Review

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Autoimmune hepatitis – Update 2015

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Introduction and history

Autoimmune hepatitis (AIH) was first described in 1951 [1] as a chronic hepatitis of young women with hypergammaglobulinemia in the absence of cirrhosis, which responds well to adrenocorticotrophic therapy (ACTH). Shortly thereafter this syndrome was described and characterized in the USA. In 1956 the association with anti-nuclear antibodies (ANA) was discovered and the term "lupoid hepatitis" was created [2]. AIH and systemic lupus erythematosus (SLE) are distinct autoimmune disorders. However, they may occur together in a given patient. Between 1960 and 1980 several prospective trials were published demonstrating the benefits of corticosteroids alone or in combination with azathioprine in severe cases of AIH. AIH became the first liver disease in which medical therapy improved survival [3]. The advent of immunofluorescence, thereafter radio, as well as enzyme-linked immunosorbent (EIA) assay technology, in addition to molecular cloning techniques allowed a molecular identification and characterization of the hepatocellular autoantigens involved in AIH (Table 1). Characterization of the humoral and cellular immune system in patients and several animal models significantly improved our knowledge of this still poorly understood autoimmune liver disease (Fig. 1). Immunosuppression and liver transplantation are our therapeutic weapons. While corticosteroids alone or in combination with azathioprine are effective and prolong survival, treatment failures to this standard of care (SOC) are still a challenge. This review on the occasion of the 30 year anniversary of the *Journal of Hepatology* and the 50 year anniversary of the European Association for the Study of the Liver (EASL) summarizes the developments over the past 50 years in AIH. We will also give an outlook on how our progress in the understanding of the molecular and cellular pathogenesis of AIH will pave the way for future therapies specifically targeting the underlying disease progress and eventually avoiding liver transplantation.

Key Points

- Autoimmune hepatitis (AIH) is a chronic self-perpetuating inflammatory disease with a female predominance occurring in all ages and races that may start with an episode of acute hepatitis and may lead to liver cirrhosis, liver cancer, liver transplantation or death
- Over the last decades molecular targets of the most relevant disease associated autoantibodies were identified and characterized. Recent investigations on the immunopathogenesis concentrated on regulatory T cells and the complex genetic background of AIH via GWAS analyses
- Immunosuppressive therapy in severe cases of AIH prolongs survival
- Standard of care includes corticosteroids alone or in combination with azathioprine to achieve normalization of transaminases and immunoglobulin G levels in serum. The topical steroid budesonide can be used in non-cirrhotic patients instead of predniso(lo)ne to reduce steroid specific side effects.
- In treatment failures mycophenolate mofetil, cyclosporine A, tacrolimus and lately anti TNF or anti CD20 monoclonal antibodies can be used as second line treatment based on a careful individual risk evaluation and should be done in experienced centers

Aetiology and pathogenesis

AIH is divided in two main types: AIH type 1 (AIH-1), positive for anti-nuclear (ANA) and/or anti-smooth muscle (SMA) antibodies, and AIH type 2 (AIH-2), positive for anti-liver kidney microsomal antibody type 1 (anti-LKM1), anti-LKM3 and/or anti-liver cytosol type 1 antibody (anti-LC1) (Figs. 2 and 3). Whether specific

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Abbreviations: AIH, Autoimmune hepatitis; AMA, Anti-mitochondrial antibody; ANA, Anti-nuclear antibodies; APC, Antigen-presenting cell; APECED, Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy; CTL, Cytotoxic T lymphocytes; DIL, Drug-induced liver injury; GWAS, Genome-wide association; HBV, Hepatitis B virus; HCV, Hepatitis C virus; HLA, Human leukocyte antigen; HSV, Herpes simplex virus; IE, Immediate early; NK, Natural killer; PBC, Primary biliary cirrhosis; PSC, Primary sclerosing cholangitis; SLE, Systemic lupus erythematosus; SMA, Smooth muscle antibody; SOC, Standard of care; SSSE, Steroid specific side effects.

Table 1. Molecular targets and disease associations for autoantibodies in liver diseases.

Autoantibodies	Target	Disease association
ANA	Multiple nuclear antigens	AIH, SLE, MTCD etc.
AMA	2-oxo-acid-dehydrogenase complex	PBC
pANCA	h-Lamp-2, proteinase 3	AIH, PSC, PBC
SMA	Actin, troponin, tropomyosin	AIH I
LKM 1	CYP 2D6	AIH II, HCV
LKM 2	CYP 2C9	Tienilic acid-induced hepatitis
LKM 3	UGT1A	AIH, hepatitis D
LM	CYP 2A6	APECED, hepatitis C
LC1	FTCD	AIH II
SLA/LP	tRNP$^{(Ser)Sec}$	AIH III?
LM	CYP 1A2	Dihydralzine-induced hepatitis, APECED
ASGP-R	Asialoglycoprotein receptor	Autoimmune liver disease, HCV

ANA, anti-nuclear antibodies; AMA, anti-mitochondrial antibodies; ANCA, antineutrophilic cytoplasmatic antibodies; SMA, smooth muscle antibodies; LKM, liver kidney microsomal antibodies; LM, liver microsomal antibodies; LC1, liver cytosolic antibodies type 1; SLA/LP, soluble liver antigen/liver pancreas antibodies; ASGPR-R, asialoglycoprotein receptor antibodies; UGT1A, UDP glucuronosyltransferase family 1 A; FTCD, formimino-transferase cyclodeaminase; AIH, autoimmune hepatitis; PSC, primary sclerosing cholangitis; PBC, primary biliary cirrhosis; HCV, hepatitis C virus; APECED, autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy.

