

## Review Article

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# The progress of autoimmune hepatitis research and future challenges

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**Abstract:** Autoimmune hepatitis (AIH) is a chronic liver inflammatory disease with various immune system manifestations, showing a global trend of increased prevalence. AIH is diagnosed through histological abnormalities, clinical manifestations, and biochemical indicators. The biochemical markers involve interfacial hepatitis, transaminase abnormalities, positive autoantibodies, etc. Although AIH pathogenesis is unclear, gene mutations and immunological factors could be the leading factors. AIH usually presents as a chronic liver disease and sometimes as acute hepatitis, making it challenging to distinguish it from drug-related hepatitis due to similar clinical symptoms. Normalizing transaminases and serum IgG levels is essential in assessing the remission status of AIH treatment. Glucocorticoids and azathioprine are the first-line AIH treatment, with lifelong maintenance therapy in some patients. The quality of life and survival can be improved after appropriate treatment. However, certain limitations jeopardize the quality of treatment, including long treatment cycles, side effects, poor patient compliance, and inability to inhibit liver fibrosis and cirrhosis. Accurate AIH animal models will help us understand the pathophysiology of the disease while providing fresh perspectives for avoiding and treating AIH. This review will help us understand AIH better, from the cellular and molecular causes to the clinical features, and will provide insight into new therapy techniques with fewer side effects.

**Keywords:** autoimmune hepatitis, pathogenic mechanism, diagnosis, treatment, animal models

## 1 Introduction

The diagnosis and treatment of non-viral hepatitis pose a significant concern in the context of chronic liver disease [1,2]. This review aims to provide a comprehensive overview of the fundamental and clinical aspects of autoimmune hepatitis (AIH), considering its insidious start, various clinical presentations, and intricate diagnostic process. AIH is a chronic inflammatory liver disease caused by hepatocytes being attacked by immune cells [3]. Circulating autoantibodies, increased hyperimmunoglobulin G and transaminases, and histological interface hepatitis characterize AIH. AIH can progress to hepatic sclerosis, liver failure, or even death without proper treatment [4]. Without efficient diagnostic biomarkers, AIH is categorized as type 1 (positive for anti-nuclear ANA, anti-smooth muscle ASM, anti-soluble liver antigen SLA, antibodies, etc.) and type 2 (anti-liver-kidney microsome antibody type 1, anti-liver cytosol type 1, anti-liver kidney microsomal type 3, etc.), based on autoantibody expression and other characteristics [5]. AIH patients are globally distributed and are predominantly female. AIH can manifest at any age or ethnicity, with most patients generally older than 40 years [6]. The prevalence of AIH is approximately 24.5 per 100,000 in the Asia-Pacific region and is significantly higher in stable populations [7,8]. AIH prevalence and clinical presentation could be associated with genetic predisposition, environmental factors, medications, and ethnicity, leading to hepatitis, decompensated cirrhosis, and liver cancer [9]. AIH severely threatens public health, seriously impacting economic and societal development.

## 2 Pathogenic mechanism

AIH causes autoimmune intolerance, resulting in autoimmune cells attacking hepatocytes [1]. However, AIH pathogenesis, which could involve genetic predisposition, environmental factors, and immune system dysregulation, remains incompletely elucidated [10,11].

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## 2.1 Genetic factors

AIH is a polygenic disease of unknown etiology, and genetic factors can influence the disease progression and clinical phenotype of AIH [12]. AIH patients are associated with extrahepatic autoimmune disease, and about 40% have an autoimmune disease history in the family [13]. Genome-wide association studies have revealed that the human leukocyte antigen (HLA) locus and some non-HLA loci are primarily associated with the AIH region [12]. AIH genetic susceptibility is related to HLA region genes on the short arm of chromosome 6, particularly with DRB1 allelic variants [14]. The diagnostic effectiveness of HLA susceptibility alleles in identifying individuals with AIH is widely recognized, leading to their inclusion in the AIH diagnostic criteria [15]. MHC class II HLA DRB1 alleles DRB1\*0301 and DRB1\*0401 encode amino acid sequences that render humans in Europe and North America more susceptible to AIH-1 [16,17]. DRB1\*15:01 can reduce the risk of AIH1 in the population [18]. Besides, DRB1\*03, DRB1\*07, and DQB1\*02:01 will make the population more susceptible to AIH-2 [19,20]. Other genes also affect AIH development since some patients with the disease do not carry HLA alleles. In addition to non-HLA genes having immunological significance, the cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) gene + 49A/G genotype was significantly correlated with AIH susceptibility [21]. The CTLA-4 gene, located on chromosome 2q33, includes a single base exchange polymorphism in exon 1, in which there is a guanine replaced by adenine at position 49, resulting in the expressed protein There is a threonine instead of alanine. A recent investigation obtained the genotypes of 155 persons diagnosed with AIH who belong to the Nordic Caucasian population and 102 healthy individuals from the same racial background. The expression of each genotype was AA = 50/155 patients vs 51/102 controls; AG = 84/155 patients vs 38/102 controls; GG = 21/155 patients vs 13/102 controls,  $\chi^2 = 8.94$ ,  $P = 0.011$ . In addition, there is a synergistic effect between CTLA-4 + 49A/G and DRB1\*03:01 on AIH-1 susceptibility [22]. A recent study showed that the GG genotype of CTLA-4 CT 60 accounted for 36.2% of AIH patients, significantly higher than that of the control group (10.0%). The strength of the correlation between the two variables, as measured by Cramer's  $V$ , was determined to be 0.341, indicating a moderate level of correlation. The bias-corrected Cramer's  $V$  demonstrated a moderate correlation, exhibiting a correlation coefficient of 0.322 [23]. The CTLA-4 GG genotype significantly increases the risk of AIH-1 when patients have HLA DRB1\*0301 in their genotype [22]. In Tunisian patients, the CTLA-4 + 49 position GG genotype was also positively associated with AIH susceptibility [24]. In addition, tumor necrosis factor- $\alpha$ -inducible protein 3 (TNFAIP3) is a genetically encoded

