Digestive and Liver Disease 42 (2010) 757–764

Contents lists available at ScienceDirect

Digestive and Liver Disease

journal homepage: www.elsevier.com/locate/dld

Review article

Current topics in autoimmune hepatitis

Luigi Muratori*, Paolo Muratori, Alessandro Granito, Giorgios Pappas, Fabio Cassani, Marco Lenzi

Department of Clinical Medicine, Alma Mater Studiorum – University of Bologna, Policlinico Sant'Orsola-Malpighi, Padiglione 11, Bologna, Italy

ARTICLE INFO

Article history:
Received 31 March 2010
Accepted 31 May 2010
Available online 7 July 2010

In memoriam of our magister ludi, the late Professor Francesco Bianco Bianchi (1939–2010).

Keywords:
Autoantibodies
Autoimmune hepatitis
Diagnosis
Liver
Therapy

ABSTRACT

Autoimmune hepatitis is a chronic liver disease of unknown aetiology characterized by interface hepatitis, hypergammaglobulinaemia and circulating autoantibodies. In the last decade a number of advancements have been made in the field of clinical and basic research: the simplified diagnostic criteria, the complete response defined as normalization of transaminase levels, the molecular identification of the antigenic targets of anti-liver cytosol antibody type 1 and anti-soluble liver antigen, the detection of anti-actin antibodies, the description of *de novo* autoimmune hepatitis after liver transplantation for non-autoimmune liver diseases, the characterization of autoimmune hepatitis with overlapping features of primary biliary cirrhosis or primary sclerosing cholangitis, the preliminary experience with novel treatment strategies based on cyclosporine, mycophenolate mofetil and budesonide, the role played by "impaired" regulatory T cells and the development of novel animal models of autoimmune hepatitis.

1. Introduction

Autoimmune hepatitis (AIH) is a chronic liver disease of unknown aetiology which can affect patients of all ages, sex, and race, characterized by interface hepatitis, hypergammaglobulinaemia – mostly of the IgG class – and circulating autoantibodies [1]. In the last decade a number of aspects of the disease have been clarified, especially in diagnosis, serological markers, pathogenesis, and treatment. The aim of this review is to highlight the areas of clinical and basic research where most of the progress has been made at the dawn of the new millennium.

2. Diagnostic criteria

AIH is mainly a clinical diagnosis, but the need to compare patients in different series led to the definition of a set of clinical, biochemical, immunological and histological criteria, first issued in 1993 [2], and revised in 1999 [3]. Only in 2008 a simplified scoring system has been devised for wider applicability in routine clinical practice [4]. This simplified scoring system includes just four criteria: autoantibody detection, IgG levels, liver histology, and exclusion of viral hepatitis (Table 1). Liver histology is an absolute prerequisite for making the diagnosis. So far, the simplified scoring system has been validated only retrospectively: the median overall sensitivity for "probable AIH" (6 points) was 91% and the median overall specificity was 94%; for "definite AIH" (7 or more points), the median overall sensitivity was 75.5% and the median overall specificity was 100% [5–8]. In the future, prospective studies are needed to validate this simplified scoring system. In addition, in everyday practice the response to immunosuppressive treatment is considered the post hoc clinical hallmark of AIH, therefore it has been suggested that treatment response might be considered as a further criterion [7].

3. Definition of treatment response

Clinical and biochemical response to immunosuppressive drugs is a key feature of AIH, and the trials performed in the early 1970s demonstrated the life-saving properties of steroids for patients with AIH. The definition of "response" has long been considered the acheivement of normal aminotransferases (or less than twice the upper normal limit) [9]. According to this definition of treatment response, nearly 80% of the AIH patients achieve remission within 3 years. However, this definition has been questioned [10] on the basis of several recent studies demonstrating that patients treated to only near-normal transaminases do have an increased risk of relapse [11,12], whereas persistent normalization greatly improves the long-term prognosis [13,14]. The suggestion to re-define remission as normalization of transaminases [15,16] has been formalized in the new AASLD guidelines on AIH [17], where normal transaminase levels are required to fulfill the definition of remission. With the application of these stricter and more severe criteria, the gen-

* Corresponding author. Tel.: +39 51 6363868; fax: +39 51 340877.
E-mail address: luigi.muratori@unibo.it (L. Muratori).

1590-8658/$36.00
doi:10.1016/j.dld.2010.05.019

Table 1
Simplified criteria for the diagnosis of autoimmune hepatitis (modified from Hennes et al. [4]).

Variable	Cutoff	Points
ANA or SMA	≥1:40	1
ANA or SMA	≥1:80	2
Anti-LKM1	≥1:40	2
Anti-SLA	Positive	2
IgG	>UNL	1
IgG	>1.1 times UNL	2
Liver histology	Compatible with AIH	1
Liver histology	Typical of AIH	2
Absence of viral hepatitis	Yes	2

Maximum of points achievable with autoantibodies: 2.
UNL: upper normal limit.
Typical AIH histology: 1. interface hepatitis (lymphocytic/lymphoplasmocytic infiltrates in portal tracts and extending into the lobule); 2. emperipolesis (active penetration by one cell into and through a larger cell); 3. hepatic rosette formation. To be considered typical, each of the three features must be present.
Compatible AIH histology: a picture of chronic hepatitis with lymphocytic infiltration without all the features considered typical.
Cumulative score = 6: probable AIH; ≥7: definite AIH.

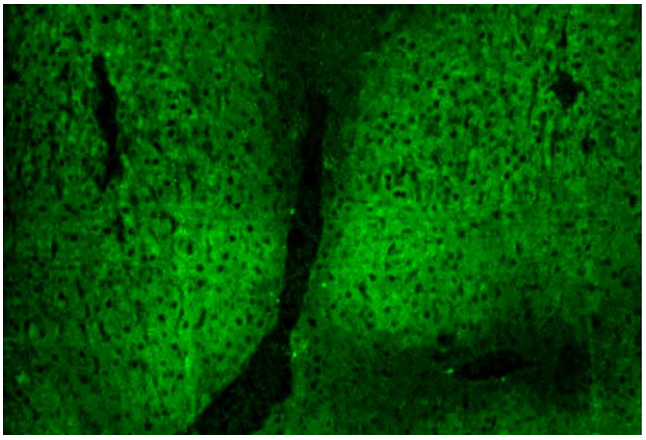

Fig. 1. Anti-liver cytosol type 1 (anti-LC1) pattern by indirect immunofluorescence on rat liver. The cytoplasmic staining of the hepatocytes is unevenly distributed throughout the liver lobule, and the perivenular layers are spared. Immunofluorescence is negative on rat kidney and stomach (not shown).

eral figures of treatment response are likely to be greatly modified (in our own experience from 73% using the 2002 AASLD criteria, to 26% with the new AASLD guidelines [14]).

Future clinical studies should also evaluate the potential role of transient elastography (FibroScan), a new non-invasive tool for measuring liver stiffness correlated to the histological stage of liver fibrosis, in order to assess the therapeutic response and to evaluate the prognosis of the disease during follow-up. To date FibroScan has been mostly validated in chronic hepatitis C [18] and B [19], whereas only scarce data are available in non-viral chronic liver disorders [20,21], therefore great caution is recommended in the interpretation of FibroScan results particularly in the setting of AIH [22], and larger studies are required before firm conclusions can be drawn.

