Autoimmune Liver Disease

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Juvenile autoimmune liver disease has been recognized only recently in the history of medicine. Autoimmune hepatitis in young women was first described in the 1950s, juvenile autoimmune sclerosing cholangitis in the 1980s and de novo autoimmune hepatitis after liver transplantation in the 1990s. This chapter explores the peculiarities of autoimmune liver disease in children and adolescents, their possible pathogenic mechanisms, their management and their outcome.

Key Points

- There are three forms of juvenile liver disease with an autoimmune component to their pathogenesis: autoimmune hepatitis (AIH), autoimmune sclerosing cholangitis (ASC) and de novo autoimmune hepatitis (de novo AIH) after liver transplantation (LT).
- AIH is in turn divided into two types: AIH-1, positive for anti-nuclear (ANA) and/or anti-smooth muscle (SMA) autoantibodies, and AIH-2, positive for anti-liver-kidney microsomal type 1 (anti-LKM1) and/or anti-liver cytosol type 1 (anti-LC1) autoantibodies.
- The typical histological feature, common to AIH, ASC and de novo AIH after LT, is interface hepatitis.
- ASC is serologically (ANA/SMA) and histologically similar to AIH-1 but in addition has bile duct damage demonstrable by cholangiography usually already at presentation.
- The International Autoimmune Hepatitis Group (IAIHG) scoring systems do not allow differentiation between AIH and ASC; a scoring system specific for juvenile autoimmune liver disease has been proposed by an ESPGHAN Hepatology Committee Position Statement.
- Both in AIH and ASC, the parenchymal inflammation responds satisfactorily to standard immunosuppressive treatment with steroids ± azathioprine, but in ASC the bile duct disease progresses in about 50% of cases, leading to LT.
- ASC is more frequently associated with inflammatory bowel disease than AIH, and deterioration of liver disease, as well as the risk of ASC recurrence after transplant, is correlated to the activity of the gut disease.
- Those patients with AIH or ASC, who do not respond to standard treatment, or who relapse frequently should be offered alternative immunosuppression in specialized centres (including in order of priority mycophenolate mofetil, calcineurin inhibitors, rituximab, anti-TNF-α).
- Relapse occurs in about 40% of patients while on treatment and is frequently due to drug non-adherence, particularly in adolescents.
- Both AIH and ASC can recur after LT, recurrence being more common in ASC than AIH.
- In both AIH and ASC, regulatory T cells defective in number and/or function are likely to play a major role in the loss of tolerance that leads to autoimmune liver damage.
- De novo AIH after LT for non-autoimmune conditions responds to the classical treatment of AIH, but not to standard antirejection treatment.

Research Needed in the Field

- New specific biomarkers for the diagnosis and the monitoring of autoimmune liver disease.
- Understanding T cells, B cells and innate immunity interplays in the causation of the autoimmune damage.
- Understanding the role of the gut-liver axis and of the microbiome in the pathogenesis of liver disease.
- Randomized controlled studies to identify the most effective second-line treatments in children who fail standard therapy.

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10.1 Definition

Autoimmune liver diseases are inflammatory liver disorders characterized histologically by a dense mononuclear cell infiltrate in the portal tract (interface hepatitis; Fig. 10.1a) and serologically by high transaminase and immunoglobulin G (IgG) levels and positive autoantibodies. All other known causes of liver disease must be excluded. Autoimmune liver diseases typically respond to immunosuppressive treatment, which should be instituted as soon as the diagnosis is made [1].

In paediatrics, there are three liver disorders in which liver damage is deemed to arise from an autoimmune attack: autoimmune hepatitis (AIH), autoimmune sclerosing cholangitis (ASC) and de novo autoimmune hepatitis after liver transplantation (de novo AIH).

10.2 Autoimmune Hepatitis

AIH affects mainly girls and is divided into two main types according to the autoantibody profile: AIH type 1 (AIH-1) is positive for anti-nuclear (ANA) and/or anti-smooth muscle (SMA) antibodies and AIH type 2 (AIH-2) is positive for anti-liver-kidney microsomal antibody type 1 (anti-LKM-1) and/or anti-liver cytosol type 1 (anti-LC-1) antibodies.

10.2.1 Epidemiology

AIH occurs worldwide, but its prevalence is unknown. Initial epidemiological information including adult and juvenile AIH was obtained for AIH-1 before the introduction of the IAIHG diagnostic scoring system [2, 3], therefore without standard criteria for patient inclusion. Early prevalence reports range from 1.9 cases/100,000 in Norway [4] and 1/200,000 in the US general population [5] to 20/100,000 in females over 14 years of age referred to a tertiary centre in Spain [6]. A study from a UK secondary referral centre reported an AIH annual incidence of 3.5/100,000 [7]. Two studies using standardized criteria for the diagnosis of AIH published in 2002 and 2010 report a point prevalence of 24.5/100,000 in New Zealand [8] and of 34.5/100,000 in Alaskan natives [9]. Though AIH prevalence and incidence are reported to be lower in the Asia-Pacific area than in Europe and America [10], a better awareness of its clinical characteristics has led to an increased frequency in the diagnosis of AIH in China, where this condition was considered very rare [11]. Also in Japan the incidence and prevalence of AIH may be higher than previously thought [12]. Studies on the largest patient cohorts come from Northern Europe. A population-based investigation in Denmark reports an incidence rate of 1.68 per 100,000 populations per year, which doubled during the 1994–2012 period of observation [13]. In a large Swedish cohort, AIH point prevalence was reported as 17.3/100,000 inhabitants in 2009, with a yearly incidence of 1.2/100,000 inhabitants between 1990 and 2009 [14]. A large Dutch study reports an AIH prevalence of 18.3 per 100,000 [15].

All these epidemiological figures are likely to be underestimates, since AIH, particularly in adults, may remain undiagnosed for several years and present eventually with decompensated liver disease attributed to 'cryptogenic' cirrhosis.

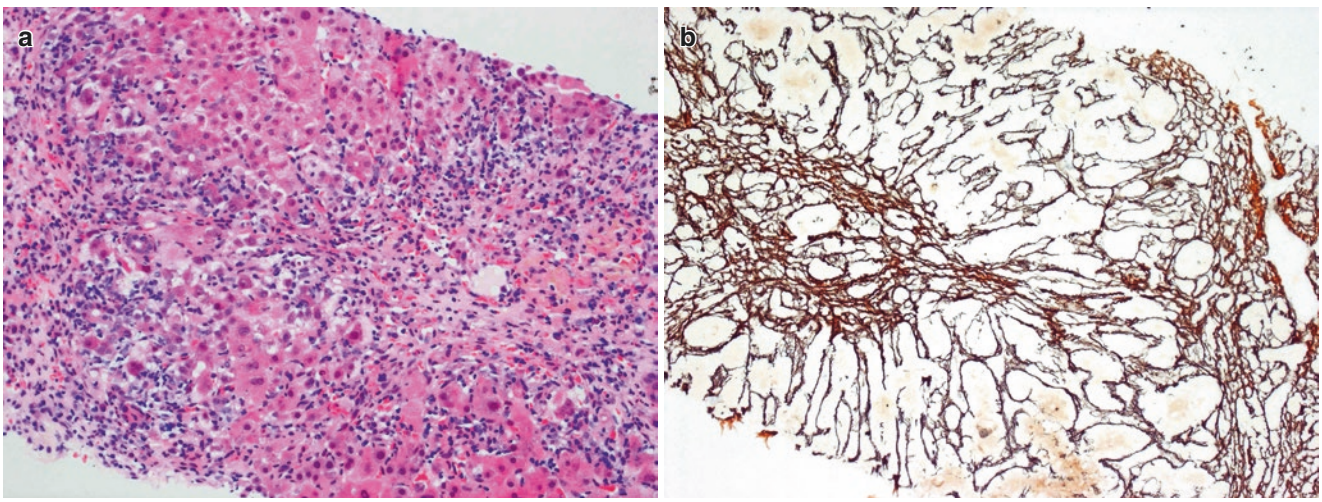

Fig. 10.1 Autoimmune hepatitis presenting acutely: (**a**) Portal and periportal lymphocyte and plasma cell infiltrate, disrupting the limiting plate (interface hepatitis) and extending into the parenchyma. Swollen hepatocytes, pyknotic necrosis and acinar inflammation are present (haematoxylin-eosin, original magnification 40×); (**b**) Reticulin staining showing connective-tissue collapse resulting from hepatocyte death and expanding from the portal area into the lobule (Images kindly provided by Dr. Alberto Quaglia)

The prevalence of AIH-2, which affects mainly children and young adults, is unknown, also because the diagnosis is probably often overlooked. Intriguingly AIH-2 has been reported more frequently in Europe that in the United States [16], possibly because of the under-testing for anti-LKM-1 antibodies in the latter, due to the unsubstantiated belief that AIH-2 is rare in Northern America and therefore that testing for anti-LKM-1 antibodies is not cost-effective [17]. In a study in Canada including 159 children/adolescents with AIH the annual incidence was 0.23 per 100,000 children, AIH-1 being diagnosed 5.5 times more frequently than AIH-2 [18].

Data collected at the King's College Hospital Paediatric Hepatology tertiary referral centre show a sixfold increase in the yearly incidence of juvenile AIH between the 1990s and 2000s [19], and a large study in Denmark shows a twofold increase in the incidence of adult AIH in the same period of time [13], suggesting either a better awareness of this condition, leading to an increased referral rate and diagnosis, or a real increase in the incidence of autoimmune liver disease.

10.2.2 Aetiology and Pathogenesis

The aetiology of AIH is unknown, although both genetic and environmental factors are involved in its expression [20].