ubiquitininating enzyme whose rs10499194 T allele and CT genotype are associated with an elevated risk of developing AIH. Thus, the rs10499194 polymorphism is an AIH locus for candidate susceptibility [25,26]. Patients in the Japanese population who had detrimental mutations in TNFAIP3 had an increased risk of cirrhosis-related AIH [27]. The receptor for Fas was found to be significantly elevated in lymphocytes from AIH patients [28]. Furthermore, the surface expression of Fas in CD4+ and CD8+ T lymphocyte subsets was significantly higher in AIH patients compared with normal subjects. Expanded Fas + T cells also reflect the presence of persistent antigen-specific or non-specific activation in the body and abnormalities in the peripheral loss of activated lymphocytes [29]. A Japanese study observed that Fas polymorphisms and AIH development were strongly correlated with genetic factors. And the Fas-670A allele carriers showed an elevated risk of developing AIH [30]. Inflammatory cytokines are also involved in AIH development [31]. For instance, the IL-4-590 C/T polymorphism and the IL-4-33 TT genotype increase AIH susceptibility [32,33]. *In vivo* intervention with recombinant human interleukin-1 receptor antagonist (rhIL-1Ra) can reduce the secretion of TNF-alpha and interleukin-17 (IL-17) and the infiltration of inflammatory cells in the liver thereby inhibiting ConA-induced hepatitis [34]. CCN1 promotes inflammation by upregulating IL-6 production in ConA mice through the  $\alpha 6\beta 1/PI3K/Akt/NF-\kappa B$  pathway [35]. Recently, miRNAs have also demonstrated a crucial impact on AIH development [36–38]. A study comparing serum miRNAs showed significant differences between healthy individuals and AIH patients [39]. Compared with controls, serum levels of miR-29a were decreased, miR-378, let-7b, miR-122 and miR-192 were increased, and miR-574-3p, miR-193a-5p and miR-148a were unchanged. This indicates that the combination can be utilized to differentiate between people with AIH. Furthermore, a substantial correlation was found between miR-557 and a higher chance of recurrence. Additionally, it might also distinguish AIH patients from hepatitis C (HCV) patients, primary biliary hepatitis patients with cholangitis, and non-alcoholic steatohepatitis patients [40]. The expression level of miR-155 was significantly elevated in liver specimens of AIH patients but was reduced in peripheral blood mononuclear cells. In the CoA model, the level of miR-223 in Kupffer cells of mice with early liver damage was significantly increased, and transfection of miR-223 mimics could inhibit the activation of Kupffer cells after Con A stimulation [41].

## 2.2 Mechanisms of organismal immune imbalance

The liver is constantly exposed to various antigens due to its location and function, indicating the tolerance or

responsiveness of the hepatic immune system [42]. Cytokines of immunosuppressive cells provide tolerance in the liver, migrating with immune mediators through the hepatic sinusoids to the liver parenchyma [43,44]. Under normal circumstances, the liver's tolerance can result from the joint action of immune cells, parenchymal cells, epithelial and endothelial cells, and the microenvironment. The concentration of the immunosuppressive cytokine IL-10 is normally high in the liver. Besides, most liver antigen-presenting cells, including DC cells and liver sinusoidal endothelial cells, have low levels of MHC class II and co-stimulatory molecules CD80/CD86, thereby inducing an immune tolerance phenotype [15]. In addition, the proliferation of *Foxp3*<sup>+</sup> Tregs in the liver mediated by the Notch signaling pathway can also release IL-10 and enhance the immunosuppressive environment [45]. Moreover, CD8<sup>+</sup> T cells generated in lymph nodes have a strong intrahepatic antigen-specific immune response, while CD8<sup>+</sup> T cells activated in the tolerogenic environment of the liver are more defective in inducing cytotoxic immune responses and have a short half-life [46]. During autoimmune diseases such as AIH, liver tolerance often collapses. Recent studies have revealed that the administration of anti-CD20 antibodies has the potential to considerably diminish the expression of MHC class II and CD80 molecules on B cells. Consequently, this intervention can decrease the capacity of B cells to deliver self-antigens to T cells, resulting in a reduction in T cell activation and proliferation. Additionally, this intervention alleviates inflammation in the liver and mitigates the breakdown of hepatocytes [47]. Chronic hepatitis is mainly caused by the interaction of multiple immune cells, such as lymphocytes, macrophages, natural killer T cells (NKT), etc. [48]. These cells secrete TNF $\alpha$ , interferon-gamma (IFN- $\gamma$ ), and other pro-inflammatory cytokines [49]. The liver inflammatory process could be initiated by presenting autoantigenic peptides to helper T-cell receptors, leading to Th1, Th2, and Th17 cell recruitment into the tissue [50]. These effector cells begin an immune response cascade depending on the release of cytokines. The regulatory T cells (Tregs) recruitment process involves lymphocytes interacting with surface molecules present in endothelial cells [51]. In the liver, Treg secretes the immunosuppressive cytokines IL-10 and transforming growth factor beta to inhibit the effector cell proliferation and action [52].  $\gamma\delta$  T cells are innate lymphocytes recognizing non-peptide antigens and stress-induced ligands into two distinct subpopulations, including IFN- $\gamma$  or IL-17-induced  $\gamma\delta$  T cell production [53]. Moreover, IL-17A, synthesized by  $\gamma\delta$  T cells, controlled hepatocyte injury in  $\text{J}\alpha 18$  knockout mice, an AIH model [54,55].  $\gamma\delta$  T cells can also secrete IL-17 and cause damage to the liver [56]. Intraepithelial  $\gamma\delta$  lymphocytes are directly responsible for the cytolysis of effector cells and antigen-presenting cells through the granzyme-perforin,

Fas-Fas ligand, and lymphotoxin pathways. They are a key population in the regulation of immune responses in tissues [57]. Both relative and absolute counts of  $\gamma\delta$  T cells were elevated in the peripheral blood of patients with AIH, which is characteristic of patients with AIH. Selective enrichment of the V81 subpopulation of  $\gamma\delta$  T cells has also been found in Takayasu arteritis and systemic sclerosis, indicating that  $\gamma\delta$  T cells are involved in the pathogenesis of autoimmunity [58–60]. In addition, in another study, it was confirmed that the number of  $\gamma\delta$  T cells increased significantly during the active phase of AIH, and the production of its effector molecule granzyme B also increased significantly, which was consistent with the levels of liver damage markers alanine aminotransferase (ALT) and bilirubin. Consistent with the increase, the proportion of the V81 subpopulation and the ability of this cell population to produce IFN- $\gamma$  increased [61]. This process can promote B cells to stimulate plasma cells to release autoantibodies, causing damage to liver cells. In other autoimmune diseases, imbalances in the physiological levels of  $\gamma\delta$  T cells are also associated with inflammatory processes [59,60]. A recent study found that AIH models could also be generated by transferring  $\gamma\delta$  T cells from TOX-deficient mice into new mice [62]. It is well known that invariant natural killer T (iNKT) cells (also called type I NKT cells) express and characterize a semi-invariant T cell receptor (TCR). The TCR region of all iNKT cells contains the canonical  $\text{V}\alpha 14\text{--J}\alpha 18$  TCR $\alpha$  chain. Thus,  $\text{J}\alpha 18$  knockout can hinder the synthesis of TCR and lead to the lack of NKT cells. In addition, the TCR of human and mouse NKT cells contains  $\text{V}\alpha 14\text{--J}\alpha 18$  chain and  $\text{V}\alpha 24\text{--J}\alpha 18$  chain, respectively. Therefore,  $\text{J}\alpha 18$  knockout mice have extremely important reference significance for the study of human  $\text{J}\alpha 18$  [63]. The current consensus among immune cells in AIH is that this autoimmune disease is caused by the signal presentation of self-antigens to uncommitted T helper (Th0) through HLA II molecules on antigen-presenting cells. At this time, Th0 cells are activated and differentiate into Th1, Th2, and Th17 cells according to different cytokines. When the cytokine environment is rich in IL-12, Th0 cells differentiate into Th1 cells. When the cytokine environment is rich in IL-4, Th0 cells differentiate into Th2 cells, while in the presence of IL-1 $\beta$ , IL-6, and TGF- $\beta$ , Th0 cells differentiate into Th17 cells [64]. When differentiated into Th1 cells, these cells can secrete IL-2 and IFN- $\gamma$ . The former cytokine can activate macrophages to secrete IL-1 and TNF- $\alpha$ , thereby damaging liver cells. The latter stimulates cytotoxic lymphocytes and enhances the expression of HLA class I molecules on APCs and HLA class II molecules on hepatocytes [65]. When differentiated into Th2 cells, IL-4, IL-10, and IL-13 can be produced, which can induce B cells to mature into plasma cells and then produce autoantibodies [20]. Finally, when the environment is rich in IL-6 and TGF- $\beta$ , these