4. Additional serological markers of AIH

Anti-nuclear antibodies (ANA), anti-smooth muscle antibodies (SMA) and liver/kidney microsomal antibody type 1 (anti-LKM1) are the classical serological markers of AIH [23–25]. Anti-liver cytosol antibody type 1 (anti-LC1) and anti-soluble liver antigen (anti-SLA) are additional markers originally described in the 1980s [26,27], but characterized in detail only in recent years. In addition, anti-actin antibodies can be detected using new immunofluorescence substrates.

4.1. Anti-LC1

The anti-LC1 staining pattern has been first recognized and described by indirect immunofluorescence on rat liver sections: anti-LC1 homogeneously stains the cytoplasmic compartment of periportal, but not perivenous, hepatocytes (Fig. 1), and is mainly detected in children or in young patients with AIH [14,26]. Anti-LC1 and anti-LKM1 are very often coexistent, and in such an occasion, the distinctive immunofluorescence of anti-LC1 is obscured by the reaction pattern of anti-LKM1, therefore other techniques such as immunodiffusion, counterimmunoelectrophoresis, or immunoblotting are required to detect anti-LC1 [28]. It is noteworthy that isolated anti-LC1, especially in the paediatric setting, often represents the only detectable autoantibody supporting the diagnosis of AIH and the need for early immunosuppressive therapy [29,30]. At variance with other autoantibodies in AIH, anti-LC1 seems to correlate with disease activity and may be useful as a marker of residual hepatocellular inflammation [29]. The molecular identity of the cytosolic target of anti-LC1 has been identified as formiminotransferase cyclodeaminase (FTCD) [31]. FTCD is a mammalian metabolic enzyme involved in the conversion of histidine to glutamic acid, and it is most highly expressed in the liver. It is bifunctional and is composed of distinct formiminotransferase (FT) and cyclodeaminase (CD) domains connected by a short linker. The FT domain transfers a formimino group from N-formimino-L-glutamic acid to tetrahydrofolate to generate glutamic acid and 5-formiminotetrahydrofolate, and the CD domain then converts the 5-formiminotetrahydrofolate to 5,10-methenyl tetrahydrofolate and ammonia. Native FTCD is an octamer with eight identical subunits arranged in a planar ring. In patients with AIH multiple regions of FTCD trigger a polyclonal anti-LC1 autoimmune response, which is mainly directed to conformation-sensitive epitopes located in the FT domain of FTCD [32]. Linear epitopes are located exclusively in the C-terminal 146 amino acids, in particular two specific linear epitopes are recognized at positions 428–434 (NTPEEKD) and 440–447 (LQEGLRRA) of human FTCD [33]. The suggestion that anti-LC1 reactivity may have pathogenic implications [29] has been recently supported by a murine model of AIH generated by DNA immunization against FTCD [34]. Using very sensitive assays such as immunoblotting and immunoprecipitation, anti-LC1 has been detected by a single group also in a large proportion of patients with hepatitis C [35].

4.2. Anti-SLA

Anti-SLA antibodies are detectable by radioimmunoassay and enzyme linked immunosorbent assays (ELISA), but cannot be revealed by immunofluorescence. SLA is not organ- or species-specific, however, the corresponding antigens are found in the 100,000 × *g* supernatant of a human liver homogenate [27]. The target antigen of anti-SLA is a 422-amino acid protein identified as UGA serine tRNA–protein complex [36–38]. Ironically, Gelpi et al. had described this autoantigen in the early 1990s; however, at that time it was not correlated to anti-SLA reactivity [39]. Epitope mapping identified a dominant immune reactivity directed to peptide p395–414 and a less prominent immune response to 2 other epitopes adjacent to the dominant epitope [40]. Anti-SLA has been originally proposed as the marker of a specific form of AIH, however, since ANA/SMA and anti-SLA positive patients share most biochemical, histological, and prognostic features, such a distinction is not clinically useful [41]. On the other hand, testing for anti-SLA may help in diagnosing AIH in rare patients without classical autoantibodies such as ANA, SMA and anti-LKM1. Recent studies with more sensitive serological tests revealed that anti-SLA are also detectable

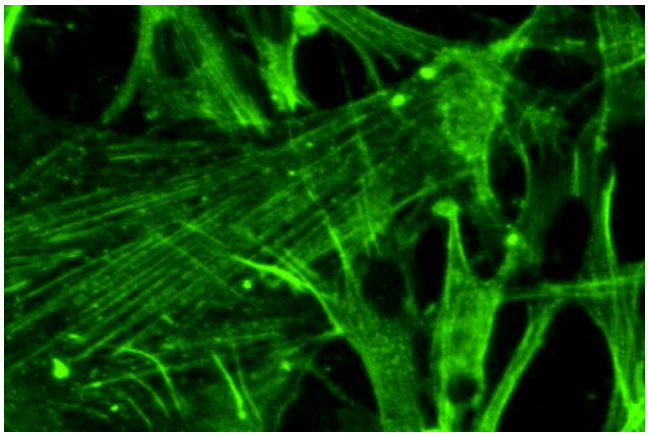

Fig. 2. Anti-actin antibodies pattern by indirect immunofluorescence on cultured fibroblasts. Microfilaments, which are extremely rich in polymerized actin (stress fibres) are easily recognized.

in children with autoimmune cholangitis [42] and in patients with HCV infection [43]. From the clinical standpoint, it is noteworthy that anti-SLA reactivity against conformational epitopes identifies patients with a worse prognosis [42].

4.3. Anti-actin antibodies

The staining of arterial vessels (V), glomerular mesangium (G) and peritubular structures (T) of rat kidney tissue detected by indirect immunofluorescence is the immunomorphological aspect of SMA, which are highly specific for AIH [24]. Attempts to characterize the antigen of SMA have provided circumstantial evidence which identify polymerized filamentous actin (F-actin) as such target antigen [44]. The availability of commercial ELISAs based on purified F-actin as antigenic source offered the opportunity to compare the diagnostic performance of the classical immunofluorescence assay (detection of the SMA pattern) with more standardized ELISAs for the detection of anti-F-actin antibodies. Even if sensitivity is the same, anti-F-actin antibodies are also found in nearly 20% of the controls. Using a higher cut-off, which improves the specificity of the test, the ELISA's sensitivity drops below that of SMA [45]. Actin is the main component of microfilaments and a very strict correlation between the SMA pattern and microfilament staining in various cell lines has been proven. Anti-actin antibodies are therefore easily and accurately detected using cultured human fibroblasts, HEp-2 cells, or vascular smooth muscle cell lines [46], as shown in Fig. 2.