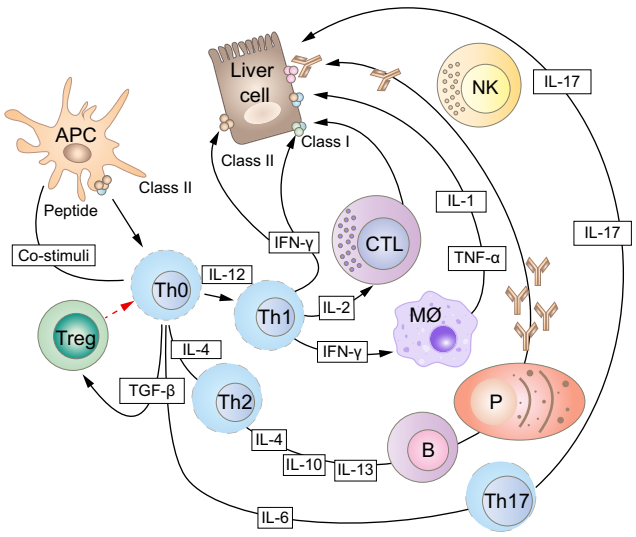

Fig. 1. Molecular pathogenesis of autoimmune hepatitis. An autoantigenic peptide is presented to an uncommitted T helper (Th0) lymphocyte within the HLA class II molecule of an antigen-presenting cell (APC). Th0 cells become activated and, according to the cytokines present in the microenvironment and the nature of the antigen, differentiate into Th1, Th2, or Th17 cells, initiating a series of immune reactions determined by the cytokines they produce: Th2 secrete mainly IL-4, IL-10, and IL-13, and direct autoantibody production by B lymphocytes; Th1 secrete IL-2 and IFN-γ, which stimulate cytotoxic T lymphocytes (CTL), enhance expression of class I and induce expression of class II HLA molecules on hepatocytes and activate macrophages; activated macrophages (MØ) release IL-1 and tumour necrosis factor alpha (TNF-α). Regulatory T cells (Treg) are derived from Th0 in the presence of transforming growth factor (TGF-β). If Tregs do not oppose, a variety of effector mechanisms can be activated: liver cell destruction could derive from the action of CTL; cytokines released by Th1 and recruited macrophages; complement activation or engagement of Fc receptor-bearing cells such as natural killer (NK) lymphocytes by the autoantibody bound to the hepatocyte surface. The role of the recently described Th17 cells, which arise in the presence of TGF-β transforming growth factor beta (TGF-β) and IL-6, is under investigation. Of note, TGF-β is highly expressed in the inflamed liver, dwindling during remission [67].

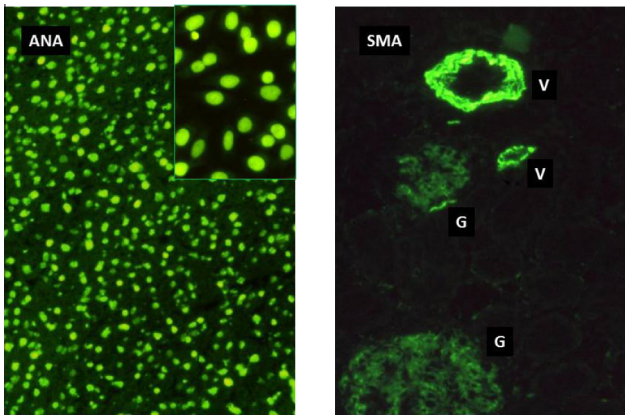

Fig. 2. Indirect immunofluorescence pattern of anti-nuclear (ANA) (left panel) and smooth muscle (SMA) (right panel) autoantibodies. Immunofluorescence pattern of anti-nuclear autoantibody (ANA) on rodent liver and Hep2 cells (inset), which, having a large nucleus, allow pattern recognition. The homogeneous pattern is the most common in autoimmune hepatitis. Immunofluorescence pattern of smooth muscle (SMA) autoantibody on rodent kidney. SMA stains the smooth muscle of arterial vessels (V) and the glomeruli (G).

autoantibody profiles determine aetiologically distinct entities of AIH remains to be proven [4].

The aetiology of autoimmune hepatitis is unknown, though both genetic and environmental factors are likely to be involved. An immune response targeting liver autoantigens is thought to initiate and perpetuate the liver damage. Several genetic factors interact to influence susceptibility to AIH, clinical manifestations, response to treatment and overall prognosis.

The strongest genetic associations are found within genes of the human leukocyte antigen (HLA) region (the human major histocompatibility complex, MHC) – located on the short arm of chromosome 6 – which are involved in the presentation of antigenic peptides to T cells, and are therefore implicated in the initiation of an adaptive immune response [5].

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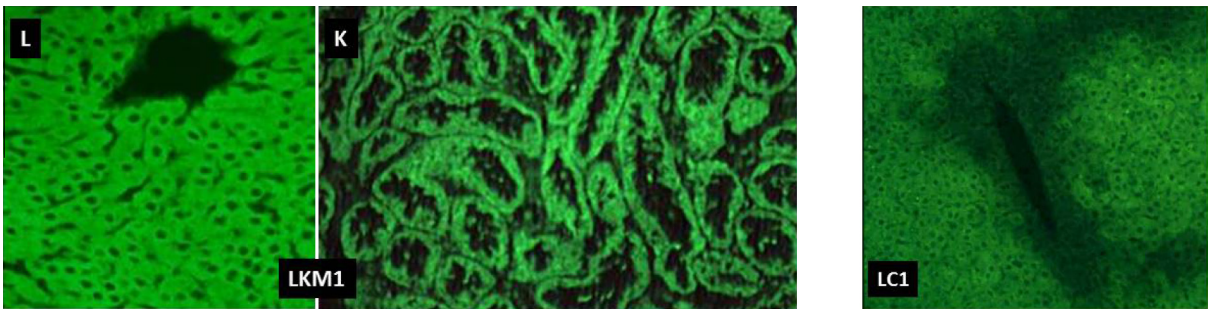

Fig. 3. Immunofluorescence pattern of anti-liver kidney microsomal type 1 antibody (anti-LKM-1) (left and middle panel) and anti-liver cytosol type 1 antibody (anti-LC-1). Immunofluorescence pattern of anti-liver kidney microsomal type 1 (LKM1) autoantibody on liver and renal rodent sections: anti-LKM1 stains the cytoplasm of hepatocytes and proximal renal tubules. Immunofluorescence pattern of anti-liver cytosol type 1 (anti-LC1) antibody on a rodent liver section: the antibody stains the cytoplasm of hepatocytes with a weakening of the staining around the central vein.

There are particularly strong associations within the HLA-DRB1 locus [6], with the HLA DR3 (DRB1*0301) and DR4 (DRB1*0401) molecules conferring susceptibility to AIH-1 in Europe and North America. The associations with HLA DR3 and DR4 are considered strong enough to contribute to the diagnosis of AIH according to the revised diagnostic scoring system designed by the International Autoimmune Hepatitis Group (IAIHG) [7].