cytokines will inhibit the function of Treg cells and induce the differentiation of Th0 cells into Th2 cells [64]. In addition, NKT cells have been shown to be significantly reduced in AIH patients, which leads to reduced secretion of IL-4, an inhibitor of Th17 development [66]. Th17 will secrete IL-17, IL-22, and TNF $\alpha$ , causing damage to liver cells and inducing hepatocytes to secrete IL-6. The secreted IL-6 will further stimulate Th17 cells to produce corresponding cytokines [67]. During this process,  $\gamma\delta$  T cells can also secrete IL-17 to play a similar role [56]. Humoral immunity is also associated with liver injury in AIH [68]. As we know, anti-nuclear autoantibodies (ANA) and/or anti-smooth muscle autoantibodies (SMA) are positive in AIH-1, while anti-liver-kidney microsomal and/or anti-hepatic cytoplasmic antibodies are present in AIH-2. The autoantigens targeted by AIH-2 autoantibodies are anti-liver and kidney microsomal antibody type 1 (LKM1) cytochrome P450 2D6, CYP2D6, and anti-lc1 formyl aminotransferase-ring deaminase. Stimulation of CYP2D6 favors Th1 responses as its peptide aa 305–324 can induce high levels of interferon production [20]. In models of AIH caused by CYP2D6 plasmids, there was a persistent elevation in transaminases, chronic inflammation, and liver fibrosis. These changes were accompanied by increased Th1 responses and decreased liver-infiltrating Treg cells [69]. In addition, CYP2D6 can be inhibited by LKM1-positive serum *in vitro*, leading to hepatocyte damage, which was mentioned in the manuscript. LKM1 could be a diagnostic marker for AIH type 2. Cytochrome P4502D6 (CYP2D6) protein can be found on the surface of human liver cells. This cytochrome can be inhibited by LKM1-positive serum *in vitro*, leading to hepatocyte damage [5].

### 2.3 Non-immunomodulatory factors

Autoimmune diseases exhibit heterogeneity, manifesting as the involvement of specific or several organ systems. A fundamental association exists between environmental exposures and the likelihood of experiencing adverse health outcomes, wherein the influence on the immune system surpasses genetic abnormalities [70]. Studies have shown that gender is one of the influencing factors for the occurrence of AIH. Whether it is type 1 or type 2 AIH, women's incidence rate is higher than men's [71]. 40% of type 1 and 80% of type 2 AIH cases occur before the age of 18 years [72,73]. Postmenopausal women are also in high-risk groups [74]. When the iodine content in the environment is high, the overall incidence of AIH is significantly higher than that in areas with low iodine content [75]. Viruses with hepatophilia significantly induce autoimmunity due to inflammatory responses in the local regions. The generated cellular

immune response can eradicate the pathogen. For instance, anti-LKM1 antibodies have been identified in HCV against CYP2D6 [5]. A study from Brazil assessed AIH frequency in chronic HCV patients. Epidemiologically, HCV infection is correlated with AIH and is also a triggering factor [76]. SLA/LP shares high sequence similarity with a non-homologous Rickettsiae protein, driving autoimmune responses through CD4+ T cell autoantigen recognition and concomitant humoral immunity [77]. A New Zealand-based study assessed the impact of environmental factors on the risk associated with AIH. Moreover, the univariate analysis of the study revealed that alcohol consumption reduced AIH risk [78]. Lifestyle factors, childhood factors, and family history of 72 AIH cases and 144 controls, who lived in Canterbury, New Zealand, between July 1, 2011 and June 30, 2012, were collected. Analysis performed by comparing AIH with drinking concluded that alcohol consumption was independently associated with a reduced risk of developing AIH, with OR 0.43, 95% CI 0.28–0.68,  $p < 0.01$  [78]. In another study, it can also be found that the alcohol consumption of AIH patients is lower than that of alcoholic liver disease (ALD) patients and AIH/ALD patients [79]. Upon evaluating the correlation between vitamin D levels and histological characteristics and therapy response in patients with AIH, it was shown that the AIH group exhibited notably lower levels of vitamin D compared to the control group. Furthermore, a strong correlation was observed between interfacial hepatitis and fibrosis scores and low levels of vitamin D. It was shown that patients with these characteristics exhibited inadequate responses to treatment [80]. Intestinal microbial ecology is critical to developing intestinal and systemic immune responses [81]. The gut microbiota could be a rich antigen reservoir, initiating and maintaining the autoimmune reaction to AIH. Due to its unique anatomical location, the liver is closely associated with the gut microbiota, playing a significant role in the development of AIH. Recent research has indicated an observed modification in the gut microbiome of individuals with AIH and primary biliary cholangitis. This finding suggests a potential association between the alteration of gut microbiomes and the development of AIH [82]. Liu *et al.* found that treating AIH model mice with *Bifidobacterium* and *Lactobacillus* can significantly increase Treg cell infiltration, inhibit the production of Th1 and Th17 cells and inflammatory factors, and inhibit the activation of Toll-like receptor 4 (TLR4)/NF- $\kappa$ B pathway to alleviate the progression of AIH [83]. The role of gut microbiota in humans has also been studied accordingly. Liwinski *et al.* found that compared with control group, biodiversity in the gut microbiota of AIH patients was reduced. Consistent with the results obtained in the AIH mouse model, the abundance of *Bifidobacterium* flora was

also significantly reduced in AIH patients and affected the AIH progression [84]. The abundance of *Bifidobacteria*, *Lactobacillus*, *Bacteroides*, and *C. leptum* in the gut microbiota of AIH patients was also significantly reduced, while the abundance of *Escherichia coli* was increased [84]. After FMT treatment in the Abx experimental autoimmune hepatitis (EAH) mouse model, the liver injury and bacterial translocation of the mice were reversed to varying degrees [85]. Thus, molecular mimicry can describe the process behind inducing autoimmunity through microbes [86]. Compared to normal structures in healthy controls, one study indicated altered intestinal tight junctions and inflammatory infiltrates within the lamina propria of AIH cases [87].