Genetics. AIH is a 'complex-trait' disease—i.e. a condition not inherited in a Mendelian autosomal dominant, autosomal recessive or sex-linked fashion. The mode of inheritance of a complex-trait disorder is unknown and involves one or more genes operating alone or in concert to increase or reduce the risk of the trait and interacting with environmental factors [21].

Susceptibility to AIH is imparted by genes in the histocompatibility leukocyte antigen (HLA) region on the short arm of chromosome 6, especially those encoding DRB1 alleles. These class II major histocompatibility complex (MHC) molecules are involved in peptide antigen presentation to CD4 T cells, suggesting the involvement of MHC class II antigen presentation and T-cell activation in the pathogenesis of AIH. The prominent predisposing role of genes encoded in the HLA region has been confirmed in the largest genome-wide association study performed to date in AIH [22].

In Europe and North America, susceptibility to AIH-1 in adults is conferred by the possession of HLA DR3 (*DRB1*0301*) and DR4 (*DRB1*0401*), both heterodimers containing a lysine residue at position 71 of the DRB1 polypeptide and the hexameric amino acid sequence LLEQKR at positions 67–72 [23, 24]. In Japan, Argentina and Mexico, susceptibility is linked to *DRB1*0405* and *DRB1*0404*, alleles encoding arginine rather than lysine at position 71, but sharing the motif LLEQ-R with *DRB1*0401* and *DRB1*0301* [25]. Thus, K or R at position 71 in the context of LLEQ-R may be critical for susceptibility to AIH, favouring the binding of autoantigenic peptides, complementary to this hexameric sequence.

The lysine-71 and other models for AIH-1 cannot explain the disease completely, since in European and North American patients, for example, the presence of lysine-71 is associated with a severe and mainly juvenile disease in those who are positive for *DRB1*0301*, but to a mild and adult onset disease in those who are positive for *DRB1*0401*. Other genes inside and/or outside the MHC are therefore likely to be involved in determining the phenotype. The cytotoxic T lymphocyte antigen-4 (CTLA-4) [26], the tumour necrosis factor-alpha (TNF-α) gene promoter [27] and Fas [28] are notable examples.

A possible other candidate is the MHC-encoded complement gene, mapping to the class III MHC region, as patients with AIH, whether positive for anti-LKM-1 or ANA/SMA, have isolated partial deficiency of the HLA class III complement component C4, which is genetically determined [29].

In Northern Europe, paediatric AIH-1, similar to adult AIH, is associated with the possession of the human leukocyte antigen (HLA) *DRB1*03*. In contrast to adult patients, possession of *DRB1*04* does not predispose to AIH in childhood and can even exert a protective role [30]. Susceptibility to AIH-2 is conferred by the possession of HLA DR7 (*DRB1*0701*) and, in DR7 negative patients, with possession of DR3 (*DRB1*0301*), those patients positive for *DRB1*0701* having a more aggressive disease and a more severe prognosis [31]. In Egypt AIH-2 appears to be associated also with possession of *HLA-DRB1*15* [32]. In Brazil and in Egypt, the primary susceptibility allele for AIH-1 is *DRB1*1301*, but a secondary association with *DRB1*0301* has also been identified [32, 33]. Interestingly, in South America, possession of the HLA *DRB1*1301* allele not only predisposes to paediatric AIH-1 but is also associated with persistent infection with the endemic hepatitis A virus [34, 35]. Homozygosity for DR3 plays a major role in the predisposition to juvenile autoimmune liver disease [36]. The combination of HLA DRB1*1301 and a specific functional form of the killer cell immunoglobulin-like receptor (KIR2DS4-FL) imparts a strong predisposition to paediatric AIH-1 in South America [37]. Susceptibility to, and severity of, AIH-2 has been linked to alleles encoding the DRB1*0301 and DRB1*0701 molecules in the United Kingdom and Brazil. Allelic variation within HLA-DRB1 has been linked to differences in the autoantibody seropositivity profiles of AIH-2 patients [38].

A form of AIH serologically resembling AIH-2 affects some 20% of patients with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED). APECED is a monogenic autosomal recessive disorder caused by homozygous mutations in the AIRE1 gene and characterized by a

variety of organ-specific autoimmune diseases, the most common of which are hypoparathyroidism and primary adrenocortical failure, accompanied by chronic mucocutaneous candidiasis [39, 40]. Interestingly there are neutralizing autoantibodies to type 1 interferons, perhaps accounting for the associated immune deficiencies. APECED has a high level of variability in symptoms, especially between populations. Carriers of a single AIRE1 mutation (heterozygotes) do not develop APECED. However, although the inheritance pattern of APECED indicates a strictly recessive disorder, there are anecdotal data of mutations in a single copy of AIRE1 being associated with human autoimmunity of a less severe form than classically defined APECED [39, 40].

The role of the AIRE1 heterozygote state in the development of AIH-2 remains to be established, though heterozygous AIRE1 mutations have been reported in three children with severe AIH-2 and extrahepatic autoimmune manifestations [41].

Immune mechanisms [42]. Immunohistochemical studies have shown that the majority of the cells infiltrating the portal tract and invading the parenchyma in the typical AIH histological picture of interface hepatitis are T lymphocytes mounting the α/β T-cell receptor. Among the T cells, the majority are positive for the CD4 helper/inducer phenotype, and a sizable minority are positive for the CD8 cytotoxic phenotype. Lymphocytes of non-T-cell lineage are fewer and include (in decreasing order of frequency) natural killer cells (CD16/CD56-positive), macrophages and B lymphocytes. Natural killer T cells, which simultaneously express markers of both natural killer (CD56) and T cells (CD3), appear to be involved in liver damage in an animal model of autoimmune hepatitis.

Powerful stimuli must lead to the formation of the massive inflammatory cell infiltrate that is present at diagnosis in both AIH and ASC. Whatever the initial trigger, it is most probable that such a high number of activated inflammatory cells cause liver damage.

There are different possible pathways that an autoimmune attack can follow to inflict damage on hepatocytes (Fig. 10.2). Liver damage is probably orchestrated by CD4 T lymphocytes recognizing a self-antigenic liver peptide. To trigger an autoimmune response, the peptide has to be embraced by an HLA class II molecule and presented to uncommitted (naïve) $CD4^+$ T-helper (Th0) cells by professional antigen-presenting cells (APC), such as dendritic cells (DCs), macrophages and B lymphocytes. The liver is home to several specialized APC

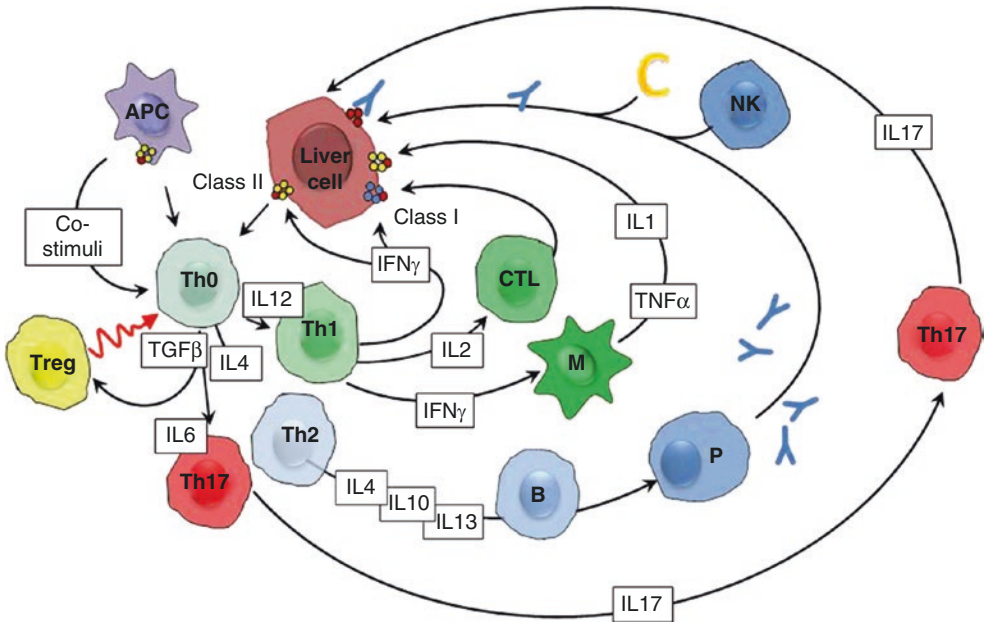

Fig. 10.2 Autoimmune attack to the hepatocyte: An autoantigen is presented to uncommitted T-helper (Th0) lymphocytes within the HLA class II molecule of an antigen-presenting cell (APC) either in the regional lymph nodes or within the liver itself. Activated Th0 cells differentiate into Th1 or Th2 cells in the presence of interleukin (IL)-12 or IL-4, respectively, and according to the nature of the antigen. This triggers a series of immune reactions determined by the cytokines they produce. Th1 cells secrete IL-2 and interferon (IFN)-γ, which are cytokines that stimulate cytotoxic T lymphocytes (CTL), enhance expression of class I HLA molecules, induce expression of class II HLA molecules on the liver cells and activate macrophages. Macrophages (M) release IL-1 and tumour necrosis factor (TNF). TH2 cells secrete mainly IL-4, IL-10 and IL-13 and stimulate autoantibody production by B lymphocytes. Regulatory T cells (T-reg) are derived from Th0 in the presence of transforming growth factor (TGF)-β. In the presence of defective T-reg, hepatocyte destruction ensues from the engagement of damaging effector mechanisms, including CTL, cytokines released by Th1 and by activated macrophages, complement activation or adhesion of natural killer (NK) cells to autoantibody-coated hepatocytes through their Fc receptors. Th17 cells produce the inflammatory cytokine IL-17 and derive from Th0 cells in the presence of TGF-β and IL-6. They are the focus of current investigations

