Review Article

Autoimmune Hepatitis: Clinical Review with Insights into the Purinergic Mechanism of Disease

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Abstract

Autoimmune hepatitis (AIH) is an important disorder that predominantly results in inflammatory liver disease in genetically predisposed women. The clinicopathological picture is characterized by symptoms associated with both systemic inflammation and hepatic dysfunction, and with increased serum aminotransferases, elevated IgG, autoantibodies, and interface hepatitis on liver biopsy. AIH usually results in liver injury as a consequence of chronic hepatitis and cirrhosis. However, rarely, patients may present with fulminant liver failure. Early diagnosis is important in all instances because the disease can be highly responsive to immunosuppressive therapeutic options. Left untreated, the disease is associated with high morbidity and mortality. Here we provide an overview of the current state of knowledge on AIH and summarize the treatment options for this serious condition in adults. We also discuss the pathogenesis of the disease as a possible consequence of autoimmunity and the breakdown of hepatic tolerance. We focus on regulatory T cell impairments as a consequence of changes in CD39 ectonucleotidase expression and altered purinergic signaling. Further understanding of hepatic tolerance may aid in the development of specific and well-tolerated therapies for AIH.

Introduction

In 1950, Jan Waldenstrom described the first case of autoimmune hepatitis (AIH) in a woman with hepatic dysfunction and hypergammaglobulinemia.[1,2] Since then, AIH has become a well-established clinical entity, albeit uncommon, with an estimated annual incidence of 1.9 per 100,000, and a prevalence of 10–20/100,000.[3] Early diagnosis is important, as AIH usually responds to immunosuppressive treatment. However, if left untreated, AIH can progress to liver failure, cirrhosis, and death.

In this updated review, we explore the pathogenesis of AIH, consider the immunological basis for the pathogenesis of liver-directed immune injury, and present new concepts in the understanding of immune tolerance that seem to be perturbed in AIH. We also comment on various developments in innovative treatment modalities.

Clinical presentation

AIH may present with a variety of clinical manifestations, ranging from asymptomatic disease to fulminant liver failure. Although up to 25% of patients may be asymptomatic at diagnosis,[4] the condition most commonly presents in an insidious manner with non-specific complaints in young or middle-aged women.[5] Approximately 30% of patients may have evidence of advanced liver disease and cirrhosis at the time of diagnosis.[6] Extrahepatic manifestations of AIH may include inflammatory bowel disease, thyroiditis, type-1 diabetes mellitus, and celiac disease.[7,8]

There are no pathognomonic features of AIH. Therefore, the diagnosis depends on a set of clinicopathological, histological, biochemical, and immunological criteria. Interface hepatitis is the histological hallmark of AIH (see Fig. 1) and is present in 84–98% of cases.[8] Biopsy findings of cirrhosis and/or bridging necrosis carry a poorer prognosis than those lacking these features.[5,8,9]

Multiple biochemical derangements can be found in AIH. Most commonly, elevated aminotransferases with or without elevated bilirubin and alkaline phosphatase are frequently seen. Serum immunoglobulins, notably IgG, are elevated in approximately 85% of cases.[8]