The drug-induced acute liver failure manifestations could be similar to AIH. Drugs could induce AIH through genetic susceptibility and molecular mimetic mechanisms, depicting the etiology of drug-induced liver injury (DILI) associated with autoimmune features [88]. Minocycline and furantoin are associated with AIH among the studied drugs and compounds [89]. The drug-induced AIH mechanisms include drug metabolite production that binds to receptors and acts as antigenic complexes. These stimulate autoantibody production, primarily CYP1A2 and CYP2A6, and lymphocyte sensitization [90]. The tissue and interaction feature of AIH characterize DILI with autoimmune features. In contrast to medication-induced AIH, autoimmune DILI manifests symptoms of liver damage and jaundice during a brief incubation time. A liver biopsy reveals inflammation within an infiltrate primarily composed of lymphocytes and eosinophils. Following withdrawal, there is a gradual recovery with a danger of relapse from re-administration [91].

### 3 Histopathology

AIH pathology is diverse and depends on inflammatory responses of hepatic parenchymal cells due to an autoimmune imbalance [92]. The characteristic clinical manifestations include portal and periportal lymphoplasmacytic infiltration and interfacial hepatitis. Thus, single or clusters of plasma cells could be observed in the lobules [93]. However, nearly 1/3 of AIH patients have scarce or absent plasma cells [94]. AIH commonly involves ballooning degeneration, punctate hepatocyte necrosis, and apoptotic vesicles. Moreover, the inflammation and necrosis severity varies from mild and active hepatitis to bridging even extensive necrosis [95]. The regenerating hepatocytes had a rosette-like structure (2–3 aqueous degenerated hepatocytes developing a pseudo glandular system possessing dilated capillaries in the center, depicting a rosette) [96]. The reticular fibrous

scaffold collapses in the necrotic area due to further progression. Thus, hepatic stellate cell activation and pseudobullet formation lead to hepatic fibrosis [97]. Around 65% of patients suffer penetration (lymphocyte entry into hepatocytes), leading to apoptosis, and its appearance correlates with inflammation degree and fibrosis in the liver [98]. In some AIH cases, central lobular injury with significant hepatocyte necrosis and mononuclear inflammation could be related to periportal activity and interfacial hepatitis [99]. Thus, chronic hepatitis injury could represent an early disease stage because the central lobular necrosis injury pattern can become a more typical portal-based form [100]. Nevertheless, this pattern of necroinflammatory injury to the central lobules is not specific to AIH; instead, it is more frequently encountered in hepatitis caused by viruses or drugs [101]. Bile duct disruption is not observed in AIH. However, up to 12% of biopsies indicate bile duct disruption; another 12% demonstrate lymphocytes infiltrating the bile duct epithelium [102].

### 4 Clinical manifestations and diagnostic criteria

AIH has an insidious onset and a variety of clinical manifestations, mostly with chronic non-specific symptoms, such as malaise, fatigue, general malaise, etc. Signs such as hepatosplenomegaly and ascites may be present [103]. Patients with AIH without obvious clinical symptoms account for 25%. About 1/3 of patients have progressed to the stage of liver fibrosis or cirrhosis by diagnosis [104]. The presentation of acute AIH may be similar to the symptoms of acute hepatitis due to other causes. It may present with elevated ALT, aspartate aminotransferase (AST), and jaundice [105]. Patients with AIH may also present with symptoms of intrahepatic cholestasis, and such patients may present with a failure to respond to glucocorticoid therapy. About 10% of patients are negative for autoantibodies [106]. These patients often show mildly elevated or normal IgG levels and rely on histological features for diagnosis. AIH is often combined with other autoimmune diseases, such as inflammatory bowel disease and Hashimoto's thyroiditis. Although we can diagnose AIH through the above diagnostic criteria, we still need to pay attention to the differential diagnosis of some diseases during implementation [107]. First, we must rule out acute and chronic viral hepatitis caused by viruses (such as HAV, HBV, EBV, or CMV). The syndromes of primary biliary cirrhosis and primary sclerosing cholangitis partially overlap with AIH. These can all be distinguished by testing blood samples for the corresponding antibodies [108]. Second, Wilson's disease is a differential disease that cannot be

bypassed. It is biochemically, serologically, and histologically similar to AIH, but it can be initially identified by measuring liver copper concentration and 24 h urinary copper excretion [109,110]. Leishmaniasis and other hepatic infectious diseases may also need to be excluded based on local factors or travel history [111]. Furthermore, a novel approach that has surfaced recently involves using the exchangeable copper assay to determine the presence of copper overload and potentially differentiate individuals with Wilson's disease [112]. KF ring is a classic ophthalmic manifestation of Wilson's disease and can be examined by slit lamp. The clinical manifestations of DILI are similar to AIH and it is quite difficult to identify these diseases. There are currently several methods available for differential diagnosis. First, it is necessary to exclude medication history. Additionally, the utilization of the RUCAM scale and M&V scale is employed to ascertain the causal association between pharmaceutical substances and hepatic injury. Ultimately, the acquisition of histological specimens via liver biopsy serves as a means to achieve a pathological diagnosis [113,114]. In addition, non-alcoholic fatty liver disease is also a disease that needs to be differentially diagnosed with AIH. The two are mainly distinguished through the NASH and liver tissue biopsy diagnostic criteria [115].

AIH diagnosis is based on clinical, biochemical, serological, and histological features [116]. The diagnostic criteria for AIH were established and revised by the international autoimmune hepatitis group (IAIHG), and the system grades the clinical, laboratory, and histological features of AIH [117]. Initially, it was designed to standardize AIH diagnosis, but later, it was applied in a clinical setting. The IAIHG scoring system involves the difference between a definite and probable AIH diagnosis, primarily associated with the increased serum gamma globulin or IgG, ANA, ASMA, or anti-LKM-1 levels, and exposure to alcohol, drugs, or infections causing liver damage [118]. Treatment response improves AIH diagnosis and is a part of the scoring system. As is shown in Table 1, there are differences in the scoring categories before and after treatment. Before treatment, clinical characteristics, laboratory tests, and liver histopathology need to be combined to score. If these scores are higher than 16 points, the diagnosis can be confirmed, and 10–15 points indicate the possibility of AIH. If the score is less than 10 points, the diagnosis of AIH can be ruled out. After treatment, the patient's response to treatment must also be considered for scoring in addition to the above items. If the score is higher than 18 points after treatment, the diagnosis is definite, and 12–17 points is a possible diagnosis [119].

Although the diagnostic criteria of AIH are relatively clear, its diagnosis is still challenging. To some extent, AIH

is still a disease of exclusion, which needs to be differentiated from many similar diseases. In the case of acute hepatitis after infection with acute autochthonous hepatitis E virus, it behaves similar to AIH [120]. In addition, Wilson's disease, Leishmaniasis, and other infectious diseases of the liver also need to be excluded. Due to differences in the preparation process, the diagnostic antibodies for AIH cannot be consistent in terms of false positives and false negatives. The expression of AIH-specific antigens has been observed in animals' kidneys, livers, and stomachs. This poses significant challenges in establishing a unified standard for immunofluorescence detection [121]. Although anti-SLA antibody has high specificity in patients, it is still powerless in the face of the AIH subgroup and SLA positive but negative for other antibodies [122]. Moreover, corresponding antibodies only emerge during further disease progression and may be absent in acute AIH. Therefore, retesting for antibodies is encouraged. On the other hand, autoantibodies (ANA and SMA) are frequently detected in almost any type of acute severe hepatitis, including DILI [123]. There remain several pressing diagnostic issues that require immediate resolution. A more precise diagnosis can also offer valuable insights for clinical treatment, enabling patients to achieve enhanced therapeutic outcomes.