populations, including liver sinusoidal endothelial cells (LSECs), Kupffer cells and DCs, where antigen presentation to both CD4 and CD8 effector T cells can occur in situ, perhaps averting the need for trafficking to the regional lymphoid tissues [43, 44]. $CD4^+$ T-cell activation is promoted by interaction of two ligands, CD28 on Th0 cells and CD80 on APC. Th0 cells then become activated and differentiate into functional phenotypes according to the cytokines prevailing in the microenvironment and the nature of the antigen and initiate a cascade of immune reactions determined by the cytokines these activated T cells produce. Th1 cells, arising in the presence of the macrophage-produced interleukin 12 (IL-12), secrete mainly IL-2 and interferon gamma (IFN-γ), which activate macrophages, enhance expression of HLA class I (increasing liver cell vulnerability to a CD8 T-cell cytotoxic attack) and induce expression of HLA class II molecules on hepatocytes. Th2 cells, which differentiate from Th0 if the microenvironment is rich in IL-4, mainly produce IL-4, IL-10 and IL-13, which favour autoantibody production by B lymphocytes. Physiologically, Th1 and Th2 antagonize each other. Th17 cells arise in the presence of transforming growth factor-β (TGF-β) and IL-6 and have an important effector role in inflammation and autoimmunity. The process of autoantigen recognition is strictly controlled by regulatory mechanisms, such as those exerted by $CD4^+CD25^+FOXP3^+CD127^-$ regulatory T cells, which are derived from Th0 in the presence of TGF-β but in the absence of IL-6. If regulatory mechanisms fail, the autoimmune attack develops and persists.

Various aspects of the above pathogenic scenario have been investigated during the last 40 years:

- *Regulatory T cells* [45]. Autoimmunity arises against a background of defective immunoregulation, and this has been repeatedly reported in AIH. Early studies showed that patients with AIH have low levels of circulating T cells expressing the CD8 marker and impaired suppressor cell function, which segregates with the possession of the disease-predisposing HLA haplotype *B*08/DRB1*03* and is correctable by therapeutic doses of corticosteroids. It is possible, although not formally tested, that these early characterized CD8 T cells with a suppressor function represent the later described $CD8^+CD28^-$ suppressor T cells. Furthermore, patients with AIH have been shown to have a defect in a subpopulation of T cells controlling the immune response to liver-specific membrane antigens. More recent experimental evidence confirms an impairment of the immunoregulatory function in AIH. Among T-cell subsets with potential immunoregulatory function, CD4 cells constitutively expressing the IL-2 receptor alpha chain (CD25) (T-regulatory cells, T-regs) represent the dominant one. These cells, constituting 5–10% of all peripheral CD4 cells in health, control innate and adaptive immune responses by limiting the proliferation and effector function of autoreactive T cells. They act by direct contact with the target cells and, to a lesser extent, by releasing immunoregulatory cytokines, such as IL-10 and tissue growth factor beta 1. Besides CD25, which is also present on T cells undergoing activation, T-regs express additional markers, including the glucocorticoid-induced tumour necrosis factor receptor, CD62L, CTLA4 and the forkhead/winged-helix transcription factor FOXP3, whose expression has been associated with the acquisition of regulatory properties. Importantly, they express little or no CD127, the IL-7 receptor. In children with AIH, T-regs are defective in number and function in comparison with normal controls, and this impairment relates to the stage of disease, being more evident at diagnosis than during drug-induced remission. The percentage of T-regs inversely correlates with markers of disease severity, such as anti-soluble liver antigen (anti-SLA) and anti-LKM-1 autoantibody titres, suggesting that a reduction in regulatory T cells favours the serological expression of autoimmune liver disease. Importantly, several studies show that T-regs from AIH patients at diagnosis are impaired in their ability to control the proliferation of CD4 and CD8 effector cells compared to T-regs isolated from AIH patients at remission or from healthy subjects. Effector CD4 T cells isolated from patients with AIH are less susceptible to the regulatory control exerted by T-regs. This defect is linked to reduced expression of the inhibitory receptor T-cell-immunoglobulin-and-mucin-domain-containing-molecule-3 (Tim-3), which upon ligation of galectin-9 present on T-regs induces effector cell death. If loss of immunoregulation is central to the pathogenesis of AIH, treatment should concentrate on restoring the T-regs' ability to expand, with a consequent increase in their number and function. This is at least partially achieved by standard immunosuppression, since numbers of T-regs increase during remission.
- *Autoreactive T cells* [42]. As mentioned above, to trigger an autoimmune response, a peptide embraced by an HLA class II molecule has to be presented to uncommitted T-helper (Th0) cells by professional APCs (Fig. 10.2). Given the impaired regulatory function described above, it is suspected that in AIH, an autoantigenic peptide is indeed presented to the helper/inducer T cells, leading to their sustained activation.

Major advances in the study of T cells have occurred in AIH-2, since the main autoantigenic target of anti-LKM-1 has been identified as cytochrome P4502D6 (CYP2D6), making it possible to characterize both CD4 and CD8 T cells targeting this cytochrome. One study has shown that CD4 T cells from patients with AIH-2 who are positive for the

predisposing HLA allele *DRB1*0701* recognize seven regions of CYP2D6, five of which have later been shown to be also recognized by CD8 T cells. High numbers of antigen-specific interferon gamma-producing CD4 and CD8 T cells are associated with biochemical evidence of liver damage, suggesting a combined cellular immune attack.

What triggers the immune system to react to an autoantigen is unknown. A lesson may be learned by the study of humoral autoimmune responses during viral infections. Thus, studies aimed at determining the specificity of the LKM-1 antibody—present in both the juvenile form of AIH and in some patients with chronic hepatitis C virus (HCV) infection—have shown a high amino acid sequence homology between the HCV polyprotein and CYP2D6, implicating a mechanism of molecular mimicry as a trigger for the production of anti-LKM-1 in HCV infection. It is therefore conceivable that an as yet unknown viral infection may be at the origin of the autoimmune attack in AIH.

The possible role of Th17 cells in the pathogenesis of AIH is under investigation. Th17 cells contribute to autoimmunity by producing the pro-inflammatory cytokines IL-17, IL-22 and TNF-α and inducing hepatocytes to secrete IL-6 [46], which further enhances Th17 activation. An elevated level of Th17 cells has been reported in both blood and liver of patients with AIH [46, 47].

10.2.3 Clinical Features (Table 10.1)

As mentioned above, AIH is divided into two forms according to its autoantibody profile: AIH-1 is positive for ANA and/or SMA and AIH-2 for anti-LKM-1 and/or anti-LC-1. Three quarters of patients with either type of AIH are female. AIH-1 affects all ages, with two peaks, one in childhood/adolescence and the other in adulthood around the age of 40 years. AIH-2 affects mainly children and young adults, being rare, though not absent, in older individuals. In paediatrics, AIH-1 accounts for at least two-thirds of the cases and presents usually during adolescence, while AIH-2 presents at a younger age, including infancy. IgG are usually raised at onset in both types, though 15% of children with AIH-1 and 25% of those with AIH-2 have levels within the normal range, particularly when the disease presents acutely, [1]. Interestingly, also these children with IgG within the normal range experience a reduction in levels during treatment. Partial IgA deficiency is common in AIH-2, affecting some 40% of patients [30, 48]. While most adult patients with AIH-1 have a chronic disease course with non-specific symptoms such as fatigue, nausea, abdominal pain and arthralgia [49], juvenile AIH has a more aggressive phenotype. The clinical course has been mainly described in patients of European origin [30, 50, 51–56], individuals from other ethnic groups being considered rarely affected by this condition. AIH, however, is being increasingly reported in children and adolescents of non-Caucasoid descent, probably because the diagnosis of autoimmune liver disease was previously overlooked in view of the presence of epidemic viral hepatitis B and/or C. Reports from India [57, 58], Malaysia [59], Pakistan [60], Bahrain [61], Iran [62], Egypt [63], Jamaica [64], and Mexico [65] on cohorts including between 5 and 181 (median 34) patients indicate a clinical presentation and response to immunosuppressive treatment similar to those described in Caucasoid patients, but an overall worse response to treatment and outcome, possibly related to delay in referral to specialized centres and diagnosis.

