extracellular complement biosynthesis.59 Once synthesised and activated, components of this conventional complement, in turn, contribute to the maintenance of hepatocytes normal function and regeneration process.60–63 Unsurprisingly, the circulating complement is also involved in a wide range of hepatic disorders, from infectious diseases (hepatitis B, hepatitis C) to non-infectious conditions (liver ischemia, reperfusion and transplantation; liver fibrosis; alcoholic fatty liver disease, autoimmune hepatitis).64–78 NAFLD manifests as a pathological nexus where perturbations in metabolic processes and hepatic dysregulation intersect. Expectedly, the role of the complement system in the development and progression of NAFLD is supported by a growing body of evidence.

In steatosis, the first and defining hallmark of NAFLD, the involvement of the complement system has been extensively demonstrated. In adult population studies, elevated serum C3 levels were independently associated with NAFLD prevalence.79–83 Similarly, serum C3a levels were significantly associated with estimated liver fat amount in adults NAFLD patients.84 Notably, elevated serum C3 levels were demonstrated to be an independent risk factor of and have a causal relationship with NAFLD development.79,85 In addition, other complement components including C3f, C5a, and FD were also documented to be significantly higher in NAFLD patients.86–88 Hepatic activation of complement components, including C3, C1q, C4d, and MBL, was detected in NAFLD patients, indicating
activation of classical and lectin pathways. Animal studies further confirmed the involvement of the complement system in NAFLD, showing increased serum complement activity and upregulation of complement genes (C3, C1s, C1r, C8a, C4a, C8b, C6, C2, FB) in diet-induced NAFLD models. Interestingly, C5 or FD deficiency in a high-fat diet mouse model reduced steatosis. Additionally, C3a receptor (C3aR) deficiency protected against NAFLD and affected adipose tissues, where C3aR is highly expressed. However, the specific mechanism through which the complement system is involved in this process is still not clear. In consideration of existing evidence, an emerging hypothesis is that in NAFL, the overactivation of the complement system, including AP, is involved in insulin resistance and lipid metabolism disorder, resulting into the over-accumulation of triglycerides in hepatocytes. Specifically, insulin resistance leads to the release of FFAs into circulation, which are taken up by the liver and contribute to TG synthesis. Hyperinsulinemia and high FFAs levels enhance de novo lipogenesis, while suppressing fatty acid oxidation. This imbalance in hepatic lipid metabolism leads to the over-deposition of lipid droplets in hepatocytes. NAFL, in turn, can initiate apoptosis, in which AP may be involved, in steatotic cells. This apoptotic process can further activate the complement system in the liver by the recognition of C1q (CP) and MBL (LP), thereby perpetuating the cycle of liver damage.

Unlike NAFL, NASH is characterised not only by the accumulation of triglycerides in hepatocytes but also by the infiltration of inflammatory cells, elevated levels of inflammatory cytokines, and liver injury, including cell ballooning. Intriguingly, the complement system appears to drive this progression. Higher serum C3 levels were associated with increased NAFLD severity, and among NAFLD patients, NASH was more prevalent in subjects with hepatic C3a, C4d, C1q and C9 deposition. A higher levels of C3 and ASP were observed in patients with NASH compared to controls. C activation status (C3c/C3 ratio) was higher in NASH patients, particularly in those with increased lobular inflammation scores. Furthermore, the enhanced crosstalk between activated C3 and pro-inflammatory cytokines was observed in patients with higher expression levels of interleukin-8 and interleukin-6 in subjects with hepatic C3a deposition. In a recent meta-analysis, elevated levels of complement components, such as C3, C5, FB, and ASP, are linked to a higher likelihood and greater severity of NAFLD. Moreover, the complement system may interact with other components of the host immunity in NASH. Immune cells, including T cells, macrophages, Kupffer cells and neutrophils, have been frequently reported to be involved in NASH. Given the fact that the complement system can regulate these immune cells, it is reasonable to speculate that the complement system may participate in NASH by modulating host immune cells. The major complement effectors (C3 and C5) stimulate their respective receptors expressed by host immune cells, triggering a chain of immune responses and inflammation within these cells, thereby shaping the progression of NAFLD. Supporting this perspective, the receptor for C3a was found to be overexpressed in macrophages in white adipose tissue and Kupffer cells in the liver of HFD-fed mice. C3a receptor deletion mitigated HFD-induced hepatic injury, along with enhanced insulin sensitivity, attenuated macrophages infiltration, and suppressed the pro-inflammatory effect of M1-like macrophages. Additionally, C5 deficiency in a high-fat diet mouse model reduced liver inflammation and expression of pro-inflammatory cytokines. Besides, by using HFD-induced obese mouse model, Julia Philer and et al. showed that C5a/C5aR axis mediates the accumulation of macrophages and macrophages polarisation towards pro-inflammatory phenotype in obese white adipose tissue. For neutrophil, in NASH patients, the accumulation of properdin, an AP component, was demonstrated in neutrophil infiltrated areas around steatotic hepatocytes. The accumulation was co-localised with C3c – an activation product of C3. C3aR and C5aR signalling was also indicated to regulate other immune cells, such as T cells and neutrophils, which potentially may play an important role in NAFLD pathogenesis. These lines of evidence imply that in the stage of NASH, the complement system drives this process by regulating the abundance and function of immune cells, such as macrophages, T cells and neutrophils. However, there remains a paucity of evidence in this area, and future studies are needed.