## 5 Treatment

Successful treatment can reduce AIH inflammation and prevent progressive fibrosis while minimizing the therapeutic side effects [124]. The current standard treatment for AIH is corticosteroids with or without azathioprine (AZA) [125,126]. Most patients respond well to this standard treatment. Only a minority of patients need alternative treatment modalities because they cannot tolerate the regimen or withdraw therapy due to side effects. Although corticosteroids are very effective in inducing remission, the usage must be tapered gradually and eventually discontinued after about 12 months due to side effects [127]. Budesonide has the potential to serve both as a primary and secondary therapeutic agent in the induction of mild early AIH. The efficacy and safety of MMF and tacrolimus as second-line agents have been extensively investigated. However, the available data consist of minimal retrospective trials with varying characteristics [128].

Since the 1950s and 1960s, prednisone combined with AZA has been the standard of care [129]. Most patients respond well to standard therapy; others are intolerant or non-responders and require alternative regimens. Around 10–15% of patients discontinued standard immunosuppressive treatment

**Table 1:** IAIHG revised diagnostic scoring system

Parameters/clinical characteristics	Scoring	Parameters/clinical characteristics	Scoring
Female	+2	Histological examination of the liver	
Ratio of ALP to AST (or ALT)	+2	Interface hepatitis	+3
<1.5	0	Mainly lymphoplasmacytic infiltration	+1
1.5–3.0	-2		
>3.0		Hepatocytes show rosette-like changes	+1
ANA, ASMA, or anti-LKM-1 antibody titers	+3	No such manifestations	-5
>1:80	+2		
1:80	+1	Bile duct changes	-3
1:40	0	Other changes	-3
<1:40	-4	Other immune diseases	+2
AMA positive		Other available parameters	
		Positive for other specific autoantibodies (anti-SLA/LP antibody, anti-LC-1 antibody, ASGPR, pANCA)	+2
Hepatitis virus markers	-3		
Positive	+3		
Negative			
Drug history	-4	HLA-DR3 or DR4	+1
Positive	+1	Response to treatment	
Negative		Complete	+2
Average ethanol intake (g/day)	+2	Relapse	+3
<25	-2		
>60			
Explanation of total points			
Before treatment		After treatment	
Definite AIH	≥16	Definite AIH	≥18
Possible AIH	10–15	Probable AIH	12–17

ALP, alkaline phosphatase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; IgG, immunoglobulin G; ANA, antinuclear antibody; SMA, anti-smooth muscle antibody; anti-LKM-1 antibody, anti-liver and kidney microsomal antibody type I; AMA, anti-mitochondrial antibody; anti-LC-1 antibody, anti-hepatocyte cytoplasmic antibody type 1; ASGPR, anti-desialytic acid Glycoprotein receptor antibody; pANCA, perinuclear type anti-neutrophil cytoplasmic antibody; HLA, human leukocyte antigen.

due to intolerable side effects, which became 18% in jaundiced AIH patients [130]. The relapse rate after discontinuing standard immunosuppressive therapy was >50%. Prednisone can effectively induce remission and, under ideal circumstances, should be tapered and used after 12 months. However, a UK analysis indicated that 55% of patients still require hormone maintenance therapy [131]. Current maintenance therapy with long-term low-dose steroid therapy maintains the immunosuppressive effect, minimizing the probability of relapse [132]. The long-term low-dose steroid medication administration in maintenance therapy effectively sustains the immunosuppressive impact, reducing the likelihood of recurrence. Meanwhile, the extended use of hormones harms patients' overall well-being with AIH, regardless of the specific hormone used, dosage, or the patient's biochemical remission status [133]. Prednisone and AZA have significant side effects during treatment. Corticosteroids can significantly reduce the patient's activity ability and increase anxiety, depression, and fatigue [134]. Prednisone can also induce hepatic insulin resistance in AIH patients and increase the risk of diabetes, with about 6–20%

of new diabetic patients. In addition, patients may also face side effects such as hypertension, moon face, acne, cataract, psychosis, and low trauma fracture [131]. In addition, previous studies have shown that when corticosteroids are completely discontinued from medication in AIH patients, the average body weight per patient can be reduced by 6 kg [135]. Besides, AZA can induce myelosuppression, hepatotoxicity, and pancreatitis, which are at higher risk in patients with cirrhosis [136]. Withdrawal can be caused by a variety of adverse events. Approximately 13% of patients withdrew prematurely due to treatment-related side effects. These adverse effects included intolerable body shape changes or obesity (47%), osteoporosis with spinal compression (27%), brittle diabetes (20%), and peptic ulcer (6%) [137]. Although AZA can be used with corticosteroids, AZA must be discontinued if cholestatic hepatitis, pancreatitis, progressive leukopenia, or gastrointestinal discomfort occurs during the medication [137].

Budesonide has a 90% first-pass hepatic clearance, showing a more anti-infective effect than prednisone, with minimal adverse effects [138]. A prospective, double-blind,

randomized trial observed that budesonide combined with AZA was superior to prednisone and AZA in treating non-cirrhotic AIH patients. However, there was a significantly lower incidence of steroid-specific adverse reactions. In contrast, emerging case reports after the trial indicated that AIH reactivation occurred during budesonide monotherapy [139]. A German retrospective study observed a biochemical response in 70% of patients after switching monotherapy from prednisolone to budesonide after 12 months. Later, a biochemical response was observed in 67% of patients after 24 months [140]. During the last follow-up (mean 63 months), 25% of patients were switched to prednisolone due to a poor budesonide response or related side effects.

Mycophenolate mofetil (MMF) inhibits purine production necessary for the *ab initio* lymphocyte synthesis during proliferation, thereby reducing inflammatory cytokines and elevating Treg cells. However, MMF has low systemic bioavailability, is teratogenic, and is not advised during pregnancy [141,142]. Saitis *et al.* reported that 88% of patients achieved biochemical remission after the combined treatment of MMF with prednisone for 26 months in 59 primary AIH patients [143]. Another study treated 109 patients with a combination of prednisolone and MMF and compared them with 22 patients treated with a combination of prednisolone and AZA. Around 72% of MMF group patients showed complete remission, which had a higher remission probability than the AZA group. In various small retrospective studies, the biochemical remission rates of MMF ranged between 31 and 73%, while the discontinuation rates ranged between 13 and 34% [144]. MMF is a second-line treatment option for non-responders or those intolerant to AZA.