Several scoring systems are available to aid in making an objective diagnosis and prognostication of AIH. In 1992, the International Autoimmune Hepatitis Group (IAIHG) published the first scoring system for AIH, with a revision released in 1999.[10] A simplified scoring system was published in 2008, which was subsequently vetted and shown to have high specificity.[11,12]
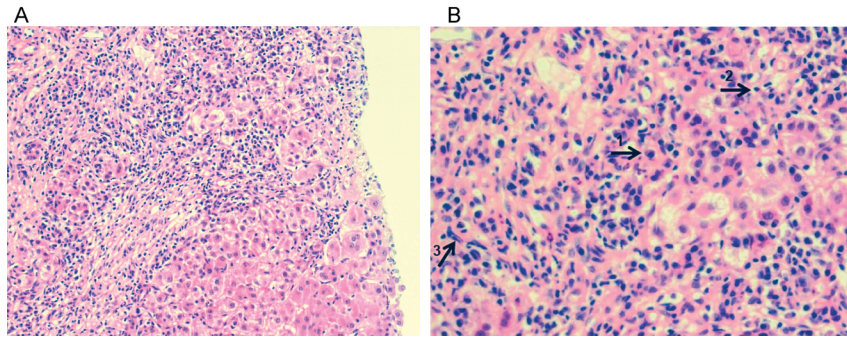
Fig. 1. Histological picture of AIH. (A) Portal and peri-portal inflammatory infiltrate, characteristic of autoimmune hepatitis, consists of (B) plasma cells, lymphocytes and monocytes/macrophages, as phenotypically indicated by arrows labeled 1, 2, and 3, respectively. There is evidence of interface hepatitis and piecemeal necrosis of hepatocytes. Hematoxylin and eosin staining, magnification $\times$ 200 (A) and x400 (B). (Credit: Dr Alberto Quaglia).

Serologic markers of disease

Autoantibodies are important in making the diagnosis of AIH, and aside from confirming the diagnosis, serologic markers may assist in subtyping the disease and determining the prognosis.[11]

Diagnostic markers

Conventional markers for AIH include anti-smooth muscle antibody (SMA) and anti-nuclear antibody (ANA), both of which characterize the classic type 1 AIH. SMA positivity appears to be more specific for AIH than ANA, which is associated with multiple sero-reactants to centromere, histones, double-stranded DNA, chromatin, and ribonucleo-protein complex components, and thus has yet to yield a specific antigenic target.[13–17] The specific antigen of SMA is also not clear, although multiple studies suggest that these autoantibodies react with actin components.[18,19]

It has also been reported that anti-nuclear and other autoantibodies are frequently noted in patients with non-alcoholic steatohepatitis. These observations may represent either nonspecific antibody responses associated with liver injury, or an autoimmune diathesis that may be linked pathogenetically to chronic inflammation, as in steatohepatitis.[20–22]

Antibodies to liver-kidney microsome type 1 (anti-LKM1) and liver cytosol type 1 (anti-LC1) characterize type 2 AIH. This subtype was described in the 1980s after the discovery of antibodies to liver-kidney microsomes.[23–25] Typically, patients are young women with more severe disease. The antigenic target of anti-LKM1 was identified as the cytochrome P450 liver enzyme CYP2D6.[26,27]

Adjunctive markers

Anti-soluble liver antigen (SLA) and liver pancreas (LP) reactivity are specific to and positive in 58% of adult patients with type 1 AIH.[1,28,29] The antigens for SLA/LP include ribonucleoprotein complex and O-phosphoseryl-tRNA:selenocysteinyl-tRNA synthase (SepSecS).[30] Anti-SLA is often associated with severe disease.[31]

Liver cytosol antibody (LC1) was described in the 1980s,[32] and is useful as an adjunct in the diagnosis of AIH type 2. The LC1 antigen is the liver-expressed enzyme formiminotransferase cyclodeaminase (FTCD), and appears to be associated with early onset of disease, concurrent autoimmunity, and rapid progression to cirrhosis.[31] LC-1 titers fluctuate with disease activity.[33,34] Rarely, anti-neutrophil cytoplasmic antibodies (ANCA) are associated with primary sclerosing cholangitis (PSC) overlap syndrome, and high rates of cirrhosis.[35–38]

Pathogenesis

The following pathogenetic model has been proposed: in a genetically predisposed host, defined environmental agent(s) catalyze(s) and trigger a series of T cell-mediated immune events directed at hepatic cellular antigens, resulting in unfettered inflammation, which ultimately culminates in fibrotic transformation of the liver, aberrant regeneration, and cirrhosis.[5,29,39]