The mode of presentation of AIH in childhood is variable (Table 10.2), and the disease should be suspected and excluded in all children presenting with symptoms and signs of prolonged or severe liver disease. Acute hepatitis episodes

Table 10.1 Comparison between autoimmune hepatitis type 1, autoimmune hepatitis type 2 and autoimmune sclerosing cholangitis

Variable		AIH-1	AIH-2	ASC
Female sex		80%	80%	50%
Male sex		20%	20%	50%
ANA or SMA[a]	≥1:20	++	+/–	++
Anti-LKM-1[a]	≥1:10	–	++	+/–
Anti-LC-1	Positive	–	++	–
Anti-SLA	Positive	+	+	+
pANNA	Positive	+	–	++
IgG	>Upper limit of normal	++	+	++
	>1.20 times upper limit of normal	++	+	++
Liver histology	Compatible with AIH	+	+	+
	Typical of AIH	+	+	+
Viral hepatitis (A, B, C, E, EBV), NASH, Wilson disease and drug exposure		–	–	–
Presence of extrahepatic autoimmunity		+	+	+
Family history of autoimmune disease		+	+	+
Cholangiography	Normal	+	+	–
	Abnormal	–	–	+
Biochemical and immunological response to steroid treatment	Yes	+	+	+
	No	–	–	–

AIH-1 autoimmune hepatitis type 1, *AIH-2* autoimmune hepatitis type 2, *ASC* autoimmune sclerosing cholangitis, *ANA* anti-nuclear antibody, *SMA* anti-smooth muscle antibody, *anti-LKM-1* anti-liver-kidney microsomal antibody type 1, *anti-LC-1* anti-liver cytosol type 1, *anti-SLA* anti-soluble liver antigen, *IgG* immunoglobulin G, *NASH* non-alcoholic steatohepatitis

[a]Antibodies measured by indirect immunofluorescence on a composite rodent substrate (kidney, liver, stomach)

Table 10.2 Modes of presentation of juvenile autoimmune hepatitis

Mode of presentation	Clinical features
Acute	Non-specific symptoms similar to viral hepatitis: malaise, nausea/vomiting, anorexia, joint and abdominal pain, followed by jaundice, dark urine, and pale stools (40–50% of patients); transaminase levels can fluctuate
Fulminant hepatic failure	Grade II to IV hepatic encephalopathy developing 2 weeks to 2 months after the onset of symptoms (~3% of patients with AIH-1 and ~20% of patients with AIH-2)
Insidious onset	Non-specific symptoms (progressive fatigue, relapsing jaundice, amenorrhea, headache, anorexia, joint and abdominal pain, diarrhoea, weight loss), lasting from months to a few years before diagnosis (~40% of patients with AIH-1 and ~25% of patients with AIH-2)
Complications of cirrhosis and portal hypertension	Haematemesis from oesophageal/gastric varices, bleeding diathesis, splenomegaly, without previous history of jaundice or liver disease (~10 of both AIH types)
Asymptomatic	Incidental finding of raised aminotransferases, without any symptoms or signs (rare in large series but real prevalence unknown)

alternating with spontaneous clinical and biochemical improvement are not uncommon, a relapsing pattern that often leads to a dangerous delay in diagnosis and treatment. Hence AIH should always be suspected when known causes of acute hepatitis are excluded.

At least one-third of patients with AIH have cirrhosis at diagnosis, irrespective of the mode of presentation [1], indicating that the disease process is long-standing. AIH patients presenting acutely have often advanced fibrosis or cirrhosis on liver biopsy.

Severity of disease is similar in the two AIH types. AIH-2, however, has a higher tendency to present as acute liver failure (ALF) and is more refractory to eventual treatment withdrawal [30, 63, 65]. In both types a family history of autoimmune disease is frequent (~40%), and some 20% of patients have associated autoimmune disorders either present at diagnosis or developing during follow-up, including thyroiditis, inflammatory bowel disease (IBD), haemolytic anaemia, vitiligo, coeliac disease, insulin-dependent diabetes, Behçet disease, Sjögren syndrome, glomerulonephritis, idiopathic thrombocytopenia, urticaria pigmentosa, hypoparathyroidism, and Addison disease (the latter mainly in AIH-2) [30, 66]. These conditions should be actively sought for prompt treatment [67]. In this context diagnoses of particular importance are thyroiditis with hypothyroidism that affects 8–23% [30, 66], coeliac disease that affects between 5 and 10% [68–71] and IBD that affects ~18% of patients [50]. Interestingly patients with AIH and coeliac disease have been reported to achieve treatment-free sustained remission in a significantly higher proportion of cases, when compared with patients with AIH without coeliac disease, suggesting a possible long-term adjuvant effect of the gluten-free diet [72].

As mentioned above, AIH-2 responsive to immunosuppressive treatment has been described in 20–30% of patients with APECED syndrome [73–75].

10.2.4 Diagnostic Criteria

The diagnosis of AIH is based on a combination of clinical, biochemical, immunological and histological features and the exclusion of other known causes of liver disease that may share serological and histological features with AIH (e.g. hepatitis B, C and E, Epstein-Barr virus infection, Wilson disease, non-alcoholic steatohepatitis [NASH] and drug-induced liver disease). Liver biopsy is needed to confirm the diagnosis and to evaluate the severity of liver damage [76, 77]. In the absence of a single diagnostic test for AIH, the International Autoimmune Hepatitis Group (IAIHG) has devised a diagnostic system for comparative and research purposes, which includes several positive and negative scores, the sum of which gives a value indicative of probable or definite AIH [2, 3]. A simplified IAIHG scoring system published more recently is better suited to clinical application [78]. However, neither scoring system is applicable to the juvenile form of the disease [79], in particular in the context of fulminant hepatic failure [80, 81]. Moreover, diagnostically relevant autoantibodies in paediatrics often have titres lower than the cut-off value considered positive in adults [82] and neither IAIHG system allows distinction between AIH and ASC (see below) [50, 83], which can only be differentiated if a cholangiogram is performed at presentation. A recent European Society of Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) Hepatology Committee Position Statement proposes a scoring system for juvenile autoimmune liver disease to differentiate between AIH and ASC [1] (Table 10.3).

10.2.5 Laboratory Findings

Characteristic laboratory findings are elevated serum transaminase and IgG/γ-globulin levels and presence of autoantibodies (ANA and/or SMA in AIH-1, anti-LKM-1 and/or anti-LC-1 in AIH-2) [82, 84]. International normalized prothrombin ratio (INR) and bilirubin and albumin levels are variably abnormal, depending on the severity and chronicity of the disease. Alkaline phosphatase and gammaglutamyl transferase (GGT) levels can vary from normal to moderately elevated. Anti-LKM-1-positive patients tend to have higher levels of bilirubin and transaminases at presentation than those who are ANA/SMA-positive, reflecting the higher incidence of acute presentation in AIH-2.

Table 10.3 Proposed scoring criteria for the diagnosis of juvenile autoimmune liver disease

Variable	Cut-off	Points	
		AIH	ASC
ANA and/or SMA[a]	≥1:20[b]	1	1
	≥1:80	2	2
Anti-LKM-1[a] or	≥1:10[b]	1	1
	≥1:80	2	1
Anti-LC-1	Positive[b]	2	1
Anti-SLA	Positive[b]	2	2
pANNA	Positive	1	2
IgG	>ULN	1	1
	>1.20 ULN	2	2
Liver histology	Compatible with AIH	1	1
	Typical of AIH	2	2
Absence of viral hepatitis (A, B, E, EBV), NASH, Wilson disease and drug exposure	Yes	2	2
Presence of extrahepatic autoimmunity	Yes	1	1
Family history of autoimmune disease	Yes	1	1
Cholangiography	Normal	2	−2
	Abnormal	−2	2

Score ≥ 7, probable AIH; ≥8, definite AIH
Score ≥ 7, probable ASC; ≥8, definite ASC
AIH autoimmune hepatitis, *ASC* autoimmune sclerosing cholangitis, *ANA* anti-nuclear antibody, *SMA* anti-smooth muscle antibody, *anti-LKM-1* anti-liver-kidney microsomal antibody type 1, *anti-LC-1* anti-liver cytosol type 1, *anti-SLA* anti-soluble liver antigen, *IgG* immunoglobulin G, *EBV* Epstein-Barr virus, *NASH* non-alcoholic steatohepatitis, *ULN* upper limit of normal
[a]Antibodies measured by indirect immunofluorescence on a composite rodent substrate (kidney, liver, stomach)
[b]Addition of points achieved for ANA, SMA, anti-LKM-1, anti-LC-1 and anti-SLA autoantibodies cannot exceed a maximum of 2 points

10.2.6 Immunoglobulins

The majority (80%) of patients has increased levels of IgG, but some 20% have serum IgG levels within the normal range for age, particularly those presenting with acute hepatic failure, indicating that normal IgG values do not exclude the diagnosis of AIH. Measurement of IgG levels is particularly useful in monitoring disease activity and response to treatment [85]. Partial IgA deficiency is common in AIH-2 (~40%).

10.2.7 Autoantibodies

Key to the diagnosis of AIH is positivity for circulating autoantibodies [2, 3, 78, 82] (Table 10.4) though autoantibodies can be present in other liver disorders and are not diagnostic in isolation. Their detection by indirect immunofluorescence on a rodent substrate not only assists in the diagnosis but also allows differentiation into the two forms of AIH. ANA and SMA characterize AIH-1; anti-LKM-1 and anti-LC1 define AIH-2 [82, 86]. The two autoantibody profiles can occur simultaneously, but not frequently. As interpretation of the immunofluorescence patterns can be difficult, guidelines have been provided by the IAIHG regarding methodology and interpretation of liver autoimmune serology [82]. A major advantage of testing for autoantibodies by indirect immunofluorescence on a freshly prepared rodent substrate that includes the kidney, liver and stomach is that it allows the concurrent detection of several autoreactivities relevant to AIH. These include ANA, SMA, anti-LKM-1 and

Table 10.4 Diagnostic autoantibodies and their targets in juvenile autoimmune liver disease

Autoantibody	Target antigen(s)	Liver disease	Conventional method of detection	Molecular-based assays
ANA	Chromatin Histones Centromeres Cyclin A Ribonucleoproteins Double-stranded DNA Single-stranded DNA Unknown	AIH-1 and ASC	IIF	ELISA, IB, LIA
SMA	Microfilaments (Filamentous actin) Intermediate filaments (Vimentin, desmin)	AIH-1 and ASC	IIF	ELISA
Anti-LKM-1	Cytochrome P4502D6	AIH-2	IIF	ELISA, IB, LIA, RIA
Anti-LC-1	Forminino-transferase cyclodeaminase	AIH-2	IIF, DID, CIE	ELISA, LIA, RIA
Anti-SLA	tRNP(Ser)Sec	AIH-1, AIH-2, ASC Prognostic of severe disease, relapse and treatment dependence	Inhibition ELISA	ELISA, IB, RIA
pANNA	Nuclear lamina Proteins	ASC and AIH-1	IIF	N/A