Cyclosporine and tacrolimus inhibit calcium-regulated neurophosphatase, resisting T-cell activation and cytokine production [145]. Children with AIH exhibit good biochemical responses with cyclosporine for 6 months. However, the efficacy and safety of cyclosporine in children have no long-term evaluation. Cyclosporine has side effects such as nephrotoxicity and neurotoxicity [146]. Tacrolimus efficacy in AIH treatment in adults has been reported differently. Combining the three studies, the biochemical response rate for patients aged 60–62 years who failed tacrolimus treatment was 92%. In contrast, only 27% achieved a complete biochemical response in a recent report on 17 adults and 6 children [147]. Another study assessed the efficacy of MMF and tacrolimus as second-line treatment. The complete remission rate (69.4%) in the MMF group was not significantly different from the tacrolimus group (72.5%). Both drugs were equally effective in patients where standard therapy failed. Tacrolimus showed a higher complete remission rate than MMF (56.5% vs 34.0%) [148]. Therefore, tacrolimus could be a second-line agent for AZA intolerance or

poor response in AIH patients, particularly those unable to use MMF [99]. Treg cells are essential in humans as loss of function leads to autoimmunity [149]. mTOR can regulate several critical cellular processes, including nutrient sensing, cell proliferation, and metabolism. mTOR controls cytokine production and T-cell differentiation in T cells. Abrogated mTORC1 and mTORC2 activities can reduce the differentiation of Th1, Th2, and Th17 and spontaneously induce Treg cells, while single abrogated mTORC1 activity will increase the differentiation of Th2 cells [150,151]. Rapamycin (mTOR) inhibitors restrict effector T cell differentiation and enhance Treg proliferation [152]. Moreover, Treg cells treated with rapamycin decreased the secretion of IL-2 and IL17, as well as the levels of glycolysis and oxidative phosphorylation [152]. In addition, mTOR signaling can regulate the stage-specific development of T cells. Lack of RAPTOR at the DN1/DN2 stage decreased T cell proliferation and increased apoptosis [153]. mTORC2 activity in T cells is also necessary to induce glycolysis and Notch-driven proliferation. Also, it has significant regulatory effects on the expression of receptors involved in thymocyte development [154,155]. Notably, AKT blocked the generation of tTreg cells in the thymus by increasing mTORC1 activity [156]. TSC1 deficiency can promote tTreg cell development by inhibiting mTORC2-dependent signaling pathways [157]. Kerkar reported six patients with failed AZA/MMF therapy after liver transplantation who were treated with sirolimus. The median biochemical response time was 4.6 months, with one patient discontinuing treatment due to infection [158]. Several therapeutic pathways are being developed, such as interventions targeting gut microbial alterations and bacterial translocation, B-cell regulation, Treg cell transplantation, and miRNAs [159]. For instance, comparing 24 AIH patients and 8 healthy volunteers showed that the patients had dysbiosis, impaired intestinal barrier function, and elevated bacterial translocation (increased plasma LPS levels) related to disease severity [160]. LPS activates TLR4 in Kupffer cells, triggering an inflammatory response. LPS also activates TLR4 in hepatic stellate cells, enhancing the secretion of chemokines and adhesion molecules by Kupffer cells with elevated chemotactic ability [161]. JKB-122 is an antagonist of TLR4 with hepatoprotective and anti-inflammatory activity. JKB-122 can reduce levels of pro-inflammatory cytokines in the serum and liver of an AIH animal. JKB-122 has a dose-dependent biochemical and histological improvement in AIH, and is currently in Phase II clinical trials [162].

## 6 Animal models of AIH

Although there are many treatment options currently available, each of these treatment options has shortcomings that

need to be overcome. Therefore, animal models are a better choice for drug development. Besides, it can provide considerable significance for the study of the mechanism of AIH and provide an early basis for further preclinical trials.

Animal AIH models are primarily divided into spontaneous (naturally occurring or knockout) and induced (biological or physicochemical factors). They provide information about hepatic immunology or cause liver damage mediated by the immune system. Due to their close connection with AIH, these models aid in evaluating novel therapeutic strategies for human diseases. The ideal experimental model should resemble the human condition closely. However, mice mimicking human autoimmune disorders have different autoantigen targets. This is due to class I and II molecule differences in MHC, with specific T-cell responses distinguishing mice from humans [163]. The animal models employed in the study of AIH are briefly described and shown in Table 2.

The first animal AIH models were developed in the early 1970s when more fundamental immune system mechanisms were discovered. During that time, AIH had a transparent immune-mediated background. Büschenfelde et al. injected liver-specific antigens LP-1 and LP-2 fractions from human liver homogenates into rabbits and various adjuvants. They observed that the large LDL LP-1 effectively induced “experimental immune hepatitis” [164]. LP-1 is a liver-specific membrane lipoprotein (LSP), identified as ASGPR. LP-1 is highly expressed on the hepatocyte surface. Anti-LSP antibodies could be observed in nearly 88% of AIH patients and can become a general marker. However, these antibodies are not for diagnostic purposes, as they are also detected in chronic hepatitis B and C, ALD, and primary sclerosing cholangitis patients [165]. Besides, Sundin et al. found that the incidence of anti-LSP antibodies detectable in the serum of nearly 55% of chronic active hepatitis patients was found by ELISA, compared with 17% in healthy controls, so it can be concluded that some healthy people also have anti-LSP antibodies [166]. The experimental rabbits synthesized antibodies against LP-1 and LP-2. However, AIH, similar to chronic hepatitis, was not triggered, demanding cellular immunity activation. In 1983, Kuriki injected homozygous liver homogenate or liver-specific lipoprotein with *Klebsiella pneumoniae* 03:K1 lipopolysaccharide as an adjuvant inside SMA mice. This led to anti hepatocyte-specific lipoprotein antibody production and monocyte infiltration in the liver [167]. The model indicated pathogenic autoimmune cells, as spleen cells were transferred to other mice after 14 days. Characteristics including liver parenchymal cell necrosis and monocyte portal infiltration were noted, which were also present in the donor mice. To create the intricate EAH model, Lohse administered homogenous liver homogenate (S100) supernatant into C57BL/6

mice, which had been emulsified with Freund’s adjuvant [168]. S100 extracts had several liver autoantigens, including CYP2D6 and SLA/LP. The EAH model induces the production of S100 protein-specific T cells and transient liver injury (perivascular infiltration and hepatocyte necrosis). Consequently, this study provides significant insights into the identification and control mechanisms of effector cells involved in immune responses specific to the liver. Its simplicity characterizes the S100 model, as it does not require transgenic mice or intricate techniques for disease induction. Nevertheless, inducing comparable chronic recurrent AIH is a significant challenge in these initial animals. Therefore, a comprehensive understanding of target antigens is necessary to track the action mechanism of hepatic autoantigen-specific T lymphocytes.