HLA DR7 (DRB1*0701) and DR3 (DRB1*0301) confer susceptibility to AIH-2. Patients positive for DRB1*0701 have a more aggressive form of the disease with worse overall prognosis [8]. HLA-DQB1*0201 has also been linked to the development of AIH-2, although this allele is in linkage disequilibrium with DRB1*0701 and DRB1*0301, both associated with AIH-2 [9].

In the first genome-wide association study (GWAS) on AIH, it was reported that AIH type 1 is associated not only with variants within the MHC region, but also with variants of SH2B3 and CARD10 [10].

A number of genes outside the MHC have also been linked to a susceptibility of developing AIH. For example, a substitution from A (adenine) to G (guanine) in exon 1 of the *CTLA-4* gene confers susceptibility to *AIH-1* in Caucasians from North America [11].

A form of AIH resembling AIH-2 has been described in some 20% of patients with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), a monogenic autosomal recessive disorder caused by homozygous mutations in the *AIRE1* gene [12,13]. *AIRE1* mutations are not increased in cases with AIH or other types of autoimmune liver diseases like primary biliary cirrhosis (PBC) or primary sclerosing cholangitis (PSC) [14]. However, *AIRE* gene mutations were observed in children with acute liver failure [15]; [16]. Therefore the APECED syndrome must be considered as a cause of acute liver failure.

In patients with increased genetic susceptibility, one potential mechanism leading to AIH is molecular mimicry, i.e. an immune response to exogenous pathogens that cross reacts with structurally similar liver autoantigens. A major linear autoepitope of CYP2D6 has sequence homology with the immediate early protein (IE) of herpes simplex virus (HSV) [17]. One patient with AIH type 2 and anti-LKM1 antibodies showed evidence of HSV infection in contrast to her identical twin sister [17]. In mice, experimental forms of AIH can be induced by a transient infection with adenovirus carrying human CYP2D6 or FTCD [18,19]. In accordance with observations in humans, experimental AIH is dependent on the genetic background of the mice too [20]. The strongest support for this model is in the context of viral hepatitis, where autoimmunity is a common feature during chronic infection. In chronic hepatitis C virus (HCV), some 10% of patients are anti-LKM1 positive, the autoantibody titre correlating with disease severity and being associated with adverse reactions to interferon treatment [21]. Within anti-LKM1 positive chronic HCV patients, reactivity against a key autoantigenic target of anti-LKM1, the epitope $CYP2D6_{193\text{-}212}$, can be seen in 50% of patients. There is evidence of cross-reactivity between anti-LKM1 and antibodies directed against homologous regions of HCV (NS5B $HCV2_{985\text{-}2990}$) and cytomegalovirus (exon $CMV_{130\text{-}135}$) [22]. One case-report describes a 10 year old girl who acquired HCV infection following a liver transplant for end-stage liver disease caused by α1-anti-trypsin deficiency. Two weeks after HCV infection immunoglobulin (Ig)-M anti-LKM1 antibodies appeared, followed by IgG anti-LKM1 antibodies. This finding is suggestive of HCV as a trigger of a primary anti-LKM1/anti-CYP2D6 autoimmune response [23]. Interestingly, 10 years after contact with HCV, the patient developed florid AIH type 2, which responded satisfactorily to immunosuppressive treatment; by that time there was no trace of the previous HCV infection. The 3–4-fold higher prevalence of antibodies against hepatitis E in patients with AIH may indicate that even mild and acute viral hepatitis may contribute to a break of hepatic tolerance [24]. On the other hand the observed increase in the prevalence of anti-HEV antibodies in AIH patients may be a consequence rather than being related to the cause of this syndrome.

Anti-LKM1 antibodies have also been detected in a patient who underwent a liver transplantation for acute Wilson disease, and thus can also occur in patients not being transplanted for an autoimmune liver disease. Suggesting they may arise as a consequence of hepatic inflammation and hepatocellular destruction during rejection episodes [25]. In addition, these observations tell us that different environmental triggers may lead to autoimmunity against identical molecular targets.

The antibiotics nitrofurantoin and minocycline [26], as well as the statins and the anti-TNF agents adalimumab and infliximab, have been reported as non-viral environmental triggers of AIH. Drug-induced liver inflammation and injury (DILI) with features of AIH can regress spontaneously after stopping xenobiotic treatment not requiring long-term immunosuppressive therapy [26].

The development of autoimmune diseases is favoured by the breakdown of self-tolerance mechanisms that, in health, prevent the majority of autoreactive T cell clones from entering the periphery. As circulating autoreactive T cells are present in health, there are both intrinsic and extrinsic peripheral tolerance mechanisms to limit autoimmune tissue damage. Key to this is the

immune suppression exerted by professional regulatory T cells (Tregs). Tregs have been investigated in AIH, detected mainly in the peripheral blood: while in the paediatric form both number and function of Tregs have been found to be impaired [27–29], the evidence in adult AIH is conflicting, as some studies could not confirm a numerical Treg deficiency in the peripheral blood. Treg activity appears to be quite notable during active disease, but apparently insufficient to effectively suppress the aberrant autoimmune response. In contrast to the peripheral blood, Tregs accumulate in the adult liver of untreated AIH [30,31]. Furthermore, intrahepatic Tregs seem to be selectively depleted under steroid and azathioprine based therapy and patients with incomplete treatment response that exhibit lower intrahepatic Treg frequencies [30].

The dense infiltrate of lymphocytes, plasma cells, and macrophages characteristic of the histological picture of AIH suggests that an autoaggressive cellular immune attack is the basis of this condition. The predominant population within the cellular infiltrate is composed of α/β T cells. Amongst these cells, the majority are CD4-positive T-helper cells, cytotoxic CD8-positive T cells accumulate with increasing histological severity of hepatitis [30]. Immunohistochemically, lymphocytes of a non-T cell lineage are relatively rare, and include natural killer (NK) cells and macrophages, [32].

The steps of an autoimmune attack on a liver cell are depicted in Fig. 1.

Diagnosis

Clinical symptoms

AIH occurs in all ages and races. It usually runs a chronic course, starting with an episode of acute hepatitis in approximately 25% of cases but may manifest as fulminant hepatitis; thus AIH must be considered in the differential diagnosis of acute liver failure [33]. In the majority of cases diagnosis is made when uncharacteristic non liver-specific clinical symptoms dominate like fatigue and arthralgias. Spider naevi, upper or lower gastrointestinal bleeding indicate advanced stages of liver disease. Jaundice may also indicate advanced liver disease. However, increased bilirubin levels may also indicate acute onset AIH, hemolysis or inborn errors of bilirubin metabolism like Gilbert syndrome. Extrahepatic autoimmune disorders like thyroiditis, arthritis, sicca or Sjogren syndrome, vitiligo, glomerulonephritis or ulcerative colitis are common and occur in all stages of liver disease.