5. *De novo* autoimmune hepatitis

In 1998 it has been reported for the first time that AIH can arise *de novo* after liver transplantation [47]. In contrast to the recurrence of disease in patients transplanted for AIH, this newly recognized condition affects patients transplanted for disorders other than AIH, usually of non-autoimmune nature. Features of this condition are identical to those of classical AIH, such as high IgG and serum autoantibodies including ANA, SMA, and typical or atypical anti-LKM antibodies (Fig. 3). The histological findings are those of interface hepatitis with an abundance of plasma cells, perivenular cell necrosis, bridging fibrosis and collapse characteristic of AIH, and different from features of acute rejection that are characterized by a lymphomononuclear cell infiltrate confined to the portal tract, venous endothelitis, and bile duct damage. *De novo* AIH after liver transplantation does not respond to antirejection treatment, that is, bolus infusions of high-dose steroids and increased dose of calcineurin inhibitors, but only to the conventional treatment

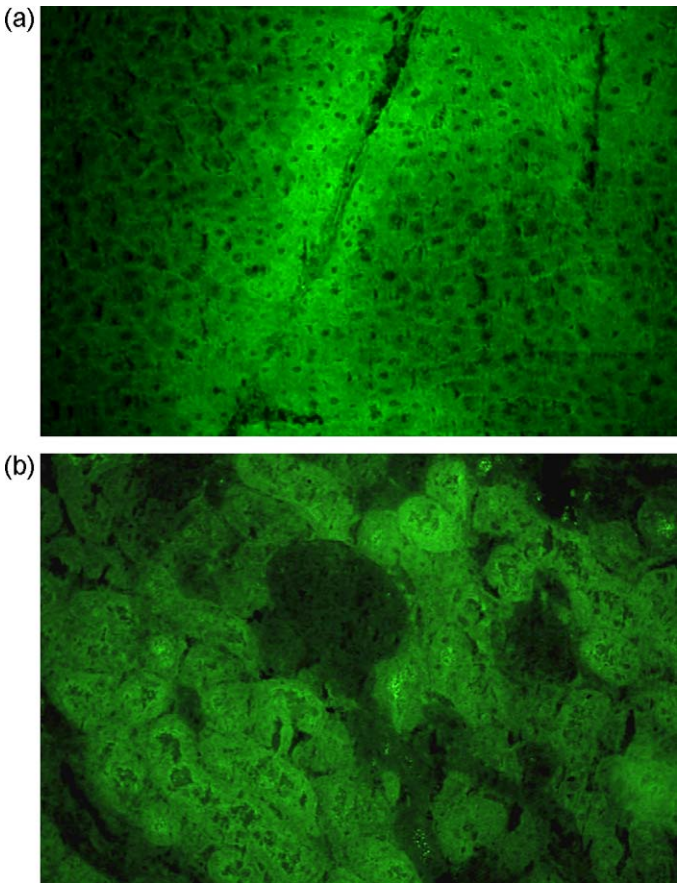

Fig. 3. Atypical anti-liver/kidney microsomal antibody (anti-LKM) pattern by indirect immunofluorescence on rat liver (panel a) and kidney (panel b). Liver staining is cytoplasmic and is mostly located in the hepatocytes around the central vein; a perimembrane reinforce is also appreciated in hepatocytes within the liver lobule (a). Kidney tubules are homogeneously stained, whereas vessels and glomeruli are negative (b).

for AIH, initially prednisolone at the dose of 2 mg/kg/day (maximum 60 mg/day) and azathioprine at a dose of 2 mg/kg/day, with tapering of the prednisolone dose until remission. After the original description, other cases of *de novo* AIH have been reported by several other groups both in the paediatric and in the adult setting [48–52]. The use of the term "autoimmune" to define hepatitis affecting an allogeneic organ has been debated, leading to alternative labels including "posttransplant immune hepatitis" or "graft dysfunction mimicking AIH" [49,50]. However, the concept of recurrence of AIH after LT in patients transplanted because of AIH has never been questioned, despite the fact that also in this condition the target organ is allogeneic. The antigenic targets for liver-specific autoimmunity are species-specific, therefore shared by both recipient and donor livers, and the graft is repopulated by dendritic cells of recipient origin. The term *de novo* AIH, with its clinical and therapeutic implications, therefore, remains the best until the pathogenesis of the condition is clarified.

The identification of glutathione S-transferase T1 (GSTT1) as target antigen of the atypical anti-LKM reactivity observed in the *de novo* AIH [53] suggests the presence of an alloimmune reaction due to a GSTT1 genetic incompatibility between donor and recipient as triggering factor of both anti-GSTT1 reactivity and the *de novo* liver disease [54,55]. However, clinically evident disease is not observed in all patients with anti-GSTT1 antibodies, and the risk of developing the disease is increased by male donor gender, non-alcoholic aetiology of original liver disease, and a high anti-GSTT1 titre [56]. In addition, in a retrospective series of 97 patients who received

Table 2
Diagnostic criteria for the AIH/PBC overlap syndrome (modified from Chazouilleres et al. [63]).

PBC criteria
1. ALP > 2× UNL, or gammaGT > 5× UNL
2. Anti-mitochondrial antibodies ≥ 1:40
3. Liver biopsy showing florid bile duct lesions
AIH criteria
1. ALT > 5× UNL
2. IgG > 2× UNL or a positive test for anti-smooth muscle antibodies
3. Liver biopsy showing moderate or severe periportal or periseptal lymphocytic piecemeal necrosis

ALP: alkaline phosphatase; gammaGT: gamma-glutalmyltranspeptidase; UNL: upper normal limit; ALT: alanine aminotransferase.
At least 2 of 3 criteria for both PBC and AIH should be fulfilled.

liver transplantation for non-autoimmune end-stage liver disease, 9 (10%) developed *de novo* AIH; of these, 7 became positive for atypical LKM, and the remaining 2 for anti-dsDNA [57].

6. Overlap syndromes

Patients with AIH may have overlapping features with concurrent primary sclerosing cholangitis (PSC) [58–61] or primary biliary cirrhosis (PBC) [60,62–65]. Overlap syndromes lack codified clinical or pathological definitions, and they do not have a particular aetiological agent or distinctive pathogenic mechanisms. The designations are arbitrary and imprecise, and the clinical phenotypes of patients with the same overlap designation may often be different. However, the recently issued EASL Clinical Practice Guidelines on cholestatic liver diseases [66] endorsed the strictest criteria for the diagnosis of AIH/primary bilary cirrhosis overlap syndrome proposed by Chazouilleres et al. [63] and reported in Table 2. From the diagnostic standpoint, the concomitant seropositivity for anti-mitochondrial antibodies and anti-double stranded DNA appears to be the peculiar immunoserological profile of the AIH/PBC overlap syndrome, as it has been reported in 47% of such patients, with a specificity of 98% [67,68].

Twenty percent of patients with AIH have antimitochondrial antibodies [69–72]; some of them (10%) may have histological features of mild bile duct injury, more pronounced biochemical cholestasis, but respond to immunosuppressive treatment similarly to classical AIH [73]. Any or all of these features suggest an overlap syndrome with PBC. Similarly, 16% of patients with AIH have concurrent inflammatory bowel disease [74]; 10% (adults) to 50% (children) have biliary changes reminiscent of PSC by magnetic resonance imaging or retrograde endoscopic cholangiography [75,76] and 13% failed to respond to corticosteroids [77]. Any or all of these features suggest an overlap syndrome with PSC. The overlap syndrome with PSC may be associated with intrahepatic bile duct changes (small duct PSC) [78,79] or extrahepatic bile duct changes with or without intrahepatic findings (classical PSC).

Management of the overlap syndromes is empirical and based on the predominant manifestations of the disease. According to the EASL guidelines [66], combined therapy with ursodeoxycholic acid (UDCA) and steroids is the recommended therapeutic option in patients with PBC–AIH overlap syndrome. An alternative approach is to start with UDCA only and to add corticosteroids if UDCA therapy has not induced an adequate biochemical response in an appropriate time span (3 months). Steroid sparing agents should be considered in patients requiring long-term immunosuppression.