Genetic predisposition

The genetic predisposition to AIH has been attributed, at least in part, to specific allelic variations in the major histocompatibility complex (MHC), located on chromosome 6 in the human leukocyte antigen (HLA) region.[31,40] Among Caucasian populations, associations between AIH type 1 and DRB1 alleles (DRB1*0301, DRB1*0401), as well as between AIH type 2 and allele DRB1*0701 have been described. Patients with DRB1*0301 tend to be younger, more likely to require liver transplant, and experience higher rates of acute liver failure and steroid treatment failure.[34,41] HLA allelic associations vary globally.[37]

Genetic risk factors outside of the MHC include polymorphisms of the gene for cytotoxic T lymphocyte antigen 4 (*CTLA-4*) in white North American and European populations. The CTLA-4 molecule interaction with antigen-presenting cells has been shown to mitigate T cell activation.

Further, a polymorphism of the gene encoding for tumor necrosis factor alpha (*TNFα2*), which is involved in the up-regulation of type 1 cytokines, is associated with more severe AIH in young white patients whose disease may be steroid-resistant.[42,43]

Molecular mimicry

Discovery and understanding of the target antigens for the autoantibodies in AIH may be important for developing specific treatments and understanding the mechanisms of

the disease. Molecular mimicry describes natural genetic homologies between autoantigens and common viral genomes (hepatitis C virus, herpes simplex virus 1, cytomegalovirus (CMV)) that spawn autoantibodies.[33,44] This genetic interplay is feasible because of incomplete specificity at CD4+ T-cell antigen receptors.[33]

Cellular immunoregulation

Immune system homeostasis is accomplished through regulation of effector CD8+ and CD4+ T cells by CD4+CD25+CD39+FOXP3+ regulatory T cells (Tregs).[45,46] A major contributor to AIH pathogenesis is the failure of immunoregulation as a result of diminished function and sheer number of Tregs, with consequent massive recruitment of inflammatory effector cells, which inflict hepatic injury.[47,48]

Tregs express unique markers including the interleukin-2Rα (IL-2Rα) chain (CD25), the glucocorticoid induced tumor necrosis factor receptor (GITR), CD62L, CTLA-4, and forkhead/winged helix transcription factor (FOXP3) as well as CD39, an ectonucleotidase responsible for extracellular nucleotide phosphohydrolysis, culminating in the production of immunosuppressive adenosine and regulated purinergic signaling.[39,47,49–53]

There is fairly recent evidence indicating that adenosine modulates effector cells by up-regulating inhibitory molecules (i.e. CTLA-4 and programmed-cell-death-protein-1 (PD1)), by decreasing IL-2 production and proliferation, and by inhibiting differentiation of effector cells into T helper 1 (Th1) and Th17 cell lineages.[54] The immunomodulating effects of adenosine are mediated by the binding of the nucleoside to A2A adenosine receptors on effector T cells. There are multiple putative Treg defects in common diseases that might contribute to a model of impaired immunoregulation in AIH and other immunological illnesses.[55] In very recent work, we and our colleagues showed that the CD39-expressing Tregs are decreased and lack the functional capacity to efficiently limit production of IL17, a pro-inflammatory cytokine elevated in the serum of patients with AIH.[56,57] The mechanism by which CD39+ Tregs limit IL17 production is unclear, although it has been previously suggested that CD39 can decrease IL17 levels by removal of ATP.[58]

Tregs in AIH exhibit slow rates of phosphohydrolysis of pro-inflammatory nucleotides compared to matched control cells. It has been proposed that defective immunoregulation in AIH is associated not only with decreased Treg number and functions, but also increased conversion of Tregs into effectors as a result of predominance of pro-inflammatory cytokines in the environment. The reasons for CD39 down-regulation or loss from Tregs in AIH are unknown, although reduced levels of transforming growth factor-β (TGF-β), an inhibitory cytokine that promotes CD39 upregulation on human leukocytes,[59] may account for this observation. Such a model of Treg CD39 dysregulation leading to autoimmune attack is depicted in Fig. 2. Such pathways may also be implicated in other autoimmune disorders of the gastrointestinal tract, such as inflammatory bowel disease.[60]

Treatment-induced remission of AIH is associated with restoration of Treg function.[61] Some groups have looked at expansion of Treg populations or diminishing effects of cytokine IL-17 as possible therapeutic interventions.[56,57,62]

Natural killer T (NKT) cells may also be involved in the pathogenesis of AIH. NKT cells are found in the vascular sinusoids, potentially providing an immunological bridge between innate and adaptive immune responses in immune liver reactions.