Biochemistry

Elevation of liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) indicate inflammatory activity, e.g. grading [34], while alterations in bilirubin, cholinesterase, thrombocytes indicate advanced stages of cirrhosis.

Elevation of gammaglobulins and in particular serum IgG levels in the absence of cirrhosis are a diagnostic hallmark of AIH. Typically there is a selective increase of IgG with IgA and IgM-levels remaining normal. This characteristic hallmark for AIH is not only an important test in making the diagnosis, but IgG levels during follow-up are an excellent, inexpensive and reliable marker of disease activity. Normalization of IgG levels together with normalization of transaminase levels has become part of the definition of biochemical remission in AIH [33].

Immunological tests

Circulating autoantibodies are key to the diagnosis of AIH and to its subdivision in two forms (see Aetiology and Pathogenesis) [7,35–37]. The two autoantibody profiles rarely occur simultaneously [37]. Autoantibodies are normally detected by indirect immunofluorescence on a rodent substrate that includes kidney, liver and stomach. This methodological approach has a major advantage in that it allows the detection of several auto-reactivities relevant to AIH including ANA, SMA, anti-LKM1 and anti-LC1, as well as anti-mitochondrial antibody (AMA), the serological hallmark of PBC [7,35,37]. Autoantibodies are considered positive when present at a dilution of 1:40 or more in adults, while in children, who are rarely positive for autoantibodies in good health, positivity at a dilution $\geqslant$1:20 for ANA and SMA or $\geqslant$1:10 for anti-LKM1 is clinically significant [38]. ANA in AIH usually has a homogeneous pattern (Fig. 2), but for its clearer and easier definition, Hep2 cells that have prominent nuclei, can be used as a substrate (Fig. 2). There are no ANA molecular targets specific for AIH. A varied profile of ANA reactivity reminiscent of that found in SLE (e.g. to nuclear chromatin, histones, centromere, double and single stranded DNA and ribonucleoproteins) has been reported in AIH, but at least a third of AIH patients positive for ANA do not react with known nuclear targets [39,40]. Interestingly, anti-double stranded DNA are shared in common only with SLE. Immunofluorescence remains therefore the gold standard for ANA testing, as recently surmised by the American College of Rheumatology ANA Task Force [41]. However, according to the AALSD clinical practice guidelines for autoimmune hepatitis practicing hepatologists in the US often use EIAs with recombinant nuclear antigens in their everyday clinical practice [33].

The immunofluorescent staining of SMA is detected in the arterial walls of rodent kidney, liver and stomach. In the kidney, SMA can have three patterns: V (vessels), G (glomeruli) and T (tubules) [42] (Fig. 2). The V pattern is present also in viral liver disease and in extrahepatic autoimmune diseases, but the VG and VGT patterns are indicative of AIH. The anti-LKM1 pattern is characterized by bright staining of the hepatocyte cytoplasm and of the P3 portion of the renal tubules (Fig. 3). Anti-LKM1 is occasionally confused with AMA but the identification of the molecular targets of anti-LKM1, cytochrome P4502D6 (CYP2D6), and of AMA, enzymes of the 2-oxo-acid dehydrogenase complexes, has allowed the establishment of immunoassays, which can be used to resolve doubtful cases (Table 1). Additional LKM reactivity has been described. Anti-LKM2 antibodies, which target cytochrome P4502C9, are only of historical interest. They were associated with a severe form of hepatitis induced by ticrynafen, a uricosuric diuretic. This drug was withdrawn from clinical use in 1980. Anti-LKM3 antibodies are specific for members of the uridine glucuronosyltransferase family 1 [43] and give an immunofluorescence pattern similar to anti-LKM1 [44]. Although anti-LKM3 is most commonly detected in patients with hepatitis D (delta), it is also present in some 10% of patients with AIH-2 and in some cases may be the only serological marker of AIH [45].

Anti-LC1 (Fig. 3), which is an additional marker for AIH-2, can be present on its own, but frequently occurs in association with anti-LKM1, and targets formimino-transferase cyclodeaminase (FTCD) [46]. Anti-FTCD antibody can be detected by commercial ELISA [37].

Anti-SLA/LP was first described in 1987 [47] and is highly specific for AIH [48]. It is detectable in 20–50% of AIH patients,

depending on the assay used. Its presence identifies patients with more severe disease and worse outcome [8]. At variance with standard diagnostic autoantibodies, anti-SLA/LP is not detectable by immunofluorescence. The molecular target of anti-SLA/LP has been identified as Sep (O-phosphoserine) tRNA:Sec (selenocysteine) tRNA synthase (SEPSECS) [49–51]. Autoantibodies to SEPSECS were previously described in severe forms of AIH [52]. Its molecular identification has allowed the establishment of molecularly based diagnostic assays. Whether these antibodies characterize a clinically distinct subgroup of AIH (type 3 AIH) is debated. The significance of anti-SLA/LP autoantibodies lies primarily in their high degree of disease specificity making them almost diagnostic by themselves in those patients positive for these antibodies.

Other autoantibodies less commonly tested, but of diagnostic importance, include perinuclear anti-neutrophil cytoplasm (pANCA) antibody and anti-asialoglycoprotein receptor antibody (ASGPR). pANCA is often detected in AIH-1, though less frequently than in PSC and inflammatory bowel disease [40] (Table 1). The pANCA found in AIH and in these conditions is referred to as atypical, since it reacts with peripheral nuclear membrane components and for this reason is also known as pANNA: peripheral anti-nuclear neutrophil antibody. In contrast to AIH-1, pANNA is virtually absent from AIH-2 [37].

Anti-ASGPR targets the main constituent of the crude liver cell extract known as liver-specific protein. Almost 90% of AIH patients are positive for anti-ASGPR antibody and its titre correlates with disease activity. However, anti-ASGPR is not specific for AIH being also detectable in viral hepatitis, drug-induced hepatitis and PBC. Moreover, since its detection requires difficult-to-prepare purified or recombinant antigen, the development of reliable molecular assays has been challenging, and its applicability to clinical practice is therefore limited [40].