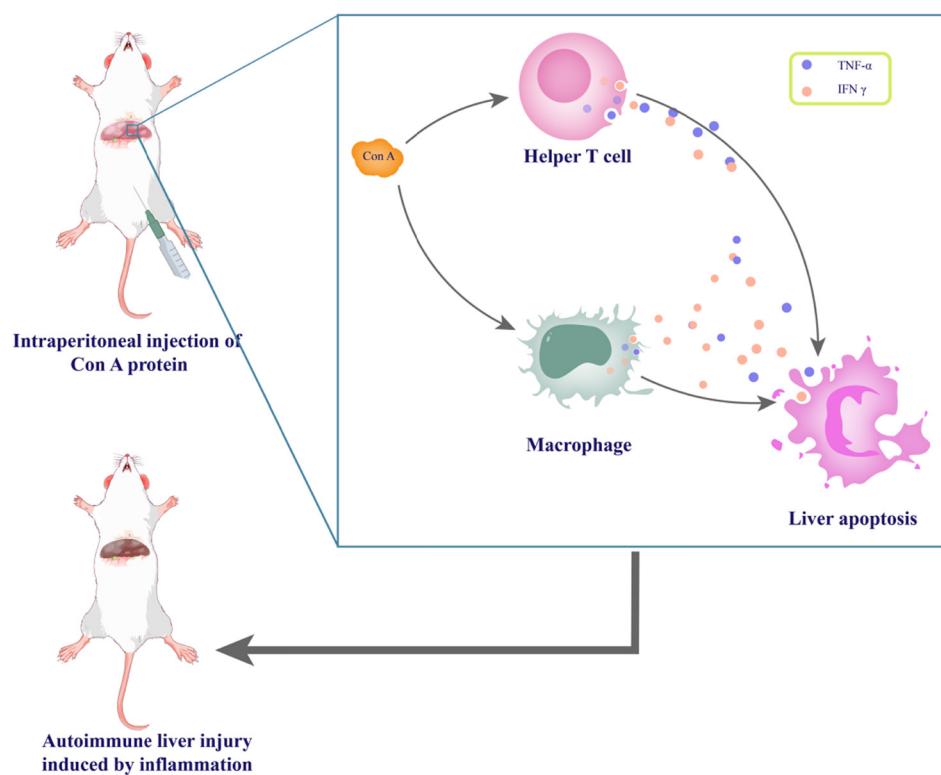
Since the early 1990s, systemic application of Ladin A (ConA) was frequently used to induce liver injury in mice. This activated non-specific T-cell antigens caused acute hepatitis. ConA, a phytohemagglutinin, is a potent mitogen causing cytokine storms. It amplifies through lymphocyte and macrophage interactions, causing inflammation, apoptosis, and liver failure [169]. Further studies indicated that the acute liver injury model in ConA-induced EAH mice primarily interacts through Th1 cytokines. Thus, NKT cells are also essential in liver injury caused by ConA [170]. The ConA hepatitis model is reliable for studying immune system-mediated liver injury. Nevertheless, this model demonstrates a more severe form of liver injury characterized by a non-dependent cytokine storm triggered by hepatic autoantigens, as opposed to long-term changes (Figure 1).

ConA is a widely used mitogen that activates T cells and depends on helper T lymphocytes (Th) and macrophages for its effects. ConA activates T lymphocytes and stimulates Th cells and macrophages to produce cytokines such as TNF- $\alpha$  and IFN $\gamma$ . TNF- $\alpha$  can directly damage hepatocytes and lead to apoptosis. After intravenous injection of ConA into mice, most of it accumulates in the liver, indicating that it is the target organ for ConA toxicity induction *in vivo*. In the inflammatory process of viral hepatitis, lymphocyte-mediated cytotoxicity is the primary immune mechanism leading to the lysis of virus-infected hepatic parenchymal cells. The adhesion between lymphocytes, antigen-presenting cells, and antigen-producing target cells is essential for the immune response process. The experimental animal model of ConA-induced liver injury in mice is pathologically characterized mainly by activation of T lymphocytes resulting in Acute immune liver injury.

The transgenic mice emergence overexpresses specific proteins or lack one or more proteins under system- or organ-specific promoters (KO mice). Many new AIH models have been developed, demonstrating new findings

**Table 2:** Mouse model of AIH

Animal model	Animal	Applications	Advantages	Disadvantages
Immunization with allogeneic or xenogeneic liver proteins	Mouse	Basic mechanisms of infiltration of AIH autoantibodies	Produced anti-LSP antibodies	Difficult to differentiate from other diseases
Immunization with syngeneic liver homogenate or liver-specific lipoproteins	SMA mouse	Basic mechanisms of infiltrations autoantibodies of AIH	Easy preparation of disease model Elucidated the relationship between genetic background and disease susceptibility	It is challenging to study the role of immune factors in AIH Lack of pathogenic T cells Induction of mild or moderate hepatitis in the liver
Immunization with syngeneic liver homogenate	C57BL/6 mouse or A/J mouse	Basic mechanisms of antibodies and T cells in AIH	Portal infiltration of mononuclear cells in the liver and necrosis of hepatic parenchymal cells Susceptible to hepatitis Production of antibodies and corresponding cytokines Presence of immunosuppressive T cells	Immune responses to AIH-unrelated antigens It is difficult to simulate the process of chronic or mild hepatitis Autoantigen complex, autoantigens with unknown components
Immunization with ConA	Mouse	Basic mechanisms of severe acute hepatitis and T cell activation	Easy preparation of disease model No transgenic animals involved	No transgenic animals involved It is challenging to simulate chronic hepatitis
Genetically modified spontaneous or induced immunization	Mouse	Role of immune response and tolerance	No complicated methods of inducing disease Exploration of antigen-independent cytokine storm-mediated liver injury Discovery of drug treatment options for liver damage	Exploration of antigen-independent cytokine storm-mediated liver injury Discovery of drug treatment options for liver damage Elucidated the link between genetic background and specific genes Identified the role of single genes in immune response/tolerance Exploration of the association between influencing factors and single genes



**Figure 1:** Schematic illustration of the main mechanisms of acute immune liver injury induced by ConA.

associated with AIH immunopathology. One model is the NTxPD-1 $^{-/-}$  mice, where the programmed cell death 1 (PD-1) gene is absent and excised from the thymus [171]. NTxPD-1 $^{-/-}$  mice do not possess adequate regulatory T cells to facilitate normal homeostasis, similar to normal PD-1-deficient mice. The thymus gland removal leads to AIH-like hepatitis development. This leads to T-cells entering the liver parenchyma, liver lobule necrosis, and ANA production. CXCL9 blockade inhibits the lethal AIH progression in NTxPD-1 $^{-/-}$  mice by reducing the CXCR3+ T cell production. Therefore, CXCL9 chemokines coordinate the movement of T-cells into the liver from the spleen. The effect of T cell priming depends on the overall antigen load. When antigen load is high, T cell priming leads to the exhaustion of T cells due to the abundant surface expression of programmed cell death protein 1 (PD-1) [172]. Loss of both Tregs and PD-1-mediated signaling leads to lethal AIH [171]. The increased expression of PD-L1 and B7-DC on Kupffer cells and hepatic sinusoidal endothelial cells can bind to PD-1 on T cells, suppressing autoreactive lymphocytes, which regulates the progression of AIH [173]. Furthermore, loss of PD-1 resulted in enhanced proliferative capacity of T cells in the viral liver [174]. PD-1 can also regulate the migration ability of T cells. Blockade of chemokine ligand 20 inhibits the migration of splenic T cells expressing chemokine receptor 6 to the liver and suppresses the development of