The purinergic receptor P2X7 recognizes extracellular ATP, and is crucial in regulating the function of NKT cells.[63] It has been proposed that the P2X7 receptor constitutes a sensor that can modulate NKT cell functions, which would also be affected by the ectonucleotidase CD39 expressed by these cells.[64] Curiously, genetic deletion in CD39, which limits Treg functions exacerbating adaptive immune responses in transplant rejection models,[65] also results in increased rates of stimulated NKT cell apoptosis in mouse models of AIH.[66] Hence, concanavalin-A hepatitis induction in Cd39 null mice results in enhanced levels of NKT cell loss and paradoxical protection from liver injury. This unexpected experimental finding illustrates the complexity of purinergic signaling in influencing diverse immune cell types (Treg vs. NKT cells) and in dictating opposing outcomes in the immune liver injury.

Treatment

Prednisone/azathioprine

The standard treatment for AIH comprises corticosteroid, and prednisone at an initial dose of 40–60 mg daily followed by combinations of prednisone in tapering doses to the lowest levels required to maintain remission with the anti-metabolite immune suppressant azathioprine (AZA) added to the therapeutic regimen. IN the USA, the daily dose of AZA is generally 50 mg, whereas in Europe, a higher dose of 1–2 mg/kg is usually preferred.[65] Several clinical trials in the 1970's demonstrated the safety and efficacy of this regimen, with remission rates of 65–80%.[5,67] However, many of the American Association for the Study of Liver Diseases (AASLD) and British Society of Gastroenterology consensus guidelines for the standardized management of AIH are based on suboptimal studies, and there remain clear uncertainties as to the management of refractory or resistant cases.[68–71]

Prolonged therapy may potentially result in a variety of adverse events that may lead to non-compliance and early cessation of therapy in a minority of patients.

Approximately 13% of patients discontinue conventional therapy because of intolerable prednisone-related side effects, with nearly half of these patients discontinuing therapy because of intolerable cosmetic issues. Similarly, long-term AZA use is associated with a constellation of potential side effects. Approximately 5% of patients treated with AZA cannot tolerate the side effects, and require early discontinuation of therapy. The most notable side effect is pancytopenia. Risk factors for this complication include malnutrition, cirrhosis, and absent (1:300) or low-level expression (10%) of thiopurine methyltransferase (TMPT).[72,73] It is increasingly accepted that TPMT testing should be performed prior to starting thiopurine drugs.

Other potential side effects of AZA include pancreatitis, cholestatic liver injury, vascular sinusoidal injury with nodular regenerative hyperplasia, and development of opportunistic infection.[74]