Scoring systems

The diagnosis of AIH is based on history, laboratory and histological features [53,54]. While in most patients the diagnosis is easily made, it can at times be difficult in view of the heterogeneity of the clinical features and the large number of differential diagnoses [55]. Moreover, in non-specialist centres the diagnosis can be overlooked or made inappropriately in patients with other forms of liver disease. In addition to clinical management problems, these difficulties have hampered the scientific evaluation of the disease. Historically this was even more so prior to the discovery of HCV and the development of reliable diagnostic assays. It was precisely this scientific dilemma that led to the formation of the IAIHG.

The first meeting was convened by Ian McFarlane during the International Association for the Study of the Liver (IASL) convention held in Brighton in 1992 and gathered a panel of experts to discuss diagnostic criteria to be used for comparative purposes in scientific publications on AIH [35]. This marked the beginning of the IAIHG, which has since met regularly and coordinated a number of projects on standardization of diagnostic aspects and terminology in autoimmune liver disease. The original diagnostic scoring system, published in the *Journal of Hepatology* [56], was largely based on expert opinion, as systematic studies were missing. In subsequent years a number of studies evaluated the utility of these criteria, leading to an extensive revision of the diagnostic scoring system published in 1999, which has since been one of the most cited papers in hepatology and the key reference for clinical studies [7], again published in the *Journal of Hepatology* (Table 2). The criteria have stood the test of time for scientific purposes, but they were not designed for daily clinical practice, as they were too numerous and complicated for bedside use [57]. Furthermore, response to treatment, which is an important component of the IAIHG diagnostic scoring system, is not available when having to decide if a trial of steroid therapy should be initiated or not [57].

These considerations led the IAIHG to devise and evaluate simplified diagnostic criteria, which were published in 2008. These only use the three features of hypergammaglobulinaemia, autoantibodies and histology, in the absence of viral hepatitis, as their basis (Table 3). The score was developed empirically with data from 11 centres in 10 countries, using both a primary cohort and a validation set [36]. This Simplified Diagnostic Scoring System has since been used widely and its reliability has been demonstrated worldwide [58–60]. While the original scoring system considered evidence of viral hepatitis an exclusion criterion for the diagnosis of AIH, the simplified score allows a diagnosis of "probable" AIH even in the presence of positive viral markers [36]. In view of the high prevalence rate of viral hepatitis in many countries, it was considered important not to overlook those patients who suffer from both viral hepatitis (more often B than C) and AIH, and who require immunosuppression, usually at the same time as anti-viral therapy. In particular, in countries such as China, with a prevalence of hepatitis B surface antigen (HBsAg) of around 10%, it is likely that one in ten AIH patients also tests positive for HBsAg. Thus, studies of large patient cohorts in China have shown the validity of the simplified scoring system [60].

Another difference between the IAIHG simplified score and revised criteria is the significance of cholestatic features. The simplified score allows the diagnosis of AIH to be made also in patients with PSC or PBC, while cholestatic features [36] exclude the diagnosis in the revised criteria score. Both approaches are correct, but have different applications. The simplified score is mainly designed to help in the decision to initiate immunosuppression in a patient with liver disease, while, for the purpose of a scientific paper, patients with cholestatic features of PBC or PSC and manifestations of AIH are likely to suffer from variants of either of the two cholestatic liver diseases and should not be classified as having AIH, as stated in the position paper of the IAIHG working group on overlap syndromes [61].

Histopathology

Histological evidence of inflammatory damage to the liver compatible with a diagnosis of AIH is an essential feature for making the diagnosis of AIH [36]. The diagnosis, however, cannot be made purely on histological findings. Just as the clinical syndrome of AIH is heterogeneous, so is its histology, though some lesions are highly suggestive of AIH. A number of typical features, though not specific for AIH, have been described: interface hepatitis (originally called piece-meal necrosis), characterized by lymphoplasmacytic infiltrates, hepatocyte rosetting and emperipolesis (i.e. endocytosed lymphocytes within hepatocytes) [62,63] (Fig. 4). Presence of at least three of these features is considered typical for AIH, and is therefore given a score of +2 in the simplified scoring system.

Distinction from other liver diseases can often be difficult also on histology. The distinction from cholestatic liver diseases, in particular PBC, can be a challenge [64]. Bile-duct damage is

Table 2. The Revised International Autoimmune Hepatitis Group Modified Scoring System [7].

Category	Score	Comments
Female sex	+2	
ALP:AST (or ALT) ratio		
<1.5	+2	
1.5-3.0	0	
>3.0	-2	
Serum globulins or IgG above normal		
>2.0	+3	
1.5-2.0	+2	
1.0-1.5	+1	
<1.0	0	
Autoantibodies (ANA, SMA, or LKM-1)		Lower titers are considered significant in children and should be scored at least +1
>1:80	+3	
1:80	+2	
1:40	+1	
<1:40	0	
Hepatitis viral markers		The patient should be tested for markers for hepatitis A, B, and C infection; tests for other viruses such as EBV and CMV may be considered
Positive	-3	
Negative	+3	
Drug history		Recent use of known or suspected hepatotoxic drugs
Positive	-4	
Negative	+1	
Average alcohol consumption		
Low (<25 g/day)	+2	
High (>60 g/day)	-2	
Liver histology		"Biliary changes" refers to bile duct patterns of injury typical of PBC or PSC with ductopenia in an adequate biopsy. "Other features" are any suggesting an alternative etiology, e.g., non-alcoholic fatty liver disease
Interface hepatitis	+3	
Lymphoplasmacytic infiltrate	+1	
Hepatocyte rosette pattern of regeneration	+1	
None of the above	-5	
Biliary changes	-3	
Other features	-3	
Other autoimmune disorders, in patient or first degree relatives	+2	
Optional parameters in patients who are seronegative for ANA, SMA, an LKM-1:		Other defined antibodies are those with published evidence of relevance to AIH, and include pANCA, anti-LC1, anti-SLA/LP, anti-ASGPR
Seropositivity for other defined autoantibodies	+2	
HLA DR3 or DR4	+1	
Response to therapy:		
Complete	+2	
Relapse	+3	
Interpretation of aggregate scores		
Pre-treatment:		
Definite AIH	>15	
Probable AIH	10-15	
Post-treatment		
Definite AIH	>17	
Probable AIH	12-17	

typically not a feature of AIH, but in severe cases it can also be observed. A follow-up biopsy may be required for a reliable distinction, as bile-duct damage is not observed in AIH in remission.