ANA anti-nuclear antibodies, *SMA* anti-smooth muscle antibodies, *anti-LKM-1* anti-liver-kidney microsomal antibody type 1, *anti-LC-1* anti-liver cytosol antibody type 1, *SLA* soluble liver antigens, *pANNA* peripheral anti-nuclear neutrophil antibodies, also known as atypical pANCA, *AIH* autoimmune hepatitis, *ASC* autoimmune sclerosing cholangitis, *IIF* indirect immunofluorescence, *DID* double-dimension immune-diffusion, *CIE* counter-immune-electrophoresis, *ELISA* enzyme-linked immunosorbent assay, *IB* immunoblot, *LIA* line-immuno-assay, *RIA* radio-immune-precipitation assay, *N/A* not applicable

anti-LC1, as well as anti-mitochondrial antibody (AMA), the serological hallmark of primary biliary cholangitis (PBC), the presence of which weighs against the diagnosis of AIH [2, 3, 78, 82], though rare cases of AMA-positive AIH have been reported, including in children [87–90].

Autoantibodies are considered positive when present at a dilution ≥1:40 in adults, while in children, who are rarely positive for autoantibodies in health, positivity at a dilution ≥1:20 for ANA and SMA or ≥1:10 for anti-LKM-1 is clinically significant [82]. Both in adults and children autoantibodies may be present at a low titre or even be negative at disease onset, particularly during acute or fulminant presentations, to become detectable during follow-up.

ANA is detectable on all rodent tissues and in AIH usually has a homogeneous pattern. For a clearer definition of the pattern, HEp2 cells that have prominent nuclei are used, but these cells are not recommended for screening purposes, because of a high positivity rate in the normal population [82, 91, 92] and in the presence of infection, particularly in children [93].

There are no ANA molecular targets specific for AIH. Though ANA reactivities similar to those found in lupus erythematosus (nuclear chromatin, histones, centromere, single−/double-stranded DNA, ribonucleoproteins) have been reported [94, 95], some 30% of AIH patients positive for ANA by immunofluorescence do not react with known nuclear targets [94]. Immunofluorescence remains therefore the gold standard for ANA testing.

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) [82]. The V pattern is present in non-autoimmune inflammatory liver disease, in autoimmune diseases not affecting the liver and in viral infections, but the VG and VGT patterns are indicative of AIH. The VGT pattern corresponds to the 'F actin' or microfilament (MF) pattern observed using cultured fibroblasts as substrate. The molecular target of the microfilament reactivity remains to be identified. Though anti-actin reactivity is strongly associated with AIH, some 20% of AIH-1 patients do not possess anti-actin antibodies [82].

The anti-LKM-1 pattern is characterized by bright staining of the hepatocyte cytoplasm and of the P3 portion of the renal tubules. Anti-LKM-1 can be confused with AMA, as both autoantibodies stain the liver and kidney, though AMA, in contrast to anti-LKM-1, also stains gastric parietal cells. The identification of the molecular targets of anti-LKM-1, cytochrome P4502D6, and of AMA, enzymes of the 2-oxo-acid dehydrogenase complexes, has allowed the establishment of immuno-assays using recombinant or purified antigens [82], which can be used to resolve doubtful cases.

Anti-LC1, an additional marker for AIH-2, can be present on its own but frequently occurs in association with anti-LKM-1 and targets formimino-transferase cyclodeaminase (FTCD) [96]. Anti-FTCD antibody can be detected by commercial ELISA [82].

Other autoantibodies less commonly tested, but of diagnostic importance, include anti-soluble liver antigen (anti-SLA) and anti-perinuclear neutrophil cytoplasm (pANCA) antibodies.

Anti-SLA is highly specific for the diagnosis of autoimmune liver disease [94, 95], and its presence identifies patients with more severe disease and worse outcome [97]. At variance with standard diagnostic autoantibodies, anti-SLA is not detectable by immunofluorescence. The discovery of the molecular target of anti-SLA as Sep (O-phosphoserine) tRNA:Sec (selenocysteine) tRNA synthase (SEPSECS) [98] and its cloning has led to the availability of molecularly based diagnostic assays.

In AIH-1, akin to ASC and IBD, pANCA are frequently detected, but they are atypical, since they react with peripheral nuclear membrane components, and are therefore also termed peripheral anti-nuclear neutrophil antibodies (pANNA). In contrast to AIH-1, pANNA are virtually absent in AIH-2 [82].

A seronegative form of AIH responsive to steroid treatment has been reported in paediatric retrospective studies, at times associated with the development of aplastic anaemia [60, 62, 99]. In these reports, however, autoantibody testing has not been performed according to IAIHG guidelines. The true prevalence of AIH negative for all the autoantibodies listed above can only be established with a rigorous prospective study.

10.2.8 Histology

Liver biopsy is necessary to establish the diagnosis (Table 10.5). The typical histological feature of AIH is interface hepatitis (Fig. 10.1a), which is however not exclusive to this condition [100]. Interface hepatitis is characterized by a dense inflammatory infiltrate composed of lymphocytes and

Table 10.5 Histological features of autoimmune hepatitis

Feature	Description
Inflammation	Dense mononuclear and plasma cell infiltration of the portal areas
Interface hepatitis	Erosion of the limiting plate and invasion of the parenchyma by plasma cell-rich mononuclear cells that surround damaged hepatocytes
Bridging collapse	Connective-tissue collapse resulting from hepatocyte death and expanding from the portal area into the lobule
Rosette formation	Hepatic regeneration with liver cells forming clusters resembling 'rosettes'
Emperipolesis	Mononuclear cells within hepatocytes
Hyaline droplets	Hyaline droplets in Kupffer cells containing IgG
Fibrosis/ Cirrhosis	New collagen deposition eventually disrupting the liver architecture

plasma cells, which crosses the limiting plate and invades the surrounding parenchyma. Hepatocytes surrounded by inflammatory cells are swollen and undergo pyknotic necrosis. Though plasma cells are characteristically abundant at the interface and within the lobule, their presence in low number does not exclude the diagnosis of AIH. When AIH presents acutely, and during episodes of relapse, a common histological finding is panlobular hepatitis with connective-tissue collapse resulting from hepatocyte death expanding from the portal area into the lobule (bridging necrosis) (Fig. 10.1b). Other features that point to the diagnosis of AIH are emperipolesis and hepatocyte rosetting [101]. These findings, however, are not present in all patients. A recent paper in a paediatric AIH cohort suggests that the finding of hyaline droplets in Kupffer cells is a useful diagnostic marker to distinguish AIH from other forms of chronic hepatitis. The hyaline droplets occur specifically in AIH regardless of the type and are positive for IgG by immunohistochemistry, correlating with a >2-fold increase in serum level of IgG [102].

Histology is also the gold standard for evaluating the extent of fibrosis and helps in identifying overlap syndromes as well as the possible presence of concomitant diseases, such as NASH [103]. Though inflammatory changes surrounding the bile ducts are present also in a small proportion of patients with classical AIH, when conspicuous they suggest an overlap with sclerosing cholangitis [50].

In contrast to patients with an insidious course, those presenting with acute liver failure (ALF) show histological damage predominantly in the centrilobular area [104] often with massive necrosis and multilobular collapse indistinguishable from other forms of acute liver failure [105]. In one paediatric study, histology did not allow distinguishing autoimmune ALF from indeterminate ALF [106]. In the presence of coagulopathy, liver biopsy should be performed by the transjugular route, which is not without risk. If transjugular biopsy is technically not available, the absence of histology should not preclude prompt initiation of immunosuppressive treatment, but liver biopsy should be performed as soon as coagulation indices permit.

10.2.9 Treatment

10.2.9.1 Definition of Remission/Relapse

In paediatric age, remission of AIH has been long defined as complete clinical recovery with transaminase levels within the normal range and is achieved in 60–90% of patients [18, 30, 57, 62, 63], the rapidity and degree of the response to treatment depending on the disease severity at presentation. In more recent years, three more criteria have been added to the definition of remission: normalization of IgG levels, negative or very low titer autoantibodies and histological resolution of inflammation [1]. The histological response, however, lags behind the biochemical response [107–109], and clinical/biochemical/immunological remission does not always reflect histological resolution, though 95% of patients have a marked histological improvement after a mean duration of 4 years of effective treatment [107]. As liver biopsy cannot be repeated frequently, for clinical purposes remission is considered complete when transaminase and IgG levels are normal, ANA and SMA are negative or low titre (<1:20) and anti-LKM-1 and anti-LC1 are negative.

Relapse is characterized by increase of serum aminotransferase levels after remission has been achieved. Relapse during treatment is frequent, occurring in about 40% of patients and requiring a temporary increase in the steroid dose. An important element in relapse is played by non-adherence, which is common, particularly in adolescents [59, 110]. In more aggressive cases, the risk of relapse is higher if steroids are administered on an alternate-day schedule, which is often instituted in the assumption that may have a less negative effect on the child's growth. Small daily doses, however, are more effective in maintaining disease control and minimize the need for high-dose steroid pulses during relapses (with consequent more severe side effects) and do not affect final height [111].