In 2010, the updated guidelines for the management of AIH redefined remission as sustained normalization of liver enzymes,[9,69,75–77] including a goal of normalized IgG/gammaglobulins.[9] Histological remission lags behind biochemical remission by 3–8 months. Although remission is achieved in the majority of patients with conventional management, 50%
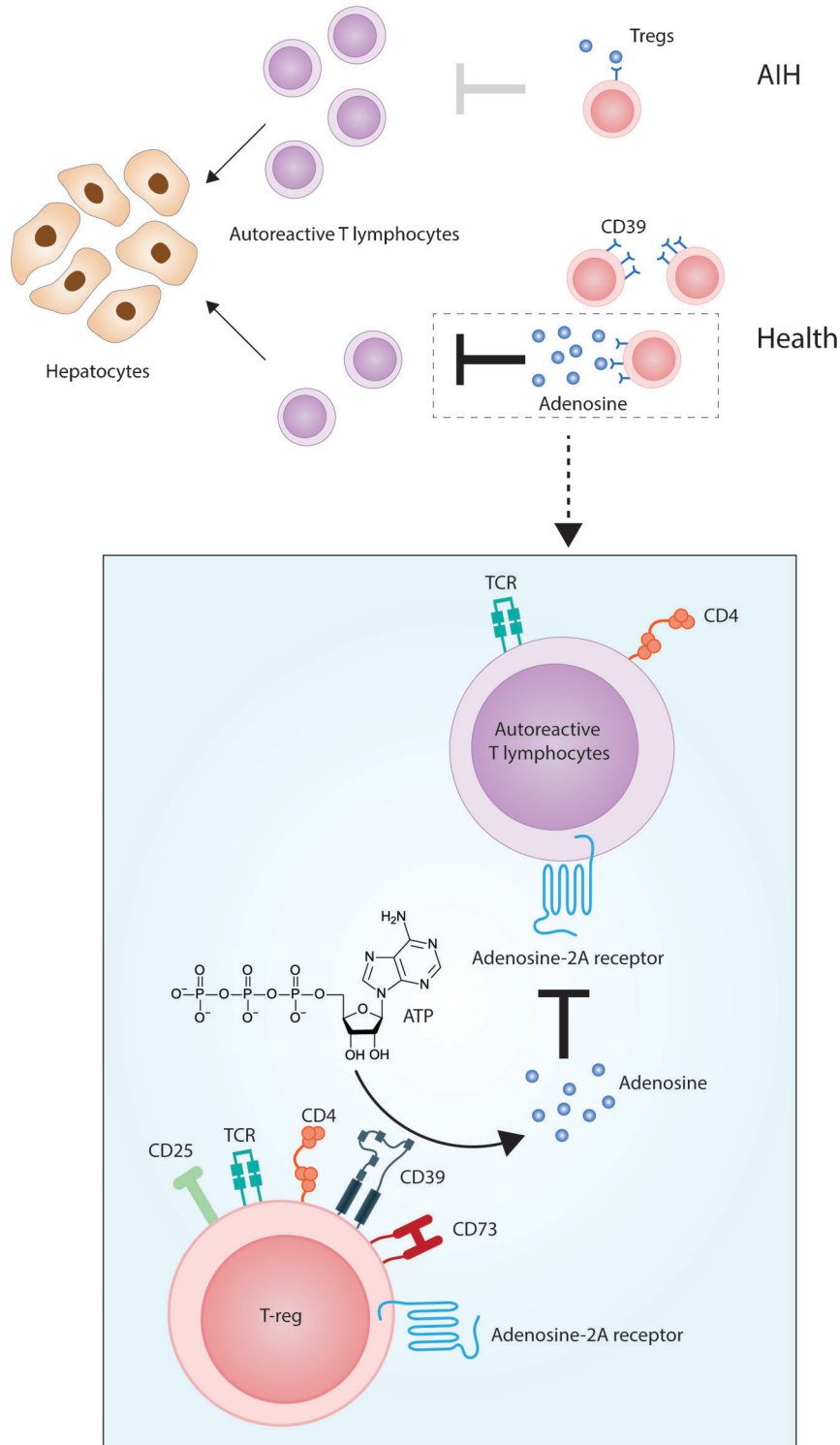
Fig. 2. Pathogenesis of liver attack in AIH: the role of CD39. In health, immunotolerance to liver autoantigens is maintained by effective control of CD4+CD25+FOXP3+Tregs over CD4+ and CD8+ autoreactive T lymphocytes. The machinery enabling Tregs to modulate effector immune responses relies on the expression of CD39, an ectonucleotidase ultimately leading to the generation of immunomodulatory adenosine. In AIH, Tregs are numerically defective and express low levels of CD39. This results in poor generation of adenosine and ineffective control over autoreactive lymphocytes, with consequent perpetuation of hepatocyte damage. Details of Treg adenosinergic suppression are depicted in the box. Adenosine is generated from ATP through the action of CD39 and CD73 ectonucleotidases in tandem, expressed by Tregs. Adenosine mediates immunomodulation by binding to A2A adenosine receptors on autoreactive T lymphocytes.