7. Novel drug therapies

In most cases, patients with AIH can be treated successfully with predniso(lo)ne, with or without azathioprine, however, a considerable number of patients tolerate azathioprine poorly, or do not respond completely to treatment [80]. Several other drugs such as cyclophosphamide [81], methotrexate [82], rapamycin [83], rituximab [84], intravenous immunoglobulin [85], deflazacort [86], ursodeoxycholic acid [87,88] and 6-thioguanines [89,90] have been proposed in AIH, but their use has been limited to single reports or small series of patients.

Cyclosporine, mycophenolate mofetil (MMF) and budesonide are the novel treatments for which more experience is available, and will be discussed more in detail.

7.1. Cyclosporine

Cyclosporine A, a lipophilic cyclic peptide of 11 residues acting on calcium-dependent signalling, appeared to be effective in a group of adult patients who were corticosteroid-resistant [91]. The principal difficulty in advocating widespread use of this drug as first-line therapy relates to its toxicity profile, particularly with long-term use (increased risk of hypertension, renal insufficiency, hyperlipidaemia, hirsutism, infection, and malignancy). A regimen of cyclosporine for 6 months followed by the administration of prednisone and azathioprine was reported as successful in inducing remission in children [92,93]. Encouraging data on long-term safety of the cyclosporine regimen have been reported, albeit on quite a small number of paediatric patients [94]. However, whether this mode of induction has any advantage over the standard treatment remains to be evaluated in controlled studies on a large number of patients stratified for disease severity.

7.2. Mycophenolate mofetil

MMF has demonstrated encouraging results in a few studies on small cohorts of patients with AIH. MMF hampers purine synthesis by acting as inhibitor of inosine monophosphate dehydrogenase. MMF treatment has been established successfully in many other conditions, such as rheumatoid arthritis or Crohn's disease. In addition, the drug has become routinely used in immunosuppressant regimens in patients who have undergone solid organ transplantation [95]. Richardson et al. [96] have reported on successful MMF treatment of seven AIH patients. Five of these patients had normal transaminases after 3 months of treatment, as well as a significant reduction in steroid dose and hepatic activity index. These findings were further supported by a series of five Canadian patients who also achieved transaminase normalization, a steroid sparing effect and histological remission [97]. Lately, Inductivo-Yu et al. [98] and Chatur et al. [99] have reported on additional 31 patients with AIH being successfully treated with MMF. In addition to the benefits observed in the earlier case report series, Inductivo-Yu et al. have also documented that the inflammatory scores and Ishak fibrosis scores were decreased. In 2008, Hennes et al. [100] reported the largest cohort to date of 36 AIH patients treated with MMF. In contrast to earlier studies, they observed a much lower frequency of response to MMF treatment, as only 14 patients (39%) experienced remission, which was still defined as aspartate transferase (AST) less than twice the upper normal limits. Twenty-two patients (61%) did not respond sufficiently to MMF. In a subset analysis, they further demonstrated that the response rate to MMF was dependent on the cause of treatment cessation of azathioprine. Hlivko et al. [101] performed a retrospective longitudinal analysis of 29 AIH patients: MMF was associated with a high rate of intolerance (34%), but most of those who could tolerate it entered remission (84%), defined as AST less than twice the upper limit of normal. Wolf et al. report their retrospective experience on 16 patients with AIH [102]: 5 of 16 patients (31%) achieved biochemical remission, defined as a reduction in ALT to less than twice normal. Seven additional patients (44%) were maintained in biochemical remission.

Two patients had an incomplete response to MMF and two patients experienced treatment failure. Only one patient discontinued MMF because of paresthesias.

In the paediatric setting MMF appears to be an effective and well-tolerated rescue therapy for children with AIH who are resistant to or intolerant of standard immunosuppression [103].

MMF certainly provides a valuable therapeutic option in patients with AIH, even if only a proportion of them may benefit from it.

7.3. Budesonide

Budesonide is a corticosteroid with the highest affinity for the glucocorticoid receptor when compared with other steroids. The drug has a high first pass metabolism, which results in a low incidence of systemic glucocorticoid-related adverse effects [104]. Budesonide can induce remission of Crohn's disease [105], even if it is less effective than conventional steroids, but also with fewer adverse events and lower adrenal suppression [106]. Over the past decade, several small studies have tested the efficacy of budesonide in inducing remission of AIH, with contrasting results [107–109]. Side effects and treatment failure were more often observed in cirrhotic patients [110,111]. Budesonide appears to be recommended for patients with AIH who are either intolerant to conventional steroids plus azathioprine, or steroid-dependent [112].

Recently, Manns et al. have compared combined budesonide and azathioprine to standard prednisolone treatment in 203 patients with AIH [113]. The primary end point of the study was complete biochemical remission (i.e., normal transaminase levels) without the typical steroid side effects (moonface, acne, buffalo hump, hirsutism, striae, diabetes, glaucoma or increased intraocular pressure). Budesonide was initiated at 3 mg thrice daily and was reduced to 3 mg twice daily upon remission. Prednisolone was initiated at 40 mg daily and tapered to 10 mg daily after 8 weeks. Azathioprine was given to both groups at a dose of 1–2 mg/kg/day. The primary endpoint (biochemical remission without steroid-related side effects) was significantly more often reached by patients in the budesonide group (47% vs. 18.4%). In the open label component of the study (for a further 6 months), 173 patients received budesonide. Again, complete biochemical remission was more frequently observed in the cohort originally randomized to budesonide (68.2% vs. 50.6%) [114]. However, for the long-term results (normalization of bilirubin and IgG over a 6-month period), budesonide was not superior to prednisolone. The main advantage of budesonide seems to be the lower incidence of side effects. However, long-term results of budesonide treatment are still unknown. Special attention needs to be given to the question of a comparable prednisolone dosage in the conventionally treated patients. Moreover, as patients with cirrhosis or a fulminant presentation were excluded from this study, the applicability of these findings is limited to non-cirrhotic patients without severe liver failure.

8. Impairment of T regulatory cells

An impairment of immunoregulatory mechanisms has been repeatedly postulated in the setting of both human and experimental autoimmunity [115,116]. Recent experimental evidence confirms an impairment of the immunoregulatory function in AIH. Thus, among recently defined T cell subsets with potential immunosuppressive function, CD4+ T cells constitutively expressing the interleukin 2 receptor alfa chain (CD25) (T regulatory cells, T-regs) have emerged as the dominant immunoregulatory lymphocytes [116]. These T-regs, which in health represent nearly 5% of the total population of peripheral CD4+ T cells, control the innate and the adaptive immune responses by preventing the proliferation and effector function of autoreactive T cells. Their mechanism of action involves mainly a direct contact with the target cells, and to a lesser extent the release of immunoregulatory cytokines, such as interleukin 10 and transforming growth factor beta 1 (TGF-beta 1). In addition to CD25, which is also present on T cells undergoing activation, T-regs express a number of additional markers such as the glucocorticoid induced tumour necrosis factor receptor, CD62L, the cytotoxic T lymphocyte associated protein-4 (CTLA-4) and the forkhead/winged helix transcription factor FOXP3, the expression of which is closely associated with the acquisition of regulatory properties [117,118].