Another crucial aspect in NAFLD pathogenesis is fibrosis and cirrhosis. In the chronic state of liver inflammation in NASH, hepatic stellate cells can be transformed to myofibroblasts. These activated cells are responsible for the excessive production of extracellular matrix, leading to the development of liver fibrosis. Approximately 25% of NASH patients progress to cirrhosis within a 10- to 20-year timeframe. An earlier investigation revealed that the C5 gene plays a role in the development of liver fibrosis. Additionally, blocking C5aR1, a receptor associated with C5, reduced the extent of liver fibrosis in mice. In the context of fibrosing NASH, the complement and coagulation cascade was down-regulated in both NASH fibrosis stage 1–4 versus control and in the fibrosis stage-based trending analysis, with a significantly lower levels of MASP2 and C5 in NASH fibrosis stage 1–4 compared to NASH without fibrosis in adult patients. In paediatric patients, a study showed that serum C3 levels were positively correlated with aspartate transaminase-to-platelet ratio, an established predictive marker for liver fibrosis. Additionally, MASp1 was elevated in methionine-choline deficient diet-induced fibrosing NASH mouse livers compared to controls. Interestingly, high levels of C7 are associated with NASH with significant/advanced fibrosis and C7 can serve as a good classifier of NASH patients with significant fibrosis. Meanwhile, C8 gamma chain was negatively correlated with fibrosis non-invasive markers such as fibrosis-4 score and liver stiffness. Intriguingly, a mechanistic study in methionine-choline deficient diet-induced fibrosing NASH mouse model has shown that C3ar1 controls the fibrosis development in NASH by modulating macrophage. The induction of C3ar1 in macrophages leads to promoting fibrosing NASH through inducing the activation of hepatic stellate cells. Hepatic stellate cells can also be activated by hepatocyte-derived MASp1 via p38 mitogen-activated protein kinase/activating transcription factor 2 signalling, thereby promoting liver fibrogenesis in NASH. However, further studies are still required to further explore the mechanisms.
underlying the role of the complement system in the occurrence and development of liver fibrosis and cirrhosis in NAFLD context.

Taken together, the complement system, particularly the central role of C3, contributes to the development and progression of NAFLD. The activation of complement pathways and their interactions with immune cells are key mechanisms underlying the pathogenesis of NAFLD (see the summary in Figure 2). Further studies are needed to expand our understanding of these complex interactions in NAFLD pathophysiology.

3 | THE COMPLOSOME AND NAFLD

3.1 | Definition and functions of the complosome

In recent years, technological advancements have facilitated the investigation of complement compartmentalization, allowing researchers to explore the physiological and pathological aspects of the complement system in greater detail. Emerging evidence indicates that complement activation and effector functions extend beyond the extracellular milieu, extending into the intracellular space. Detailed reviews of this intracellular complement system, termed the "complosome", have been previously published, including recently. Briefly, it represents a novel concept characterized by a complex protein assembly that operates intracellularly and interacts with internal danger detection and effector systems, such as inflammasomes and autophagosomes. The complosome is posited to include all complement components with intracellular expression and activity. At present, the comprehension of the complosome predominantly centres on the alternative pathway, but emerging evidence also indicates the significance of classical and lectin pathways (see Figure 1B). An increasing number of cell types has been identified as the harbouring cells for the complosome, including both immune cells and non-immune cells (depicted in Figure 1C). Various intracellular locations including the cytoplasm, lysosome, endoplasmic reticulum, outer membrane of mitochondria, and nucleus have been identified as sites for the complosome proteins. Generally, intracellular complement proteins exert their effects exclusively within specific sub-compartments of cells; however, in certain scenarios, they can be derived from the extracellular environment and internalised intracellularly, such as C3, C3a, FH, FH related