Distinction from DILI can also be very difficult. Acute cases of AIH may present with centrilobular necrosis and other features considered typical of DILI. Such cases were first described in Japan, but have now increasingly also been observed in the Western world [65,66].

Histology does not only have a role in making the diagnosis, but also in the management of the disease [33]. This applies both to the initial biopsy, which by providing information on the grading of inflammatory activity and staging of the fibrosis helps

Table 3. Simplified scoring system for autoimmune hepatitis of the International Autoimmune Hepatitis Group (IAHG) [36].

Feature	Cut-off	Points
ANA or SMA +	≥1:40	1
ANA		
or SMA +	≥1:80	2*
or LKM	≥1:40	
or SLA/LP	Positive	
IgG	>upper limit of normal	1
	>1.10 times upper limit of normal	2
Liver histology	Compatible with AIH	1
	Typical AIH	2
Absence of viral hepatitis	Yes	2
		≥6: probably AIH
		≥7: definite AIH

*Addition of points achieved for all antibodies (maximum, 2 points).

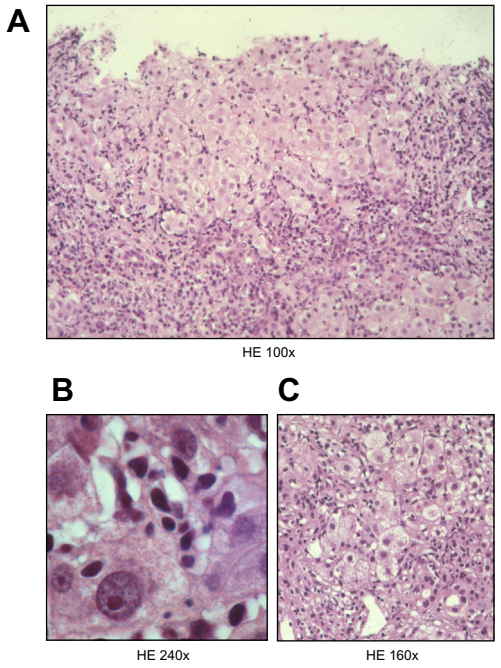

Fig. 4. Liver histology showing typical features of autoimmune hepatitis as described in [61]. (A) Typical histopathology of autoimmune hepatitis with portal/periportal predominance of necroinflammatory lesion and broad interface hepatitis. (B) Emperipolesis with a lymphocyte in the cytoplasm of a damaged hepatocyte. (C) Typical rosetting of hepatocytes in the area of interface hepatitis. Histopathology pictures were provided by Hans Peter Dienes, University of Vienna, Austria.

to guide treatment decisions, and to follow-up biopsies, which may be needed to assess response to treatment in difficult to treat patients. In addition, assessment of remission by liver biopsy is often recommended prior to a trial of treatment withdrawal, as remaining inflammatory activity reliably predicts relapse after cessation of immunosuppressive therapy [33].

The treatment aim in AIH is halting progression of fibrosis, and in many cases even regression of fibrosis can be achieved. Progression of fibrosis depends on the remaining inflammatory activity [67]. Persistence of interface hepatitis despite treatment is predictive of progressive fibrosis, and calls for more intensive immunosuppression. Complete histological remission, or minimal inflammatory activity as measured by a hepatitis activity index (HAI-score) of 3 or less, should be the aim of therapy [33]. Current guidelines define remission in AIH as normalization of the transaminase and IgG levels as well as histological remission as described above. Whether or not follow-up biopsy to demonstrate remission is required is a matter of debate. As most adult patients with repeatedly normal results for ALT and IgG do not display strong histological inflammatory activity, follow-up biopsy may not be necessary [68]. Furthermore liver biopsy and histomorphology are important in the differential diagnosis to exclude other causes of liver disease as well as comorbidities.

Treatment

Standard of care

The overall goal of treatment is achieving normalization of both transaminases (ALT/AST) and IgG. Otherwise disease progression cannot be avoided. For practical purposes induction of remission is distinguished from maintenance of remission. Once remission is achieved it is maintained with the lowest dose of immunosuppression possible. If complete remission is achieved, i.e. normalization of ALT/AST plus IgG, for at least 2–3 years immunosuppression may be terminated if histology does not show any inflammatory activity (see above) [33]. Such a complete remission is only achieved in about 25% of patients as reported by some authors [4,69,70], while in juvenile AIH complete remission is reported in over 80% of patients. However, results for complete remission under treatment must be distinguished from successful withdrawal after cessation of therapy. Only about 20% of patients maintain long-term remission after complete withdrawal of immunosuppression, documented normal transaminases and IgG levels as well as absence of any inflammatory activity on follow-up biopsies.

Medication to induce remission consists of either high dose corticosteroids alone or in combination with azathioprine (Table 4). Combination with azathioprine reduces steroid dose. Whether addition of azathioprine allows faster tapering of corticosteroids remains to be demonstrated. While steroids rapidly induce remission of symptoms, transaminases and IgG azathioprine needs 6 to 8 weeks to achieve optimum immunosuppression. Doses are given in Table 4. Starting dose of prednisolone is 60 mg as monotherapy while a lower dose of 30 mg is used if combined with azathioprine. European centres tend to give higher doses of prednisolone (i.e. 0.5–1.0 mg/kg body weight) from the start even when combined with azathioprine. European centres usually use azathioprine at a dose of 1–2 mg/kg body weight while in the US azathioprine is traditionally given at a flat dose of 50 mg [33]. TMPT genotyping may predict azathioprine toxicity. However, routine use of pretreatment TMPT genotyping is usually not recommended [33]. If diagnosis of AIH is uncertain or tolerability to azathioprine is in question, patients may be started on corticosteroid monotherapy and azathioprine is added as a corticosteroid sparing agent during the course of treatment (Fig. 5). In the case of uncertain diagnosis response to corticosteroid monotherapy is a diagnostic criterion [7].

The topical steroid budesonide may be used as an alternative to predniso(lo)ne in order to reduce steroid specific side effects

Table 4. Standard therapy for autoimmune hepatitis.

	Monotherapy	Combination therapy			
		Steroid		Azathioprine	
	Predniso(lo)ne (mg/d)	Predniso(lo)ne (mg/d)	Budesonide In non-cirrhotic patients (mg/d)	USA (mg/d)	Europe (mg/kg/d)
Week 1	60	30	9	50	1-2
Week 2	40	20	9	50	1-2
Week 3-4	30	15	6	50	1-2
Maintenance therapy	≤20	10	≤6	50	1-2
Reasons for preference	Cytopenia	Postmenopausal state			
	Thiopurin methytransferase deficiency	Osteoporosis			
	Pregnancy	Uncontrolled diabetes, hypertension, obesity			
	Malignancy	Acne			
	Expected therapy <6 months	Emotional lability			

Modified according to [86].