10.2.9.2 When to Treat

AIH should be suspected and sought in all children with evidence of liver disease after exclusion of infectious and metabolic aetiologies. AIH is exquisitely responsive to immunosuppression, and treatment should be initiated promptly to avoid progression of disease. The goal of treatment is to reduce or eliminate liver inflammation, induce remission, improve symptoms and quality of life and prolong life expectancy [1, 112]. Although cirrhosis is present in between 44% and 80% of children at the time at diagnosis [30, 54, 107, 112], mortality within childhood/adolescence is low, and most patients remain clinically stable and well on long-term treatment.

10.2.9.3 How to Treat

With the exception of a fulminant presentation with encephalopathy (see below), AIH responds satisfactorily to immunosuppressive treatment whatever the degree of liver impairment, with a reported remission rate of up to 90% [18, 30, 50, 58].

Standard treatment (Table 10.6)—Conventional treatment of AIH consists of prednisolone (or prednisone) 2 mg/kg/day (maximum 60 mg/day), which is gradually decreased over a period of 4–8 weeks, in parallel to the decline of transaminase levels, to a maintenance dose of 2.5–5 mg/day [1, 76, 77, 113, 114]. In most patients an 80% decrease of the transaminase levels is achieved in the first 2 months, but their complete normalization may take several months [105, 113]. During the first 6–8 weeks of treatment, liver function

Table 10.6 Immunosuppressive treatment regimens for juvenile autoimmune liver disease

	Initial regimen		Maintenance			Definition of remission	Treatment length	Before attempting treatment withdrawal
AIH	**Predniso(lo)ne** 2 mg/kg/day (up to 60 mg/daily) decreased weekly in parallel to transaminase levels decrease to a minimum maintenance dose of 2.5–5 mg daily	**Azathioprine** 1–2 mg/kg/day added gradually if transaminase levels plateau or increase Alternatively, added in all patients after 2 weeks of predniso(lo)ne treatment	**Predniso(lo)ne** 0.1–0.2 mg/kg/day or 5 mg/day	**Azathioprine** 1–2 mg/kg/day if required	**Azathioprine monotherapy (in AIH-1)** 1.2–1.6 mg/kg/day	– Normal transaminase and IgG levels – Negative or low titre (<1:20) ANA/SMA – Negative anti-LKM-1/anti-LC-1	3 years before considering suspension	Remission for at least 3 years + follow-up liver biopsy showing no inflammatory changes
ASC	**Predniso(lo)ne ± azathioprine** as above, plus **ursodeoxycholic acid** 15 mg/kg/day		**Predniso(lo)ne ± azathioprine** as above, plus **ursodeoxycholic acid** 15 mg/kg/day			As above	As above	As above

AIH autoimmune hepatitis, *ASC* autoimmune sclerosing cholangitis

tests should be checked weekly to allow frequent dose adjustments, avoiding severe steroid side effects. The timing for the addition of azathioprine as a steroid sparing agent varies according to the protocols used in different centres. In some, azathioprine is added only in the presence of serious steroid side effects or if the transaminase levels stop decreasing on steroid treatment alone, at a starting dose of 0.5 mg/kg/day. In the absence of signs of toxicity, the dose is increased up to a maximum of 2.0–2.5 mg/kg/day until biochemical control is achieved. In other centres azathioprine is added at a dose of 0.5–2 mg/kg/day after a few weeks (usually 2 weeks) of steroid treatment. Whatever the protocol, 85% of the patients eventually require the addition of azathioprine. Some centres use a combination of steroids and azathioprine from the beginning [56], but caution is recommended with this approach because azathioprine can be hepatotoxic, particularly in cirrhotic and severely jaundiced patients [77]. A recent retrospective analysis of patients treated with a combination of azathioprine and prednisolone from diagnosis reports more side effects (93%) and a higher relapse rate (67%) [115] than what observed in AIH children treated with steroid induction followed by azathioprine addition only when indicated (relapse rate 33–36%; side effects 18–38%) [30, 50].

Measurement of thiopurine methyltransferase (TPMT) activity level before initiating azathioprine therapy has been proposed as a predictor of drug metabolism and toxicity [105] though, at least in adult patients, advanced fibrosis, but not TPMT genotype or activity, was able to predict azathioprine toxicity in AIH [116]. Measurement of the azathioprine metabolites 6-thioguanine (6-TGN) and 6-methylmercaptopurine has been reported to help in identifying drug toxicity and non-adherence and in achieving a level of 6-TGN considered therapeutic for IBD [117], though an ideal therapeutic level for AIH has not been determined. In a recent retrospective review, 87% of 66 children with AIH were reported to maintain sustained biochemical remission (normal transaminase levels) in association with low 6-TGN levels ranging from 50 to 250 pmol on an azathioprine dose of 1.2–1.6 mg/kg/day [118]. Moreover, the same report shows that remission can be maintained on monotherapy with this dose of azathioprine in AIH-1 [118].

Alternative treatments (Table 10.7)—Alternative AIH treatments have been proposed (a) to induce remission at disease onset in an attempt to decrease steroid side effects and (b) to treat refractory patients, i.e. those intolerant of or unresponsive to standard immunosuppression, often referred to as 'difficult-to-treat'.

(a) **For induction of remission**—An attractive drug for the induction and maintenance of remission in AIH is budesonide, a drug with hepatic first-pass clearance of >90% of the oral dose and fewer side effects than predniso(lo)ne, representing an ideal 'topical' liver treatment, more acceptable to patients [119]. A drawback is that it cannot be used in the presence of cirrhosis, which affects at least one-third of AIH patients. In a large European trial, comprising 160 adult and 46 paediatric patients, a combination of budesonide and azathioprine was compared with a combination of prednisone and azathioprine [120]. Remission was defined as normal transaminase levels without steroid side effects. The effect of budesonide at a dose of 3 mg three times daily, decreased upon response, was compared with that of prednisone 40 mg once daily reduced per protocol, irrespective of response, for 6 months; and then budesonide was given to all patients for further 6 months. The results among the children recruited into the study were disappointing, with a similarly low remission rate of 16% for budesonide/azathioprine and 15% for prednisone/azathioprine after 6 months of treatment and of 50% and 42%, respectively, after 12 months of treatment, with similar steroid side effects in both groups, apart from a higher frequency of weight gain in children on prednisone [121]. As these remission rates are much poorer than

Table 10.7 Alternative treatments for juvenile autoimmune liver disease

Agent	Pros	Cons
Mycophenolate mofetil	Favourable toxicity profile Experience as transplant immunosuppressant	Contradictory reports regarding its efficacy Teratogenicity
Tacrolimus	Potent immunosuppressant Experience in the transplant setting	Anecdotal experience Unclear efficacy Renal toxicity
Cyclosporine	Potent immunosuppressant Experience in the transplant setting	Unclear benefit over standard treatment Cosmetic effects Renal toxicity
Budesonide	High first-pass metabolism in the liver Less side effects than prednisolone	Ineffective in cirrhotic patients Less effective as first line treatment compared to standard treatment
Rituximab	Relatively favourable toxicity profile	Infectious complications Anecdotal experience Unclear efficacy
Infliximab	Potent immunomodulatory properties Effective in inflammatory bowel disease	Unclear efficacy in liver disease Infectious complications Paradoxical development of AIH
Ursodeoxycholic acid	Putative immunomodulatory capacities Choleretic	Efficacy yet to be demonstrated

those achieved with the standard treatment schedule, caution is advisable in using budesonide to induce remission in juvenile AIH [19]. A controlled trial in a larger number of treatment-naïve paediatric AIH patients, using a study design that includes strict diagnostic criteria and drug schedules appropriate for the juvenile disease, is needed to establish whether budesonide has a role in the treatment of this condition. Nevertheless, budesonide could be considered as an alternative treatment in selected non-cirrhotic patients who are at risk of adverse effects from steroids.

Induction of remission has been obtained in treatment-naïve children using cyclosporine A alone for 6 months, followed by the addition of prednisone and azathioprine; 1 month later the cyclosporine was discontinued [122, 123]. Cyclosporine was used at the dose of 4 mg/kg/day in three divided doses, increased if necessary every 2–3 days to achieve a whole blood concentration of 250 ± 50 ng/mL for 3 months. If there was clinical and biochemical response in the first months, cyclosporine was reduced to achieve a concentration of 200 ± 50 ng/mL for the following 3 months, before discontinuing it. Whether this mode of induction has any advantage over the standard treatment has yet to be evaluated in controlled studies. Tacrolimus, a more potent immunosuppressive agent than cyclosporine with similar drug class toxicity, has anecdotally been used to induce remission in adults with AIH. Its use in the juvenile form of the disease is limited to one report [124], where tacrolimus was administered to 17 children with newly diagnosed AIH with or without the addition of prednisolone and/or azathioprine and to 3 children who had failed conventional therapy. Target tacrolimus trough levels were relatively low (2.5–5 ng/mL) and similar to those used in the maintenance of successful liver transplant. Though the study shows that monotherapy with tacrolimus is not sufficient to achieve complete remission in most cases, the calcineurin inhibitor is reported to allow reduction of the dose of prednisolone and azathioprine, avoiding their side effects.