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**FIGURE 2** The roles of the complement and complosome in NAFLD. The figure depicts the roles of the extracellular complement and the intracellular complement in NAFLD development and progression. The extracellular complement is activated upon the triggers, through various pathways (classical, lectin, and alternative) and, with the central role of C3, drives NAFLD developmental process. The induction can be commenced through interaction with host immune cells. Meanwhile, intracellularly, the complosome may participate in NAFLD by affecting lipophagy and VLDL assembly and secretion inside hepatocytes through C3–AGT16L1 interaction. Potentially, the complosome may also exert their roles through their dysregulated components in key immune cells, including CD4+ T cells, CD8+ T cells, and macrophages, generating corresponding pathological phenotypes. AGT16L1, autophagy related 16-Like 1; C5aR1, C5a receptor 1; IFN-γ, interferon gamma; IL-β, interleukin-beta; VLDL, Very low density lipoprotein.
protein-3, and C1q. Nevertheless, extracellularly derived complement components may not fully compensate for the unique structural characteristics and modifications of cell-autonomous complement proteins, which might function exclusively within cellular compartments accessible to the cell-autonomous protein sorting machinery. Furthermore, it has been observed that cells have the capability to reutilize complement C3, which they had previously synthesized and released, and can transport the components between different intracellular compartments in response to stimulation. Irrespective of their sources, complosome components are encoded by the same genes as their counterparts in the extracellular complement system.

Functionally, intracellular C3 and C5 can undergo cleavage activation by specific proteases (such as Cathepsin L) or by intracellular C3 and C5 convertases that are generated underneath the plasma membrane and on the surface of subcellular compartments. Upon the stimulation of intracellular receptors, including C3aR and C5aR1, the complosome can trigger signalling events akin to their cell surface counterparts. Nevertheless, it also has the ability to engage with distinct cellular machinery, spatially separated from extracellular complement components, by interacting with new binding partners, underscores its predominantly non-canonical functions. One of the most intriguing findings in relation to these non-canonical complement functions is that the complosome plays a crucial role in regulating fundamental cellular processes, particularly cell metabolism. Intracellularly, the engagement of complosome components leads to an increase in glycolysis, fatty acid metabolism and oxidative phosphorylation. The complosome’s activity also participates in maintaining normal mitochondrial function and fitness. Interestingly, intracellular complement components seem to contribute to cell autophagy and vesicular transport, involving the reshuffling of materials within subcellular compartments. Another intriguing function of the complosome that has been suggested is its active role in gene transcription and regulation as well as protein translation.

### 3.2 Evidence linking complosome dysregulation to NAFLD pathogenesis

The complosome has been identified to be present in a number of cell types, including hepatocytes - a major cell population that comprises up to 80% of total liver volume. Traditionally, it was believed that the impacts of the complement system on hepatic physiology and pathophysiology were solely attributed to the extracellular complement. However, the advent of the complosome has precipitated a paradigm shift, thereby instigating a nascent domain of comprehensive exploration into the intricate interplay between the complement system and liver conditions.

In NAFLD, current evidence has hinted at plausible roles of the complosome in hepatic lipid metabolism as well as in NAFLD pathogenesis. Specifically, in mouse hepatocytes, the complosome C3 has been found to have a protective role against hepatic steatosis via modulating lipophagy, a highly selective form of autophagy targeting lipid droplets in hepatic triglyceride catabolism, through interacting with autophagy-related 16 like 1. Additionally, intracellular C3 stimulates the assembly and secretion of very low-density lipoprotein, thereby reducing lipid accumulation in hepatocytes. Moreover, emerging research has indicated the complosome’s capacity to regulate insulin production within pancreatic β-cells, particularly influenced by CD59 and C3.

Such observations underscore the complosome’s involvement in diabetes, a primary instigator of NAFLD. In addition to that, in the development of atherosclerosis, a close metabolic disorder to NAFLD, cell-intrinsic C5a-C5aR1 axis in monocytes and macrophages has been reported as a crucial contributor in a high-fat diet-induced mouse model.