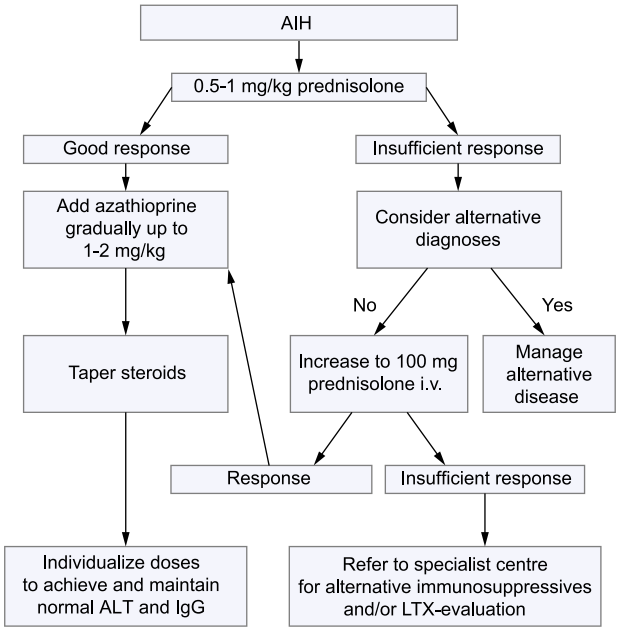

Fig. 5. Therapeutic algorithm for autoimmune hepatitis when starting with prednisolone monotherapy [54].

(SSSE) (Table 4) [71]. Budesonide for use in non-cirrhotic AIH patients has been approved in various countries including 15 member states of the European Union. Data are available from a large prospective trial using a combination of budesonide with azathioprine [69]. Budesonide together with azathioprine can induce remission with lower steroid specific adverse events. Moreover, switching from prednisone to budesonide reduces SSSE [69]. However, budesonide is acting via the same steroid receptor as predniso(lo)ne and thus budesonide should not be given to patients failing to respond to conventional steroid based therapies. Budesonide is only approved for non-cirrhotic patients. Pharmacokinetic benefits of a topical steroid are lost in patients with portal hypertension and portocaval shunting [72]. Furthermore portal vein thrombosis was reported as a severe adverse event in patients with PBC stage IV receiving budesonide in combination with UDCA as part of a clinical trial [73].

Budesonide is also effective in children and adolescents [74]. In particular, weight gain observed under prednisone plus azathioprine therapy is reversed after a switch to budesonide. However, treatment of AIH in children and adolescents may be different from adults since the disease in children seems to run a more aggressive course. Prominent centres use 2 mg/kg/day (maximum dose 60 mg) prednisolone which is decreased according to the response over a period of 8 weeks. As in adults some centres add azathioprine as a steroid sparing agent *ab initio* others prefer to add only if prednisolone does not achieve rapid remission. There is a debate whether in the prospective European Budesonide trial [74,69] steroid dosing in the prednisone/azathioprine arm was high enough which may explain higher remission rates in some reports for prednisone based AIH therapies in children and adolescents [75]. Multicentre prospective clinical trials are urgently needed to define the optimum immunosuppressive treatment regimen for AIH in children and adolescents.

Which particular regimen is used, predniso(lo)ne alone or a combination of either predniso(lo)ne or budesonide with azathioprine, depends on a careful benefit risk evaluation for the individual patient (Table 4, Fig. 5). At present EASL is preparing clinical practice guidelines on the diagnosis and management of AIH.

Remission is maintained with either predniso(lo)ne or azathioprine monotherapy or a combination of predniso(lo)ne/budesonide with azathioprine. Steroids are reduced to as low as 5 mg predniso(lo)ne or 3 mg budesonide per day [33]. A large single centre experience using azathioprine monotherapy for maintenance of remission was reported 20 years ago [76].

Management of treatment failure

If complete remission is not achieved, alternative immunosuppressive agents need to be explored. Prospective trials are usually missing. Thus evidence is mainly based on expert opinion. Second line therapies are cyclophilin inhibitors, such as cyclosporin A or tacrolimus. Side effects need to be considered. Nowadays mycophenolate mofetil (MMF) is widely used as a second line therapy for inducing and maintaining remission if azathioprine based therapies are not tolerated. Benefits of MMF as second line regimen are limited if previous azathioprine therapy failed

Table 5. Alternative therapies to corticosteroids and azathioprine.

Medication	Dose	Major side effects
Cyclosporine A	3-5 mg/kg KG/qd	Hypertension Renal insufficiency
Tacrolimus	3-5 mg bid	Hypertension Renal insufficiency Diabetes Polyneuropathy
Mycophenolate mofetil	750-1000 mg bid	GI-symptoms Diarrhoea, Leukopaenia
Anti-TNF mAb (Infliximab)	5 mg/kg body weight Every 2-8 weeks	Infections Induction of immune mediated liver injury
Anti-CD20 mAb (Rituximab)	2x1000 mg infusions Day 1 and 15	Reactivation of infections, e.g., hepatits B

Qd, once daily; bid, two times per day. Modified according to [86].

because of inefficacy [36]. Encouraging results were also reported for MMF as a first line therapy [77].