(b) **For refractory cases** (Table 10.7)—A promising drug for difficult-to-treat patients is mycophenolate mofetil (MMF), the prodrug of mycophenolic acid. In juvenile AIH patients in whom standard immunosuppression is unable to induce stable remission, or who are intolerant to azathioprine, MMF at a dose of 20 mg/kg twice daily, together with prednisolone, has been used successfully [125]. A recent meta-analysis, including data from several small, even anecdotal, studies of second-line treatments in children refractory to standard therapy suggests that calcineurin inhibitors might have the highest response rate at 6 months but also have the highest rate of adverse events; MMF was the second most effective drug with a low side effect profile, supporting the notion that MMF should be the primary choice for second-line therapy in AIH children refractory to standard treatment [126]. If there is a persistent absence of response or if there is intolerance to MMF (headache, diarrhoea, nausea, dizziness, hair loss and neutropaenia), the use of calcineurin inhibitors should be considered.

Anecdotal experience with successful use of the anti-B lymphocytes monoclonal antibody rituximab in two children with refractory AIH has been reported [127]. However, despite the relatively low adverse event profile of this drug, its use has been associated with a 2.4% rate of sepsis in children with autoimmune diseases [128].

Infliximab has been reported to be effective in the treatment of refractory AIH, including in a paediatric case [129–131]. However, its use as a rescue treatment should be carefully evaluated in view of the potential serious side effects, including infections and hepatotoxicity [129]. Moreover, anti-TNF-α-induced AIH has been reported in adults and children treated for IBD or other autoimmune conditions [132, 133]. Better understanding of the role of TNF-α in the pathogenesis of AIH is needed before recommending its use.

As patients with AIH have a defect in immunoregulation, sirolimus, a drug that selectively expands regulatory T cells in vivo and in vitro [134] has been used in four patients with refractory AIH, with short-term beneficial effect in two of them [135].

Interestingly, a recent survey on management of juvenile AIH commissioned by the IAIHG [136] has shown that within the paediatric IAIHG community there is considerable more experience with second-line therapeutic agents, than among the IAIHG adult hepatologists [137].

Fulminant hepatic failure management—The management of AIH presenting with fulminant hepatic failure (FHF), i.e. with hepatic encephalopathy, is controversial. In adults, corticosteroid therapy is reported to be of little benefit in AIH FHF and to favour septic complications [138]. In a recent paediatric cohort, prednisone treatment has led to the recovery of four out of nine children with AIH FHF referred to a transplant centre, the other five requiring liver transplant despite steroids [106]. In that paper AIH was diagnosed on the basis of positivity for autoantibodies and raised immunoglobulin G. Though liver histology was also obtained, it did not differentiate AIH FHF from cryptogenic FHF, highlighting that fact that liver biopsy in FHF is not only dangerous, because of severe coagulopathy, but also does not provide diagnostic information. Similarly good results with steroid therapy are reported in a paper from India, where 10 out of 13 patients with severe acute presentation of AIH, including encephalopathy in 6, were rescued by prednisone treatment [58].

10.2.10 When and How to Stop Treatment

In paediatric AIH, current recommendation is to treat children for at least 3 years and to attempt withdrawal of treatment only if transaminase and IgG levels have been normal and autoantibody negative (or at maximum titre of 1:20 by immunofluorescence on rodent tissue for ANA/SMA) for at least a year. A liver biopsy is advisable before deciding to attempt treatment cessation, as residual inflammatory changes, even with normal blood tests, herald relapse [1, 76, 77]. Following this protocol, successful long-term complete withdrawal of treatment was possible in 20% of patients with AIH-1, but not in AIH-2, relapse while attempting withdrawal affecting 45% [50]. A recent retrospective review, which includes also a fair proportion (21.4%) of children with AIH/sclerosing cholangitis overlap (who have a different response to treatment, see below), reports successful withdrawal of immunosuppression in some 40% of patients with AIH-1 in whom withdrawal was attempted. Failure to suspend immunosuppression successfully was associated with elevated international normalized ratio (INR), positivity for ANCA, cirrhosis and presence of non-hepatic autoimmune disorders [52]. These encouraging results in juvenile AIH contrast with reports in the adult population [139] possibly because of lack of strict criteria before attempting treatment withdrawal in the latter.

10.3 Autoimmune Sclerosing Cholangitis

Sclerosing cholangitis is a chronic inflammatory disorder that affects the intrahepatic and/or extrahepatic biliary tree leading to bile duct and liver fibrosis. The diagnosis is based on typical bile duct lesions being visualized on cholangiography. With the growing use of non-invasive biliary imaging, sclerosing cholangitis, hitherto considered rare in children, is diagnosed with increasing frequency in paediatric age. It is an important cause of morbidity and mortality, accounting for approximately 2% of the paediatric liver transplants in the United States between 1988 and 2008 [United Network for Organ Sharing (UNOS) Data Report—October 2009. http://www.unos.org/data/].

Sclerosing cholangitis in children/adolescents is widely referred to as primary sclerosing cholangitis (PSC), borrowing the adult definition. However, there are important differences between adult PSC and juvenile sclerosing cholangitis [140].

'Primary' denotes ignorance about aetiology and pathogenesis, while in paediatrics there are well-defined forms of sclerosing cholangitis, including biliary atresia and autosomal recessive neonatal sclerosing cholangitis. Other inherited conditions, e.g. mild to moderate defects in the *ABCB4* (MDR3) gene, are being increasingly recognized as a possible cause of small duct sclerosing cholangitis in both children and adults [141]. Sclerosing cholangitis may also complicate a wide variety of disorders, including primary and secondary immunodeficiencies, Langerhans cell histiocytosis, psoriasis, cystic fibrosis, reticulum cell sarcoma and sickle cell anaemia. An overlap syndrome between AIH and sclerosing cholangitis (autoimmune sclerosing cholangitis, ASC) is more common in children than in adults. Though the name ASC is not universally accepted, it is becoming increasingly more used by both the paediatric and adult hepatology community. Only in those paediatric patients in whom sclerosing cholangitis occurs without any of the above defining features the name of 'primary' would be appropriate.

The only published prospective study aiming at defining the prevalence of ASC versus AIH in children has shown that when cholangiographic studies are performed at presentation, ASC is as prevalent as AIH-1 [50]. This study shows that, in contrast to AIH, ASC affects equally males and females (Table 10.1) and that almost all patients with ASC have autoimmune serology and histological characteristics similar to AIH-1 (Fig. 10.3). The differential diagnosis between AIH and ASC is achieved only by cholangiographic studies, which show evidence of bile duct disease, usually from disease onset (Fig. 10.4). Of note, alkaline phosphatase and GGT levels—usually elevated in cholestatic disease—are often normal or only mildly increased in the early disease stages of ASC, though the alkaline phosphatase/AST ratio is significantly higher in ASC than AIH. A quarter of the children with ASC, despite abnormal cholangiograms, have no histological features suggesting bile duct involvement; conversely, 27% of the patients with AIH have some biliary features on histology (including bile duct damage, acute and/or chronic cholangitis, biliary periportal hepatitis) [50, 142]. The pathognomonic feature of adult sclerosing cholangitis—i.e. fibrous obliterative cholangitis with periductular fibrosis ('onion skin fibrosis')—is rarely seen at presentation in ASC and is a sign of advanced disease.

As mentioned above, neither the original nor the simplified IAIHG scoring systems [2, 3, 78] discriminate between AIH and ASC, as they do not include cholangiographic studies at disease onset. ASC is therefore frequently diagnosed and treated as AIH-1, and the presence of sclerosing cholangitis may be discovered during follow-up, after the appearance of an overt cholestatic biochemical profile. Hence, the ESPGHAN Hepatology Committee Position Statement proposes a new scoring system for juvenile autoimmune liver disease [1] (Table 10.3). The prospective study mentioned above shows that if treatment is started early, the parenchymal liver damage in ASC responds well in terms of normalization of biochemical and immunological parameters to the same immunosuppressive treatment used for AIH, with good medium to long-term survival. However, the bile duct disease progresses in about 50% of patients despite treatment

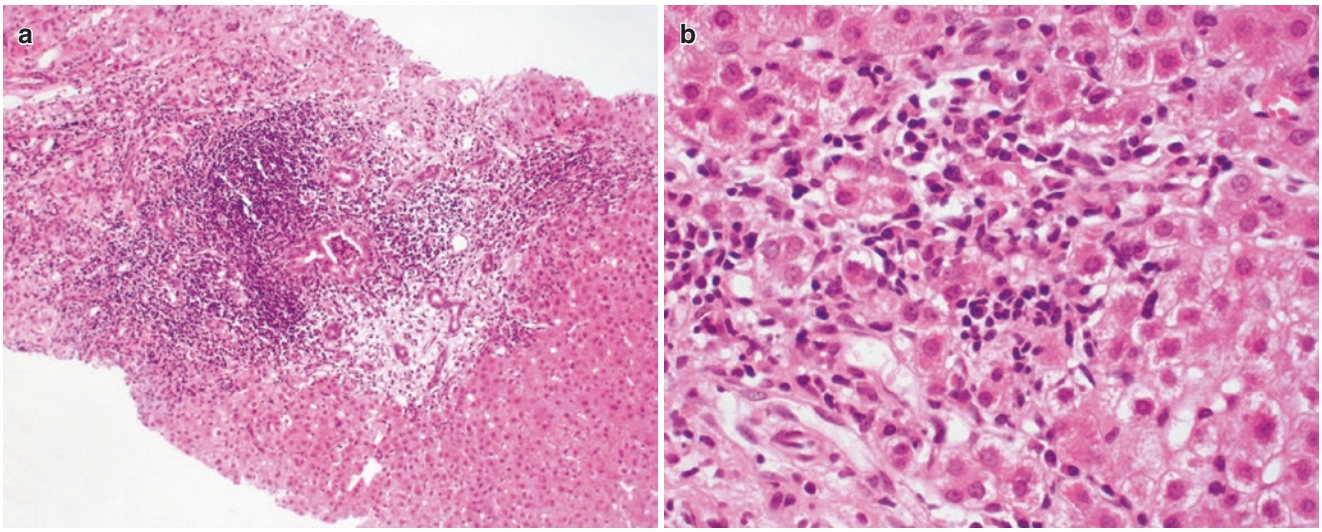

Fig. 10.3 Autoimmune sclerosing cholangitis: (**a**) Portal and periportal lymphocyte and plasma cell infiltrate, disrupting the limiting plate (interface hepatitis) and extending into the parenchyma. The picture is similar to what observed in autoimmune hepatitis; in addition in this case bile duct reduplication and cholangiolitis are observed (haematoxylin-eosin, original magnification 40×); (**b**) Higher magnification (100×) showing inflammatory infiltration with numerous plasma cells (Images kindly provided by Dr. Yoh Zen)