Furthermore, in spite of the lack of direct evidence, it is reasonable to speculate that the complosome can modulate immune cells, thereby driving the disease pathogenesis. One of the widely established characteristics of NASH is an increase in the proportion of CD4⁺ T Th1 cells in both peripheral blood and hepatic tissues. The percentage of CD4⁺ T Th1 cells producing interferon-gamma (IFN-γ) is positively correlated with insulin resistance and obesity-related inflammation. IFN-γ has pathogenic effects in the liver, including inducing hepatocyte apoptosis, cell cycle arrest, and the expression of chemokines on hepatocytes. C-X-C motif chemokine ligand 10, an IFN-γ-induced chemokine, recruits T cells expressing C-X-C Motif Chemokine Receptor 3, a driver in NASH, and its deficiency reduces liver inflammation, injury, and fibrosis in NASH. Another important T cell population in the pathogenesis of NAFLD is CD8⁺ cytotoxic T cells with a shift in their activated form confirmed in the disease group. An increased presence of CD8⁺ T cells producing IFN-γ was also observed both in the hepatic microenvironment and peripheral blood. Interestingly, CD4⁺ T cells, as one of the most extensively studied cells for the complosome, rely on intracellular C3 for their homeostatic survival signal. Additionally, the complosome’s CD46 (binding partner of C3b) emerges as a key initiator of indispensable processes facilitating IFN-γ production and the induction of CD4⁺ T Th1 immunity. Within human CD8⁺ T cells, the intrinsic engagement of CD46 facilitates the cell activation, the optimal secretion of IFNγ and exhibition of cytolysis. These lines of evidence implicate the interplay between the complosome and T cells in NAFLD. For macrophages and monocytes, they manifest their influence in NAFLD through multifaceted activities. One of those is the production of the pro-inflammatory cytokine interleukin-1 beta (IL-1β), thereby driving liver steatosis, inflammation and fibrosis. Of note, excessive activation of mitochondrial C5aR1 and heightened intracellular C5 levels within monocytes and macrophages promoted the cholesterol-induced generation of IL-1β-producing M1-like macrophages. Collectively, the complosome’s ability to influence immune cells suggests its potential involvement in NAFLD pathogenesis (illustrated in Figure 2).
Hepatic zonation, an intrinsic hallmark of liver architecture, refers to the division of liver lobules into distinct functional zones along the porto—central axis. Each zone exhibits unique metabolic and physiological characteristics, allowing for specialised functions and interactions within the liver.\textsuperscript{169} Periportal hepatocytes (zone 1) handle energy-demanding processes, such as xenobiotic metabolism, bile acid biosynthesis, and glycolysis. Central layers (zone 3), on the other hand, manage less demanding functions, including β-oxidation, cholesterol biosynthesis, protein secretion and gluconeogenesis. The middle zone (zone 2), situated between the periportal and central regions, regulates iron and other tasks.\textsuperscript{169,170} This spatially organised structure also assumes an indispensable role in various pathological processes.\textsuperscript{170} Periportal damaged conditions are associated with processes zonated in periportal area. For instance, drug-induced liver injury is thought to be caused by the toxic intermediates accumulation in the hepatocytes that express the detoxification machinery, a process of xenobiotic metabolism.\textsuperscript{171} Conversely, pericentral injury is observed in autoimmune hepatitis.\textsuperscript{171} In NAFLD, the initial development predominantly occurs in the pericentral area, with steatosis spreading along the porto—central axis, particularly in lobule regions (zone 2 and zone 3).\textsuperscript{172–175} In contrast, in NASH, inflammation typically manifests in zone 1 or fibrous bands.\textsuperscript{175}

Recent investigations have demonstrated a zonal exhibition of the complement cascade in both normal mice and healthy human hepatocytes. Transcriptomic data indicate increased cascade activity towards zone 1, while certain components such as C6 show a differential expression in zone 3.\textsuperscript{176,177} It is plausible that this pattern may undergo changes and contribute to pathological conditions. In NAFLD, given the emerging roles of the complement system as demonstrated, alternations in the complement zonal pattern are therefore suggested. Moving forward, a deeper understanding of the complement biology will also shed the light on the relationship between the complement zonal heterogeneity and the complement compartmentalization in hepatocytes in response to NAFLD. Enhancing our comprehension of the intricate interplay between these spatial factors and NAFLD trajectory is of paramount importance in developing precise and tailored therapeutic interventions.