In patients with AIH, T-regs are defective both in number and in function compared to normal controls and these abnormalities relate to the stage of disease, being more evident at diagnosis than during drug-induced remission [119–121]. If loss of immunoregulation was central to the pathogenesis of autoimmune liver disease, treatment should concentrate on restoring T-regs' ability to expand, with consequent increase in their number and function. This is at least partially achieved by standard immunosuppression, since T-reg numbers do increase during remission [119–121]. In addition, functional T-regs can be expanded ex vivo and generated ex novo in patients with AIH [122], a fundamental step in reconstituting impaired immune regulation and restoring peripheral tolerance. However, the defect in immunoregulation in AIH is multifaceted and is not confined to classical CD4+CD25hi T-cells, which also involve other cells with immunoregulatory properties such as natural killer T cells and gamma-delta T cells [123].

9. New animal models of AIH

In recent years new animal models have provided important information about the pathogenesis of the disease [124,125]. One such model has been developed by immunization of female mice with cytomegalovirus vectors that express the antigenic region of human CYP2D6 and human FTCD [34]: immunized mice developed the peak serum aminotransferase abnormalities 4–7 months after the injection, showed periportal, portal and lobular inflammatory infiltrates, produced anti-LKM1 and anti-LC1 antibodies, and had liver-infiltrating CD4+, CD8+ and B lymphocytes, including cytotoxic-specific T cells. Genetic background is an important component of this animal model of AIH, as mouse strains with different genes within and outside the major histocompatibility complex (MHC) exhibited different susceptibilities for the disease [126]. In this murine model, peripheral tolerance and development of regulatory T cells, and not sexual hormone nor central tolerance, were the main factors for susceptibility to AIH in females [127].

The most recent model uses an adenovirus expressing human CYP2D6 to infect mice, and is based on the premise that viral infections initiate autoimmunity by inducing a strong inflammatory response within the liver; the inflammatory response in turn attracts aggressive lymphocytes; molecular mimicry ensues between the viral antigens and self-antigens; and a chronic liver disease emerges as a consequence of antigen-sensitized, promiscuous T cells infiltrating the liver [128]. In this model, the viral infection produces a transient liver injury that is followed within 2 weeks by a severe hepatitis that can persist for more than 3 months [129]. The chronic hepatitis is triggered only by adenovirus expressing CYP2D6, and is characterized by histological features of AIH, high titres of anti-LKM1 antibodies recognizing the immunodominant epitope WDPAQPPRD of cytochrome P450 2D6 (CYP2D6), hepatic infiltration with CD4+ lymphocytes, and extensive hepatic fibrosis [129]. In the future, these animal models will possibly provide a tool to dissect the fine mechanisms involved in the immunopathogenesis of the autoimmune-mediated chronic

hepatic injury as seen in human AIH, and to develop innovative methods of therapeutic interference [130].

10. Conclusion and future perspectives

In conclusion, AIH still represents a diagnostic and therapeutic challenge in the new millennium, however the availability of simplified diagnostic criteria, clear definitions of treatment response, reliable and standardized serological markers with prognostic implications, and novel treatment strategies will greatly help the clinical hepatologist to promptly recognize AIH and to treat it most effectively; on the other hand, the development of novel animal models of AIH and the understanding of the key role played by the T cell-network with regulatory properties will offer exciting new paths of investigation in the pathogenesis of the disease.

Conflict of interest

None declared.