AIH in the absence of PD-1 [175]. Furthermore, anti-PD-1 antibodies in serum help differentiate AIH-1 from DILI [176]. Since mice mutations led to multi-organ inflammatory damage, the vital role of transforming growth factor receptor-1 (TGF- $\beta$ 1) in the homeostasis of immune cells is described. Balb/c TGF- $\beta$ 1 $^{-/-}$  mice are susceptible to spontaneous and extensive inflammation in the liver and hepatocyte necrosis, with increased levels of plasma ALT [177]. Balb/c TGF- $\beta$ 1 $^{-/-}$  mice inflammation and necrosis were attributed to CD4+ T cells and the secreted regulation of IFN $\gamma$ -mediated immunity, similar to humans. The AIH model was constructed with autoantigens recognized by antibodies or T cell targets in AIH patients to mimic the autoimmune response and the ability to activate autoantigen-specific of immune cells in human AIH. Besides, TGF- $\beta$  can promote the differentiation of Th0 cells into Treg cells in the immune microenvironment. In addition, under the joint action of TGF- $\beta$ , IL-1, and IL-6, the differentiation of Th0 cells into Th17 cells is enhanced [15]. Previous experiments have confirmed that TGF- $\beta$  is also an important mediator of AIH. Serum levels of TGF- $\beta$  were significantly elevated in mild AIH patients [178]. PD-1 and TGF- $\beta$  can synergistically promote the development of Treg cells. The joint action of PD-1 and TGF- $\beta$  can inhibit the phosphorylation of Smad3 mediated by cdk2, and enhance the deactivation of Smad3 in a TGF- $\beta$ -independent manner to promote the

differentiation of naive T cells into Treg cells [179]. CYP2D6 is the characteristic antigen identified using T cells and antibodies in AIH-2 patients. The CYP2D6 mice model uses the same antigen in humans as the trigger. This antigen is injected into the wild-type C57BL/6 mice liver by infecting an adenovirus with the encoded human gene. The triggered self-responses target mouse CYP2D6 homologs since mice lack human CYP2D6 [180]. Adenovirus-infected mice cause acute hepatitis, which causes chronic AIH resembling interfacial hepatitis. There is inflammatory cell infiltration, such as CD4+ and CD8+ T cells, B cells, macrophages, dendritic cells, and neutrophils, in interfacial hepatitis. Moreover, mice synthesize autoantibodies similar to LKM-1, primarily recognizing CYP2D6 epitopes similar to AIH-2 patients. T-cell epitope mapping identified one CD4+ and three distinct CD8+ T-cell epitopes, not corresponding to AIH patients due to MHC molecule differences [18]. However, the T cell epitope is in the crossover region between human CYP2D6 and murine CYP homologs. Thus, AIH pathogenesis could be associated with molecular mimicry. The CYP2D6 model can elucidate the persistent fibrosis mechanism related to AIH. Hepatic stellate cells are activated after the adenovirus infection. This increases the extracellular matrix component expression, such as the deposition of type I and type III collagen and alpha-smooth muscle actin [181]. Gil-Farina *et al.* injected adeno-associated viruses with IL-12 gene overexpression into mice and observed type I AIH characteristics [182]. However, none of the above models appropriately depicts the human AIH. Some models are better adapted to understanding immune activation or regulatory mechanisms, while others aid in evaluating innovative therapeutic methods.

## 7 Future therapy strategies

Most current studies have recognized the role of immune response and tolerance in AIH. Hence, it is imperative to select pharmaceutical agents that exhibit minimal adverse effects on prolonged immunosuppression in the management of AIH. Additionally, exploring potential alternative therapies in cases where the initial treatment fails to provide a satisfactory response is crucial. As previously stated, tacrolimus demonstrates notable efficacy in treating patients with AIH. It may be an alternative therapeutic option for those who exhibit intolerance or experience adverse events to AZA, particularly those who cannot tolerate MMF. However, its current side effects still need to be overcome. Liver-specific autoantigen epitopes recognized by TCRs have not been identified in AIH-1. Autoantibodies and clear autoantigen epitopes

of TCRs also enable new options for transgenic animal models and treatments in type 2 AIH. Thus, mechanistic exploration is still essential for AIH-1. Future treatment can start from several aspects based on the current research progress. One area of focus is the advancement of novel immunosuppressants that exhibit enhanced specificity for AIH, thereby effectively managing hepatic damage.

Additionally, there is a need to focus on advancing pharmaceutical interventions to reinstate immune regulatory mechanisms and promote antigen-specific tolerance. Furthermore, regulating autoreactive CD4 and CD8 T cell proliferation and elimination is crucial. In conclusion, there is a pressing need to enhance the efficacy of anti-fibrotic medications to mitigate the onset of liver fibrosis or maybe reverse the existing condition. These elements are anticipated to serve as novel therapeutic avenues.

## 8 Conclusion and outlook

Early diagnosis and treatment of AIH can facilitate good health and reduce the societal burden of chronic diseases. However, there are many unanswered questions in studying AIH mechanisms. These include racial differences, genetic heterogeneity, social environment, and other disease development factors. Therefore, large samples, multicentric and cross-ethnic studies, and developing animal models similar to human AIH can further elucidate AIH pathogenesis. Disease awareness and timely diagnosis are crucial, as untreated disease has a poor prognosis. AIH diagnosis is exclusionary based on various combinations. Diagnosis includes laboratory tests and diagnostic scores, but most are not disease-specific markers, such as autoantibodies, elevated IgG/gamma globulin levels, histological features, and exclusion of other liver diseases. Thus, supplementing diagnostic-specific markers and gaps in high-risk predictors is the requirement. There is still room for improvement in the sensitivity and specificity of current diagnostic methods. To maintain regular testing quality, it is recommended that a quality control system be implemented in the future, employing blind serum testing.

Moreover, the current scoring system for AIH fibrosis is mainly derived from viral hepatitis research. Therefore, deepening the recent AIH research is expected to establish a unified histological scoring system to quantify AIH fibrosis in the future. AIH patients face significant unmet clinical needs, and treating those who are intolerant or refractory to standard therapy is challenging. Future treatments should differentiate AIH from other autoimmune diseases, focusing precisely on AIH pathways to define more specific therapies. In parallel, more descriptions of the pathogenesis of AIH

should be documented to address treatment options further. Aberrant immune regulatory pathways, epigenetic genome, and intestinal microecology are essential therapeutic targets to alter AIH treatment based on an increasing understanding of disease pathogenesis. The treatment of AIH will be able to achieve the effect of removing pathogenic autoimmune cells while retaining autoprotective immunity. In order to attain this objective, it is imperative to thoroughly investigate the existing body of research about the underlying mechanism of AIH. By adopting this approach, we can better understand genetics, facilitating the implementation of genuinely personalized medical interventions. A comprehensive comprehension of triggers, immunopathogenic mechanisms, and effector processes can promote the advancement of improved and more diagnostic experimental approaches and the formulation of novel therapeutic strategies. Eventually, the tolerance of liver autoantigens will be restored, thereby changing our current treatment plan, providing patients with a better quality of life, and prolonging the prognosis of patients.

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