**5 | TARGETING THE COMPLEMENT SYSTEM IN NAFLD MANAGEMENT**

In recent years, the field of complement drug discovery has witnessed remarkable advancements in both the discovery and clinical/translational levels. Currently, a number of therapeutic agents targeting different components and pathways of the complement cascade have been developing.\textsuperscript{178,179} A handful of those, mainly targeting C3 (Pegcetacoplan) or C5/C5aR1 (Eculizumab, Ravulizumab, Avacopan), have been approved for clinical use. Furthermore, recent development of a new generation of complement therapeutics has paved the way for optimal target selection, enabling targeted interventions tailored to specific diseases. In the context of NAFLD, there is compelling evidence of complement’s significant involvement in both animal models and NAFLD patients, making it a promising therapeutic approach. Encouragingly, preclinical studies using antagonists against the receptors of the complement key effectors (C3aR and C5aR1) or complete deletion of these receptors have protective effects against steatosis, fibrosing NASH, inflammation and metabolic dysfunction.\textsuperscript{92,124,180} Therefore, while currently clinical trials evaluating the effectiveness of complement-targeted therapeutics are currently lacking, it presents an opportunity for complement modulation in NAFLD management.

Considering the rising significance of the complosome, the investigation of intracellular complement modulation represents a new and promising avenue for advancing complement-based therapies with enhanced efficacy in NAFLD therapeutic treatment. Accumulating findings have also implicated the applications of intracellular complement targeting in NAFLD. Indeed, a cell-permeable FB inhibitor has been proved to successfully intervene intracellular C3 activity in vitro.\textsuperscript{181} Given the potential role of cell-intrinsic C3 in regulating hepatocytes functions, particularly lipid metabolism and autophagy, manipulating hepatocytes complosome C3 may be a prospective approach. Additionally, the use of cell-penetrating complement inhibitors has demonstrated a modulating effect on normalising IFNγ production in CD4+ T Th1 cells and reducing IL-1β production in human monocytes—two crucial events related to NAFLD.\textsuperscript{126,128} These findings suggest the complosome’s therapeutic relevance in hepatic inflammatory processes in NAFLD.

Furthermore, another point of consideration is to target the circulating complement system in combination with the complosome intervention for the treatment of NAFLD. By doing that, a more holistic approach can be met, where both canonical and non-canonical pathways of the complement system are appropriately controlled. However, how the extracellular complement and the complosome interact in physiological and pathological conditions remains unclear. Therefore, further exploratory efforts are warranted, thereby applying in corresponding intervention. Moreover, it is also worth noting that the interconnected contribution of extracellular and intracellular complement in the pathogenesis of NAFLD may vary between patients. Thus, it necessitates comprehensive patient stratification and robust monitoring during anti-complement treatment.

The safety concerns associated with complement therapeutics have sparked debates regarding the need to balance the functional consequences of complement in terms of pathogen clearance and tissue homeostasis. However, the clinical record of currently approved anti-complement agents, such as eculizumab, has alleviated the concerns. Nonetheless, prophylactic vaccination can effectively protect patients undergoing chronic treatment. Of note, primary C3 deficiencies increase susceptibility to infections during childhood.
but are less of a concern in adulthood due to a matured adaptive immune system. Collectively, these have mitigated the original risk, allowing exploration of complement inhibitors for both acute phase and chronic applications.

6 | CONCLUSION AND FUTURE DIRECTIONS

The complement system is present and functions not only in the extracellular space but also inside immune cells and non-immune cells such as hepatocytes. The localization of the complement components and their activity exhibit their diverse functions. In NAFLD, existing evidence suggests that the intravascular complement system and its intracellular counterpart, the complosome, are intricately involved in the disease pathogenesis. Accordingly, the circulating complement system has been indicated to contribute to NAFLD developmental process by the complement activation through all three pathways (classical, lectin, and alternative pathways). Furthermore, the extracellular complement system also exerts its roles in NAFLD via interaction with other immune cells, including macrophages, Kupffer cells, and neutrophils. The emergence of the complosome has laid the groundwork for further exploring the novel impacts of the complement system in the context of NAFLD. There, intracellular complement components can directly modulate hepatocytes as well as work in tandem with other non-parenchymal cells. Furthermore, alterations in the interplay between the complement compartmentalization and hepatic zonation in response to NAFLD are also raised. These complement’s emerging roles in NAFLD draw attention to potential implications for targeting complement, extracellularly and intracellularly, in the disease management. To that end, the need for further studies is raised to elucidate the causality between the complement system and NAFLD. There, the use of modern techniques such as profiling complement transcriptomics and proteomics at single-cell resolution can provide insights into the source of complement, susceptibility of different liver cell types. Also, how the complement mechanistically exerts its impacts at molecular levels during NAFLD development and progression can be revealed. Moreover, with growing evidence obtained towards the complosome’s roles, more efforts are required to untangle its contribution in the context of liver physiology in general and NAFLD in particular, which currently remains poorly understood.

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