References

[1] Krawitt EL. Autoimmune hepatitis. N Engl J Med 2006;354:54–66.
[2] Johnson PJ, McFarlane IG. Meeting report: International Autoimmune Hepatitis Group. Hepatology 1993;18:998–1005.
[3] Alvarez F, Berg PA, Bianchi FB, et al. International Autoimmune Hepatitis Group Report: review of criteria for diagnosis of autoimmune hepatitis. J Hepatol 1999;31:929–38.
[4] Hennes EM, Zeniya M, Czaja AJ, et al. Simplified criteria for the diagnosis of autoimmune hepatitis. Hepatology 2008;48:169–76.
[5] Czaja AJ. Performance parameters of the diagnostic scoring systems for autoimmune hepatitis. Hepatology 2008;48:1540–8.
[6] Muratori P, Granito A, Pappas G, et al. Validation of simplified diagnostic criteria for autoimmune hepatitis in Italian patients. Hepatology 2009;49:1782–3.
[7] Wiegard C, Schramm C, Lohse AW. Scoring systems for the diagnosis of autoimmune hepatitis: past, present, and future. Semin Liver Dis 2009;29:254–61.
[8] Miyake Y, Iwasaki Y, Kobashi H, et al. Clinical features of autoimmune hepatitis diagnosed based on simplified criteria of the International Autoimmune Hepatitis Group. Dig Liver Dis 2010;42:210–5.
[9] Czaja AJ, Freese DK. Diagnosis and treatment of autoimmune hepatitis. Hepatology 2002;36:479–97.
[10] Yeoman AD, Longhi MS, Heneghan MA. Review article: the modern management of autoimmune hepatitis. Aliment Pharmacol Ther 2010;31:771–87.
[11] Verma S, Gunuwan B, Mendler M, et al. Factors predicting relapse and poor outcome in type I autoimmune hepatitis: role of cirrhosis development, patterns of transaminases during remission and plasma cell activity in the liver biopsy. Am J Gastroenterol 2004;99:1510–6.
[12] Montano-Loza AJ, Carpenter HA, Czaja AJ. Improving the end point of corticosteroid therapy in type 1 autoimmune hepatitis to reduce the frequency of relapse. Am J Gastroenterol 2007;102:1005–12.
[13] Miyake Y, Iwasaki Y, Terada R, et al. Persistent normalization of serum alanine aminotransferase levels improves the prognosis of type 1 autoimmune hepatitis. J Hepatol 2005;43:951–7.
[14] Muratori P, Granito A, Quarneti C, et al. Autoimmune hepatitis in Italy: the Bologna experience. J Hepatol 2009;50:1210–8.
[15] Verma S. In type 1 autoimmune hepatitis (AIH), should remission be redefined as normalization of transaminases? J Hepatol 2006;44:819–20, 820–1.
[16] Al-Chalabi T, Heneghan MA. Remission in autoimmune hepatitis: what is it, and can it ever be achieved? Am J Gastroenterol 2007;102:1013–5.
[17] Manns MP, Czaja AJ, Gorham JD, et al. Diagnosis and management of autoimmune hepatitis. Hepatology, in press.
[18] Castera L, Forns X, Alberti A. Non-invasive evaluation of liver fibrosis using transient elastography. J Hepatol 2008;48:835–47.
[19] Zhang YG, Wang BE, Wang TL, et al. Assessment of hepatic fibrosis by transient elastography in patients with chronic hepatitis B. Pathol Int 2010;60:284–90.
[20] Anastasiou J, Alisa A, Virtue S, et al. Noninvasive markers of fibrosis and inflammation in clinical practice: prospective comparison with liver biopsy. Eur J Gastroenterol Hepatol 2010;22:474–80.
[21] Obara N, Ueno Y, Fukushima K, et al. Transient elastography for measurement of liver stiffness measurement can detect early significant hepatic fibrosis in Japanese patients with viral and nonviral liver diseases. J Gastroenterol 2008;43:720–8.
[22] Romanque P, Stickel F, Dufour JF. Disproportionally high results of transient elastography in patients with autoimmune hepatitis. Liver Int 2008;28:1177–8.
[23] Bianchi FB, Muratori P, Muratori L. New autoantibodies and autoantigens in autoimmune hepatitis. Clin Liver Dis 2002;6:785–97.
[24] Vergani D, Alvarez F, Bianchi FB, et al. Liver autoimmune serology: a consensus statement from the committee for autoimmune serology of the International Autoimmune Hepatitis Group. J Hepatol 2004;41:677–83.
[25] Bogdanos DP, Mieli-Vergani G, Vergani D. Autoantibodies and their antigens in autoimmune hepatitis. Semin Liver Dis 2009;29:241–53.
[26] Martini E, Abuaf N, Cavalli F, et al. Antibody to liver cytosol (anti-LC1) in patients with autoimmune chronic active hepatitis type 2. Hepatology 1988;8:1662–6.
[27] Manns M, Gerken G, Kyriatsoulis A, et al. Characterisation of a new subgroup of autoimmune chronic active hepatitis by autoantibodies against a soluble liver antigen. Lancet 1987;1:292–4.
[28] Muratori L, Cataleta M, Muratori P, et al. Detection of anti-liver cytosol antibody type 1 (anti-LC1) by immunodiffusion, counterimmunoelectrophoresis and immunoblotting: comparison of different techniques. J Immunol Methods 1995;187:259–64.
[29] Muratori L, Cataleta M, Muratori P, et al. Liver/kidney microsomal antibody type 1 and liver cytosol antibody type 1 concentrations in type 2 autoimmune hepatitis. Gut 1998;42:721–6.
[30] Bridoux-Henno L, Maggiore G, Johanet C, et al. Features and outcome of autoimmune hepatitis type 2 presenting with isolated positivity for anti-liver cytosol antibody. Clin Gastroenterol Hepatol 2004;2:825–30.
[31] Lapierre P, Hajoui O, Homberg JC, et al. Formiminotransferase cyclodeaminase is an organ-specific autoantigen recognized by sera of patients with autoimmune hepatitis. Gastroenterology 1999;116:643–9.
[32] Muratori L, Sztul E, Muratori P, et al. Distinct epitopes on formiminotransferase cyclodeaminase induce autoimmune liver cytosol antibody type 1. Hepatology 2001;34:494–501.
[33] Lapierre P, Johanet C, Alvarez F. Characterization of the B cell response of patients with anti-liver cytosol autoantibodies in type 2 autoimmune hepatitis. Eur J Immunol 2003;33:1869–78.
[34] Lapierre P, Djilali-Saiah I, Vitozzi S, et al. A murine model of type 2 autoimmune hepatitis: xenoimmunization with human antigens. Hepatology 2004;39:1066–74.
[35] Beland K, Lapierre P, Marceau G, et al. Anti-LC1 autoantibodies in patients with chronic hepatitis C virus infection. J Autoimmun 2004;22:159–66.
[36] Wies I, Brunner S, Henninger J, et al. Identification of target antigen for SLA/LP autoantibodies in autoimmune hepatitis. Lancet 2000;355:1510–5.
[37] Costa M, Rodriguez-Sanchez JL, Czaja AJ, et al. Isolation and characterization of cDNA encoding the antigenic protein of the human tRNP(Ser)Sec complex recognized by autoantibodies from patients with type-1 autoimmune hepatitis. Clin Exp Immunol 2000;121:364–74.
[38] Volkmann M, Martin L, Baurle A, et al. Soluble liver antigen: isolation of a 35-kd recombinant protein (SLA-p35) specifically recognizing sera from patients with autoimmune hepatitis. Hepatology 2001;33:591–6.
[39] Gelpi C, Sontheimer EJ, Rodriguez-Sanchez JL. Autoantibodies against a serine tRNA–protein complex implicated in cotranslational selenocysteine insertion. Proc Natl Acad Sci U S A 1992;89:9739–43.
[40] Herkel J, Heidrich B, Nieraad N, et al. Fine specificity of autoantibodies to soluble liver antigen and liver/pancreas. Hepatology 2002;35:403–8.
[41] Ballot E, Homberg JC, Johanet C. Antibodies to soluble liver antigen: an additional marker in type 1 auto-immune hepatitis. J Hepatol 2000;33:208–15.
[42] Ma Y, Okamoto M, Thomas MG, et al. Antibodies to conformational epitopes of soluble liver antigen define a severe form of autoimmune liver disease. Hepatology 2002;35:658–64.
[43] Vitozzi S, Lapierre P, Djilali-Saiah I, et al. Anti-soluble liver antigen (SLA) antibodies in chronic HCV infection. Autoimmunity 2004;37:217–22.
[44] Bottazzo GF, Florin-Christensen A, Fairfax A, et al. Classification of smooth muscle autoantibodies detected by immunofluorescence. J Clin Pathol 1976;29:403–10.
[45] Granito A, Muratori L, Muratori P, et al. Antibodies to filamentous actin (F-actin) in type 1 autoimmune hepatitis. J Clin Pathol 2006;59:280–4.
[46] Muratori P, Muratori L, Agostinelli D, et al. Smooth muscle antibodies and type 1 autoimmune hepatitis. Autoimmunity 2002;35:497–500.
[47] Kerkar N, Hadzic N, Davies ET, et al. De-novo autoimmune hepatitis after liver transplantation. Lancet 1998;351:409–13.
[48] Andries S, Casamayou L, Sempoux C, et al. Posttransplant immune hepatitis in pediatric liver transplant recipients: incidence and maintenance therapy with azathioprine. Transplantation 2001;72:267–72.
[49] Gupta P, Hart J, Millis JM, et al. De novo hepatitis with autoimmune antibodies and atypical histology: a rare cause of late graft dysfunction after pediatric liver transplantation. Transplantation 2001;71:664–8.
[50] Heneghan MA, Portmann BC, Norris SM, et al. Graft dysfunction mimicking autoimmune hepatitis following liver transplantation in adults. Hepatology 2001;34:464–70.
[51] Hernandez HM, Kovarik P, Whitington PF, et al. Autoimmune hepatitis as a late complication of liver transplantation. J Pediatr Gastroenterol Nutr 2001;32:131–6.
[52] Miyagawa-Hayashino A, Haga H, Egawa H, et al. Outcome and risk factors of de novo autoimmune hepatitis in living-donor liver transplantation. Transplantation 2004;78:128–35.
[53] Aguilera I, Wichmann I, Sousa JM, et al. Antibodies against glutathione S-transferase T1 in patients with immune hepatitis after liver transplantation. Transplant Proc 2003;35:712.
[54] Aguilera I, Sousa JM, Gavilan F, et al. Glutathione S-transferase T1 mismatch constitutes a risk factor for de novo immune hepatitis after liver transplantation. Liver Transpl 2004;10:1166–72.
[55] Aguilera I, Sousa JM, Gavilan F, et al. Glutathione S-transferase T1 genetic mismatch is a risk factor for de novo immune hepatitis in liver transplantation. Transplant Proc 2005;37:3968–9.