of patients relapse within 6 months of cessation of immuno-suppressive therapy, and nearly 70% within 3 years.[71] Achieving histological remission reduces the frequency of relapse to approximately 28%, while evidence of even mild portal hepatitis or inflammation increases the frequency to greater than 50%.[78]

Budesonide

Budesonide is a synthetic, orally administered corticosteroid with a rate of hepatic first-pass metabolism of 80–90%.[79] The drug is metabolized in the liver to by-products that have negligible glucocorticoid activity, but a marked affinity for glucocorticoid receptors. In 1994, Danielsson and Prytz studied the use of budesonide with or without AZA[80] (see Table 1). Since then multiple small studies[81–83] have preceded the first prospective, clinical trial studying the use of budesonide in AIH by Manns et al. in 2010.[84]

There is increasing evidence supporting budesonide as an alternative to prednisone. As demonstrated in published accounts, budesonide appears to be similar in efficacy to but more tolerable than prednisone. Although the Mayo Clinic series did not find any benefit with this alternative use of budesonide, other studies have demonstrated its efficacy and favorable side effect profile.[85]

Because the drug is metabolized almost exclusively in the liver, it may be intolerable to patients with cirrhosis. Additionally, patients exposed to prednisone may experience significant corticoid-related side effects when transitioning to budesonide.[85] Further follow-up regarding sustained remission with budesonide is required.

Mycophenolate mofetil

Mycophenolate mofetil (MMF) is the prodrug of mycophenolic acid, which is a potent, irreversible inhibitor of inosine monophosphate dehydrogenase, and has prominent cytostatic effects on lymphocytes. Richardson and colleagues published the first case series investigating usage of MMF in patients resistant to or intolerant of AZA.[86] Multiple subsequent case series and retrospective reviews noted achievement of biochemical remission in the majority of refractory cases of AIH, with a significant steroid-sparing effect.[87–91] However, Czaja and Carpenter studied the use of MMF in eight patients, and found that none of the patients who previously failed conventional therapy responded to MMF as salvage therapy.[92]

In 2010, another group studied the role of MMF in treatment-naïve patients. At the end of the study there were no non-responders, while 59.3% of patients achieved a complete response, and 28.8% of patients had a complete response initially, followed by a relapse.[93]

MMF appears to be an effective agent in treatment-naïve disease. However, there is no clear consensus on the use of MMF as a second line agent in those patients who fail conventional management. It appears that MMF has a role to play in patients who were previously intolerant to conventional therapy. The role of MMF as a definitive second line

Table 1. Review of major literature on the use of budesonide in AIH

Author	Year	No. of Patients	Budesonide Dose	Study End Points	Results
Danielsson	1994	13	6–8 mg daily	Decrease in ALT, AST, IgG	Complete response: 100%
Zandieh	2008	9	3 mg every other day to 9 mg daily	**Complete response:** Normal ALT, AST	Complete response: 78% No response: 22%
Wiegand	2005	12	Day 1: 6 mg daily Day 2: 9 mg daily Upon remission: 6 mg daily	**Complete remission:** AST and ALT drop $\leqslant$ two times the upper limit of normal **Partial response:** ALT or AST $\leqslant$ two times the upper limit of normal or AST/ALT improvement $>$ 80% from baseline	Complete response: 58% Partial response: 25%
Csepregi	2006	11	9 mg daily	**Remission:** Absence of symptoms Normal ALT, ALP, IgG	Treatment-naïve: 57% Treatment-experienced: 100%
Manns	2010	203 (100 received Budesonide and 103 received prednisone)	6 mg daily or 9 mg daily	**Complete response:** Normal ALT Normal AST Absence of steroid-related side effects	Budesonide group: 47% Prednisone group: 18.4%
Czaja	2000	10	9 mg daily	**Remission:** Asymptomatic Normal or near normal AST Normal bilirubin Normal γ-globulin **Failure:** Clinical/biochemical deterioration	Remission: 30% Treatment failure: 40%