Remission is achieved in the majority of patients with such first or second line regimens. However, single cases need alternative therapies, since high inflammatory activity may lead to rapid fibrosis progression. Nowadays, biologicals interfering with signal transduction pathways are being explored although in small numbers of patients and usually in uncontrolled studies [30,33]. Examples are anti-TNF antibodies, e.g. infliximab, and antibodies to the B cell receptor CD20, e.g. rituximab [78,79] (Table 5). An individual benefit risk evaluation is mandatory since these drugs interfere with crucial pathways of the patients' immune system. Rituximab therapy may lead to reactivation of occult hepatitis B virus (HBV) infection. Therefore HBV status should be checked before starting rituximab therapy and patients positive for anti-HBc only should receive prophylactic oral anti-HBV therapy (e.g. tenofovir or entecavir) while under rituximab treatment or 6 months following last application. Furthermore treatment failure patients should be referred to tertiary referral centres with special expertise in the treatment of autoimmune liver diseases in particular when biologicals are considered in difficult to treat patients. This is important for the patients' safety and also for a state of the art scientific documentation; whenever possible as part of prospective multicentre trials. This is of particular importance for such rare diseases like AIH. Initial promising case reports demonstrated amelioration of AIH in patients where infliximab or rituximab was given because of other indications such as rheumatoid arthritis in the case of anti-TNF and B cell lymphoma or mixed cryoglobulinemia in the case of anti-CD20. Side effects of infliximab and rituximab are mainly infections (Table 5). Moreover, patients need to be tested for HBsAg since reactivation of hepatitis B may occur under rituximab therapy (Table 5). Individual cases have been successfully treated with anti-CD3 antibodies following promising results in diabetes mellitus. Recently, low dose anti-CD3 antibodies successfully induced remission in a xenoimmunized mouse model of AIH [80]. Adoptive transfer of Tregs is also being explored as a future therapy in difficult to treat AIH patients.

Transplantation

Liver transplantation is the ultimate rescue treatment for all liver diseases, but has only a minor role in AIH [81]. Approximately 4% of liver transplantations in both the US and Europe are due to AIH. The cornerstone of AIH management is in fact the avoidance of liver transplantation by timely diagnosis and adequate immunosuppressive therapy. In individual cases, however, liver transplantation may be required. Indication, timing and post-operative management can provide major challenges.

Fulminant AIH, in particular in children and young adults, may require emergency liver transplantation, as response to treatment is often too slow to allow recovery of liver function. Initial management in the case of fulminant AIH should be intravenous prednisolone therapy at high doses (up to 100 mg/d). In patients responding promptly, conservative management should be continued, but several case series have suggested that in patients not responding promptly the decision to proceed to emergency liver transplantation should be made, usually within two weeks, as prolonged high dose steroid therapy in the context of liver failure poses an unacceptable risk of fatal infections [82].

The majority of patients with AIH diagnosed at the stage of advanced cirrhosis recover sufficient liver function with immunosuppressive treatment and avoid liver transplantation. In a small minority of patients, however, recovery is not sufficient, despite immunosuppression and transplant may be the only option. Transplantation during childhood/adolescence is also required in some 20% of children presenting with AIH/sclerosing cholangitis overlap syndrome (autoimmune sclerosing cholangitis), a condition that eventually progresses to end-stage liver disease in about 50% of cases. In addition, non-adherence or inadequate immunosuppressive therapy may lead to progressive liver failure in AIH [83]. Especially in cases of non-adherence, frequently observed around puberty and during young adulthood, but which is also seen in older patients, the decision to proceed to transplantation needs to be weighed very carefully as non-adherence is a relative contraindication for liver transplantation, and the post-operative long-term management of these patients can be very frustrating.

Patients requiring liver transplantation for AIH are at particular risk of infections early after surgery [84]. Later in the post-operative period, acute rejection episodes occur more frequently than in other transplant recipients. In the ensuing months and years the recurrence of AIH presents a problem that should be prevented by adequate immunosuppressive therapy. The rules of treatment after transplantation are basically the same as prior to transplantation, and therefore these patients should receive azathioprine or MMF as part of their immunosuppressive regimen. While novel strategies for immunosuppression following liver transplantation for viral hepatitis avoid long-term use of corticosteroids they are an important component of immunosuppression for AIH patients.

De novo AIH with typical features of hypergammaglobulinaemia and autoantibodies has also been described in patients after liver transplantation was performed for other liver diseases. The exact nature of this condition is a matter of debate, as distinction from variants of rejection is difficult [85]. From the clinical point of view, this distinction may be somewhat academic, as both rejection and *de novo* AIH are characterized by a damaging immune response to liver cells requiring immunosuppressive therapy.

Conclusions and outlook

Although since the 1950s AIH has been effectively treated with immunosuppressive agents in the majority of cases, and is the

first liver disease in which medical therapy has been shown to prolong survival, it is still a disease of unknown cause. Several environmental factors are candidates to trigger this self-perpetuating disease process in a genetically susceptible individual. Several different triggers can induce a loss of tolerance towards the same molecular target(s). While between 1987 and 2000 most of the autoantigen targets of human autoantibodies have been cloned and molecularly characterized, there remains the need of more specific immunosuppressive agents with less long-term side effects. Our continued progress in the understanding of the molecular pathogenesis of AIH will lead the way to such effective and better tolerated agents, which hopefully will only need to be given for a finite period of time. At the moment Tregs are the focus of research endeavours, akin to other immune mediated diseases. However, this may change. A dream would come true if we were able to identify aetiological agent(s) triggering or maintaining the disease process. Then strategies could be developed that either prevent or cure AIH, eliminating it as an indication for liver transplantation. AIH shares the problems of all rare diseases. The AIH community must join the various "CARE FOR RARE" initiatives in order to obtain orphan drug status by the regulatory authorities for future drug development. Now that hepatitis C can be cured in over 90% of cases with rather short all-oral therapies, private and public funding will hopefully be directed to this rather neglected and potentially life-threatening disease.

Summary

AIH is a disease of unknown cause mainly occurring in women of all ages and races. The diagnosis is made based on clinical and laboratory criteria including specific circulating autoantibodies. Liver histology contributes to the diagnosis, its value mainly regarding grading and staging of damage and the exclusion of other morbidities or comorbidities. Our understanding of the molecular pathogenesis of AIH has improved in the last decades, including the identification of autoantigens at the molecular level as well as the function of TH1, TH2, and TH17 cells. In addition, GWAS in recent and upcoming years will help us to understand better disease susceptibility and may also help us to identify patients at particular risk. Therapies with corticosteroids alone, or in combination with azathioprine will be the SOC for the time being and will continue to save lives. Immunosuppressive drugs derived from transplantation medicine, like cyclosporin A, tacrolimus or MMF, offer an alternative for difficult to treat patients, novel more specific and safer immunosuppressive agents are urgently needed. Biological agents, like anti-TNF or anti-B cell antibodies, which interfere with important signal transduction pathways of the immune system have raised expectations within the AIH scientific community. Future therapies likely to enter clinical practice are the use of anti-CD3 or Tregs. However, our next goal is to eliminate AIH as an indication for liver transplantation. Here the end is at hand.

Conflict of interest

M.P. Manns; Falk Pharma consultant and clinical trial support. A.W. Lohse and D. Vergani have nothing to disclose.

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