[56] Salcedo M, Rodriguez-Mahou M, Rodriguez-Sainz C, et al. Risk factors for developing de novo autoimmune hepatitis associated with anti-glutathione S-transferase T1 antibodies after liver transplantation. Liver Transpl 2009;15:530–9.
[57] Granito A, Vannini A, Quarneti C, et al. Clinical impact and behaviour of serum autoantibodies in adult liver transplantation. J Hepatol 2010;52(Suppl. 1):S197–8.
[58] McNair AN, Moloney M, Portmann BC, et al. Autoimmune hepatitis overlapping with primary sclerosing cholangitis in five cases. Am J Gastroenterol 1998;93:777–84.
[59] Gohlke F, Lohse AW, Dienes HP, et al. Evidence for an overlap syndrome of autoimmune hepatitis and primary sclerosing cholangitis. J Hepatol 1996;24:699–705.
[60] Muratori L, Cassani F, Pappas G, et al. The hepatitic/cholestatic "overlap" syndrome: an Italian experience. Autoimmunity 2002;35:565–8.
[61] Olsson R, Glaumann H, Almer S, et al. High prevalence of small duct primary sclerosing cholangitis among patients with overlapping autoimmune hepatitis and primary sclerosing cholangitis. Eur J Intern Med 2009;20: 190–6.
[62] Lohse AW, zum Buschenfelde KH, Franz B, et al. Characterization of the overlap syndrome of primary biliary cirrhosis (PBC) and autoimmune hepatitis: evidence for it being a hepatitic form of PBC in genetically susceptible individuals. Hepatology 1999;29:1078–84.
[63] Chazouilleres O, Wendum D, Serfaty L, et al. Primary biliary cirrhosis–autoimmune hepatitis overlap syndrome: clinical features and response to therapy. Hepatology 1998;28:296–301.
[64] Talwalkar JA, Keach JC, Angulo P, et al. Overlap of autoimmune hepatitis and primary biliary cirrhosis: an evaluation of a modified scoring system. Am J Gastroenterol 2002;97:1191–7.
[65] Twaddell WS, Lefkowitch J, Berk PD. Evolution from primary biliary cirrhosis to primary biliary cirrhosis/autoimmune hepatitis overlap syndrome. Semin Liver Dis 2008;28:128–34.
[66] EASL clinical practice guidelines: management of cholestatic liver diseases. J Hepatol 2009;51:237–67.
[67] Muratori P, Granito A, Pappas G, et al. The serological profile of the autoimmune hepatitis/primary biliary cirrhosis overlap syndrome. Am J Gastroenterol 2009;104:1420–5.
[68] Efe C, Purnak T, Ozaslan E, et al. The serological profile of the autoimmune hepatitis/primary biliary cirrhosis overlap syndrome. Am J Gastroenterol 2010;105:226.
[69] Nezu S, Tanaka A, Yasui H, et al. Presence of antimitochondrial autoantibodies in patients with autoimmune hepatitis. J Gastroenterol Hepatol 2006;21:1448–54.
[70] Montano-Loza AJ, Carpenter HA, Czaja AJ. Frequency, behavior, and prognostic implications of antimitochondrial antibodies in type 1 autoimmune hepatitis. J Clin Gastroenterol 2008;42:1047–53.
[71] Mishima S, Omagari K, Ohba K, et al. Clinical implications of antimitochondrial antibodies in type 1 autoimmune hepatitis: a longitudinal study. Hepatogastroenterology 2008;55:221–7.
[72] O'Brien C, Joshi S, Feld JJ, et al. Long-term follow-up of antimitochondrial antibody-positive autoimmune hepatitis. Hepatology 2008;48:550–6.
[73] Czaja AJ, Muratori P, Muratori L, et al. Diagnostic and therapeutic implications of bile duct injury in autoimmune hepatitis. Liver Int 2004;24:322–9.
[74] Bittencourt PL, Farias AQ, Porta G, et al. Frequency of concurrent autoimmune disorders in patients with autoimmune hepatitis: effect of age, gender, and genetic background. J Clin Gastroenterol 2008;42:300–5.
[75] Abdalian R, Dhar P, Jhaveri K, et al. Prevalence of sclerosing cholangitis in adults with autoimmune hepatitis: evaluating the role of routine magnetic resonance imaging. Hepatology 2008;47:949–57.
[76] Gregorio GV, Portmann B, Karani J, et al. Autoimmune hepatitis/sclerosing cholangitis overlap syndrome in childhood: a 16-year prospective study. Hepatology 2001;33:544–53.
[77] Montano-Loza AJ, Carpenter HA, Czaja AJ. Features associated with treatment failure in type 1 autoimmune hepatitis and predictive value of the model of end-stage liver disease. Hepatology 2007;46:1138–45.
[78] Angulo P, Maor-Kendler Y, Lindor KD. Small-duct primary sclerosing cholangitis: a long-term follow-up study. Hepatology 2002;35:1494–500.
[79] Bjornsson E, Olsson R, Bergquist A, et al. The natural history of small-duct primary sclerosing cholangitis. Gastroenterology 2008;134:975–80.
[80] Czaja AJ. Current and future treatments of autoimmune hepatitis. Expert Rev Gastroenterol Hepatol 2009;3:269–91.
[81] Kanzler S, Gerken G, Dienes HP, et al. Cyclophosphamide as alternative immunosuppressive therapy for autoimmune hepatitis—report of three cases. Z Gastroenterol 1997;35:571–8.
[82] Burak KW, Urbanski SJ, Swain MG. Successful treatment of refractory type 1 autoimmune hepatitis with methotrexate. J Hepatol 1998;29: 990–3.
[83] Kerkar N, Dugan C, Rumbo C, et al. Rapamycin successfully treats post-transplant autoimmune hepatitis. Am J Transplant 2005;5:1085–9.
[84] Santos ES, Arosemena LR, Raez LE, et al. Successful treatment of autoimmune hepatitis and idiopathic thrombocytopenic purpura with the monoclonal antibody, rituximab: case report and review of literature. Liver Int 2006;26:625–9.
[85] Carmassi F, Morale M, Puccetti R, et al. Efficacy of intravenous immunoglobulin therapy in a case of autoimmune-mediated chronic active hepatitis. Clin Exp Rheumatol 1992;10:13–7.
[86] Rebollo Bernardez J, Cifuentes Mimoso C, Pinar Moreno A, et al. Deflazacort for long-term maintenance of remission in type I autoimmune hepatitis. Rev Esp Enferm Dig 1999;91:630–8.
[87] Nakamura K, Yoneda M, Yokohama S, et al. Efficacy of ursodeoxycholic acid in Japanese patients with type 1 autoimmune hepatitis. J Gastroenterol Hepatol 1998;13:490–5.
[88] Miyake Y, Iwasaki Y, Kobashi H, et al. Efficacy of ursodeoxycholic acid for Japanese patients with autoimmune hepatitis. Hepatol Int 2009;3: 556–62.
[89] de Boer NK, de Graaf P, Wilhelm AJ, et al. On the limitation of 6-tioguaninenucleotide monitoring during tioguanine treatment. Aliment Pharmacol Ther 2005;22:447–51.
[90] de Boer NK, van Nieuwkerk CM, Aparicio Pages MN, et al. Promising treatment of autoimmune hepatitis with 6-thioguanine after adverse events on azathioprine. Eur J Gastroenterol Hepatol 2005;17:457–61.
[91] Fernandes NF, Redeker AG, Vierling JM, et al. Cyclosporine therapy in patients with steroid resistant autoimmune hepatitis. Am J Gastroenterol 1999;94:241–8.
[92] Alvarez F, Ciocca M, Canero-Velasco C, et al. Short-term cyclosporine induces a remission of autoimmune hepatitis in children. J Hepatol 1999;30:222–7.
[93] Cuarterolo M, Ciocca M, Velasco CC, et al. Follow-up of children with autoimmune hepatitis treated with cyclosporine. J Pediatr Gastroenterol Nutr 2006;43:635–9.
[94] Sciveres M, Caprai S, Palla G, et al. Effectiveness and safety of cyclosporine as therapy for autoimmune diseases of the liver in children and adolescents. Aliment Pharmacol Ther 2004;19:209–17.
[95] Ritter ML, Pirofski L. Mycophenolate mofetil: effects on cellular immune subsets, infectious complications, and antimicrobial activity. Transpl Infect Dis 2009;11:290–7.
[96] Richardson PD, James PD, Ryder SD. Mycophenolate mofetil for maintenance of remission in autoimmune hepatitis in patients resistant to or intolerant of azathioprine. J Hepatol 2000;33:371–5.
[97] Devlin SM, Swain MG, Urbanski SJ, et al. Mycophenolate mofetil for the treatment of autoimmune hepatitis in patients refractory to standard therapy. Can J Gastroenterol 2004;18:321–6.
[98] Inductivo-Yu I, Adams A, Gish RG, et al. Mycophenolate mofetil in autoimmune hepatitis patients not responsive or intolerant to standard immunosuppressive therapy. Clin Gastroenterol Hepatol 2007;5:799–802.
[99] Chatur N, Ramji A, Bain VG, et al. Transplant immunosuppressive agents in non-transplant chronic autoimmune hepatitis: the Canadian Association for the Study of Liver (CASL) experience with mycophenolate mofetil and tacrolimus. Liver Int 2005;25:723–7.
[100] Hennes EM, Oo YH, Schramm C, et al. Mycophenolate mofetil as second line therapy in autoimmune hepatitis? Am J Gastroenterol 2008;103:3063–70.
[101] Hlivko JT, Shiffman ML, Stravitz RT, et al. A single center review of the use of mycophenolate mofetil in the treatment of autoimmune hepatitis. Clin Gastroenterol Hepatol 2008;6:1036–40.
[102] Wolf DC, Bojito L, Facciuto M, et al. Mycophenolate mofetil for autoimmune hepatitis: a single practice experience. Dig Dis Sci 2009;54:2519–22.
[103] Aw MM, Dhawan A, Samyn M, et al. Mycophenolate mofetil as rescue treatment for autoimmune liver disease in children: a 5-year follow-up. J Hepatol 2009;51:156–60.
[104] Spencer CM, McTavish D, Budesonide. A review of its pharmacological properties and therapeutic efficacy in inflammatory bowel disease. Drugs 1995;50:854–72.
[105] McKeage K, Goa KL. Budesonide (Entocort EC Capsules): a review of its therapeutic use in the management of active Crohn's disease in adults. Drugs 2002;62:2263–82.
[106] Seow CH, Benchimol EI, Griffiths AM, et al. Budesonide for induction of remission in Crohn's disease. Cochrane Database Syst Rev 2008 [CD000296].
[107] Danielsson A, Prytz H. Oral budesonide for treatment of autoimmune chronic active hepatitis. Aliment Pharmacol Ther 1994;8:585–90.
[108] Czaja AJ, Lindor KD. Failure of budesonide in a pilot study of treatment-dependent autoimmune hepatitis. Gastroenterology 2000;119:1312–6.
[109] Wiegand J, Schuler A, Kanzler S, et al. Budesonide in previously untreated autoimmune hepatitis. Liver Int 2005;25:927–34.
[110] Geier A, Gartung C, Dietrich CG, et al. Side effects of budesonide in liver cirrhosis due to chronic autoimmune hepatitis: influence of hepatic metabolism versus portosystemic shunts on a patient complicated with HCC. World J Gastroenterol 2003;9:2681–5.
[111] Csepregi A, Rocken C, Treiber G, et al. Budesonide induces complete remission in autoimmune hepatitis. World J Gastroenterol 2006;12:1362–6.
[112] Zandieh I, Krygier D, Wong V, et al. The use of budesonide in the treatment of autoimmune hepatitis in Canada. Can J Gastroenterol 2008;22:388–92.
[113] Manns MP, Bahr MJ, Woynarowski M, et al. Budesonide 3 mg tid is superior to prednisone in combination with azathioprine in the treatment of autoimmune hepatitis. J Hepatol 2008;48:S369.
[114] Manns MP, Woynarowski M, Kreisel W, et al. Budesonide 3 mg bid in combination with azathioprine as maintenance treatment of autoimmune hepatitis—final results of a large multicenter international trial. Hepatology 2008;48:376A–7A.
[115] Shevach EM. Regulatory T cells in autoimmunity. Annu Rev Immunol 2000;18:423–49.
[116] Piccirillo CA, Shevach EM. Naturally-occurring CD4+CD25+ immunoregulatory T cells: central players in the arena of peripheral tolerance. Semin Immunol 2004;16:81–8.