salvage agent requires further studies in the form of prospective controlled trials. The most common adverse reactions with MMF are gastrointestinal side effects, and significant thrombocytopenia, leukopenia, and rarely CMV infection. Considering that the treatment duration is often measured in years, the adverse effects associated with prolonged use of MMF in this population are yet to be determined. Additionally, judicious use of MMF must be employed given its greater cost compared to conventional treatment.[89]

Cyclosporin

Cyclosporin is a calcineurin inhibitor that acts by binding to cyclophilin, thus creating a complex.[94] Mistilis and colleagues first reported the use of cyclosporin in the management of AIH in 1985.[95] Since then, several isolated case reports and case series have described the successful use of cyclosporin in adults intolerant or non-responsive to conventional management.[96–100] Malekzadeh and colleagues in 2001 published the largest case series reporting the use of cyclosporin as an alternative to steroid-based treatment in AIH.[101] Although cyclosporin appears to have a promising role in the treatment of AIH, the potential long-term adverse effects and nephrotoxicity of the drug have yet to be studied in this particular patient population.

Tacrolimus

Tacrolimus is a macrolide with a similar mechanism of action to cyclosporin but with greater immunosuppressive potency. Tacrolimus binds to the FK506 binding protein, thus inhibiting phosphatase activity, which is required for cytokine gene transcription and T-cell activation. The net result of tacrolimus activity is inhibition of both T and B cells.[102]

Treatment of AIH with tacrolimus was first proposed in 1995.[103] In an open label study, 15 of 21 patients demonstrated biochemical improvement. Multiple follow-up single-center studies on patients who were either steroid-refractory or steroid-intolerant demonstrated similar results.[104,105] A review of the literature suggests that tacrolimus has a role to play in the management of patients intolerant and/or refractory to conventional therapy, and may be effective as a second line therapy in AIH. Adverse effects include nephrotoxicity, hypertension, bone marrow toxicity, diabetes, neurotoxicity, and opportunistic infections.[106,107]

Alternative therapies

Several isolated case reports have investigated other agents as potential alternatives to conventional management, including ursodeoxycholic acid, infliximab, etanercept, methotrexate, rapamycin and rituximab; however, rigorous supportive data is lacking.

Transplantation

Orthotopic liver transplantation (OLT) is reserved for those patients who have failed medical therapy or who present with acute fulminant hepatitis that is too advanced for medical management.

AIH is the indication for OLT in approximately 4–6% of adult transplants occurring in the USA and Europe.[108] Outcomes are excellent in patients who undergo OLT for treatment of AIH, with 5-year and 10-year survival rates close to 75%. Recent studies indicate that the rate of recurrence of AIH post-OLT is approximately 23%.[109] In these patients, modification of the immunosuppressive regimen and close follow-up is mandatory.[9]

Conclusions

AIH remains clinically challenging despite decades of awareness of this complex disease. Although standard therapy with prednisone and AZA frequently results in an excellent treatment response, the need for novel and steroid-sparing treatments remains. The goal is to optimize care for the wide spectrum of patients afflicted with this condition. Further exploration of the underlying immunologic processes in AIH, particularly those which, at least in part, involve purinergic signaling, should be undertaken. This will lead to a deeper understanding of how the usual mechanisms of hepatic tolerance are rendered incompetent in this dangerous and yet fascinating liver disease.

Conflict of interest

None

Author contributions

Reviewing literature, conceiving concepts and developing the review (NY, JTH, MSL, SCR)

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