[117] Piccirillo CA. Regulatory T cells in health and disease. Cytokine 2008;43:395–401.
[118] Piccirillo CA, d'Hennezel E, Sgouroudis E, et al. CD4+Foxp3+ regulatory T cells in the control of autoimmunity: in vivo veritas. Curr Opin Immunol 2008;20:655–62.
[119] Longhi MS, Hussain MJ, Mitry RR, et al. Functional study of CD4+CD25+ regulatory T cells in health and autoimmune hepatitis. J Immunol 2006;176:4484–91.
[120] Longhi MS, Ma Y, Bogdanos DP, et al. Impairment of CD4(+)CD25(+) regulatory T-cells in autoimmune liver disease. J Hepatol 2004;41:31–7.
[121] Longhi MS, Ma Y, Mitry RR, et al. Effect of CD4+ CD25+ regulatory T-cells on CD8 T-cell function in patients with autoimmune hepatitis. J Autoimmun 2005;25:63–71.
[122] Longhi MS, Meda F, Wang P, et al. Expansion and de novo generation of potentially therapeutic regulatory T cells in patients with autoimmune hepatitis. Hepatology 2008;47:581–91.
[123] Ferri S, Longhi MS, De Molo C, et al. A multifaceted imbalance of T-cells with regulatory function characterizes type 1 autoimmune hepatitis. Hepatology, in press.
[124] Christen U, Hintermann E, Jaeckel E. New animal models for autoimmune hepatitis. Semin Liver Dis 2009;29:262–72.
[125] Hardtke-Wolenski M, Jaeckel E. Mouse models for experimental autoimmune hepatitis: limits and chances. Dig Dis 2010;28:70–9.
[126] Lapierre P, Beland K, Djilali-Saiah I, et al. Type 2 autoimmune hepatitis murine model: the influence of genetic background in disease development. J Autoimmun 2006;26:82–9.
[127] Lapierre P, Beland K, Martin C, et al. Forkhead box p3(+) regulatory T cell underlies male resistance to experimental type 2 autoimmune hepatitis. Hepatology 2010;51:1789–98.
[128] Christen U, Holdener M, Hintermann E. Cytochrome P450 2D6 as a model antigen. Dig Dis 2010;28:80–5.
[129] Holdener M, Hintermann E, Bayer M, et al. Breaking tolerance to the natural human liver autoantigen cytochrome P450 2D6 by virus infection. J Exp Med 2008;205:1409–22.
[130] Czaja A.J. Emerging opportunities for site-specific molecular and cellular interventions in autoimmune hepatitis. Dig Dis Sci 2010 [Epub ahead of print].