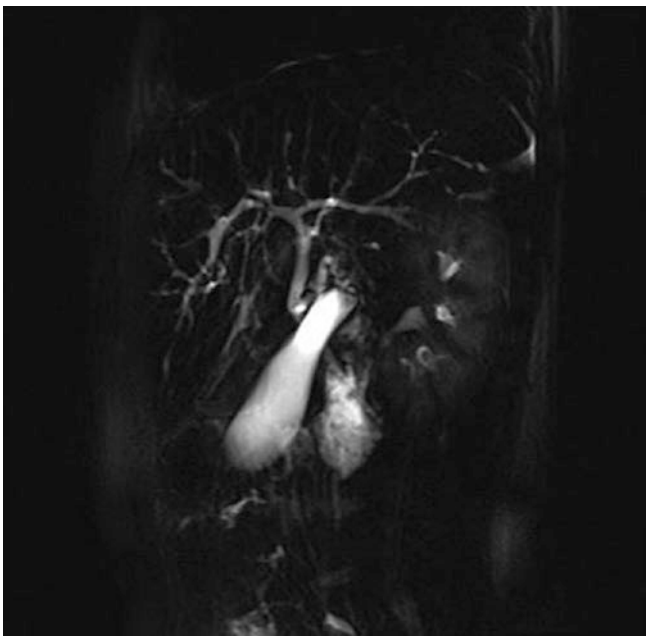

Fig. 10.4 Autoimmune sclerosing cholangitis: Magnetic resonance imaging showing diffuse cholangiopathy with ductal changes in both liver lobes

[50], particularly in those with associated difficult-to-control IBD. In a retrospective study aiming at comparing the response to treatment and outcome of children with AIH and ASC, no difference is reported between the two groups of patients, with a good response to prednisolone ± azathioprine in both [143]. However, in contrast to the prospective study, in this paper the diagnosis of ASC was only made in those patients developing cholestatic manifestations during follow-up, no cholangiographic studies having been performed at presentation, making the comparison between the two studies impossible.

Ursodeoxycholic acid (UDCA) treatment was added to immunosuppression in the prospective study [50], but whether it has any role in arresting the progression of the bile duct disease remains to be established. In adults with PSC, high-dose UDCA was reported as more beneficial than standard doses [144], but a randomized double-blind controlled study shows that high-dose UDCA has a negative long-term effect [145]. It is prudent, therefore, to use doses not higher than 15 mg/kg/day.

Most of the other published series of paediatric sclerosing cholangitis are retrospective studies from single centres, based on small patient numbers, with the exception of a recently published retrospective multicentre large cohort of juvenile sclerosing cholangitis [146]. In these reports the incidence of the various clinical forms of sclerosing cholangitis differs depending upon the year of publication and the centre where the study was conducted, reflecting different study designs, patterns of referral and diagnostic protocols. In all these retrospective series, cholangiographic studies were prompted by biochemical and/or histological features of cholestatic disease. In all, boys are more affected than girls; 20–40% of patients have intrahepatic cholangiopathy with normal extrahepatic bile ducts, and there is a strong association with IBD, which is described in 60–90% of cases according to study design. More than two-thirds of the patients have ulcerative colitis, the others having indeterminate colitis or Crohn disease. IBD can precede the diagnosis of liver disease by many years, be diagnosed at the same time or develop during follow-up.

In all retrospective studies, a variable proportion of patients have ASC, but while in some this condition is reported to respond favourably to treatment with immunosuppression, having a better prognosis than PSC [53, 147–149], in others the prognosis of ASC is reported to be severe and not ameliorated by immunosuppressive treatment [150] or similar to that of PSC irrespective of treatment [146, 151–153]. Major limitations of all these retrospective studies are uneven diagnostic protocols and lack of accurate information on the treatment of IBD before the diagnosis of sclerosing cholangitis, as immunosuppression for IBD has an effect also on the presentation and course of the liver disease. Thus, as shown by the prospective study, which is often cited negatively to support a worse prognosis for ASC compared to AIH, immunosuppressive treatment is effective in controlling both parenchymal and biliary disease in 50% of ASC cases [50], suggesting that the real prognosis of ASC compared to PSC cannot be adequately established in retrospective cohorts with variable diagnostic approaches and treatment protocols.

Recently, it has been suggested that the chronic IBD associated with ASC may represent a distinct nosologic entity, different from classic ulcerative colitis and Crohn disease, being characterized by right-sided colitis with frequent rectal sparing, and small bowel mucosal breaks on capsule enteroscopy [154].

Multicentre prospective studies are needed for defining hepatic and intestinal phenotype of ASC, for establishing diagnostic criteria and for exploring pathogenic mechanisms with the aim of devising more effective forms of treatment.

10.4 Outcome

10.4.1 Autoimmune Hepatitis

Once remission is achieved, the medium- to long-term prognosis of AIH is good. Frequent relapses herald progression of disease. Evolution to cirrhosis is more common in AIH-1 than in AIH-2 [30]. A more severe disease and a higher tendency to relapse are associated with the possession of antibodies to soluble liver antigen (SLA), which are present in about half of patients with either type of AIH at diagnosis [97].

A recent study on 30 children with autoimmune liver disease (AIH, PSC and ASC) reports a decreased health-related quality of life score in patients compared to healthy controls, the worse scores being found in those with complications of chronic liver disease, in particular ascites [155]. In this study, however, 73% of the 30 patients investigated had advanced liver disease. It would be interesting to assess a larger and more representative cohort, including a higher proportion of those patients on long-term immunosuppression without liver-related complications, who represent the majority.

Overall, pregnancy and childbirth appear to be safe for both child and mother, even in women with compensated liver cirrhosis, without the need to withdraw azathioprine [156–160]. For women who are concerned about the use of azathioprine in pregnancy, treatment with steroids alone can be considered. One large series from Sweden reports an increased risk of gestational diabetes, preterm birth and low-birth-weight infants compared with the general population [161]. Clinical improvement and disease exacerbation have been observed in relation to pregnancy, the latter particularly in the post-partum period [159], indicating that high-quality antenatal and postnatal care is essential for women with AIH and their infants.

Despite the efficacy of standard immunosuppressive treatment, severe hepatic decompensation in patients with AIH may develop even after many years of apparently good biochemical control, leading to transplantation 10–15 years after diagnosis in 10% of the patients [30].

10.4.2 Autoimmune Sclerosing Cholangitis

The medium- to long-term prognosis of ASC is worse than that of AIH because of progression of bile duct disease despite treatment in some 50% of patients, with 20% eventually requiring liver transplantation. Reactivation of the liver disease often follows flares of the intestinal disease in patients with IBD. It is therefore essential to control the bowel pathology to avoid progression of liver disease. A beneficial effect of oral vancomycin (500 mg tds) has been reported in patients with sclerosing cholangitis and IBD [162]. All patients showed improvement of liver function tests and erythrocyte sedimentation rate, which was more marked in those without cirrhosis. These results await confirmation in a larger number of patients. Whether vancomycin acts through its antibiotic, choleretic or immunomodulatory properties remains to be elucidated.

Fat-soluble vitamin supplements are required if cholestasis develops. As in AIH, measurement of autoantibody titres and IgG levels is useful in monitoring disease activity and the response to treatment [85]. Evolution from AIH to ASC has been documented, suggesting that AIH and ASC may be part of the same pathogenic process [50].

10.4.3 Neoplasia

Long-term immunosuppressive treatment could be associated with the development of malignancies since extrahepatic cancers, including non-Hodgkin lymphoma and skin cancer, are reported to be more frequent in patients with AIH than in age-matched and sex-matched normal populations [163–166]. The risk of developing primary hepatocellular

carcinoma (HCC) in AIH is associated with the presence of cirrhosis, akin to other chronic liver diseases [164, 165, 167–169]. Both the American Association for the Study of Liver Disease (AASLD) and European Association for the Study of the Liver (EASL) Autoimmune Hepatitis Guidelines recommend active surveillance for HCC [76, 77].

Cholangiocarcinoma (CC) is a very rare complication of paediatric sclerosing cholangitis. While in adult patients with PSC, the incidence and prevalence of CC are reportedly between 5–36% and 0.6% per year, respectively [170]; in paediatrics, there are only three cases of CC described in patients with PSC, two by Deneau et al. [53] and one by Ross et al. [171]. The three patients were 17.9, 18 and 14 years of age at the time of CC diagnosis, all had ulcerative colitis and developed CC 6, 4.2 years and 14 months after the diagnosis of PSC, respectively. None of the patients with ASC enrolled in the prospective study mentioned above [50] has developed CC over an observation period of 30 years. Long-term follow-up of cases identified in paediatric age is needed to establish the incidence and prevalence of CC in juvenile sclerosing cholangitis.

10.5 Implications for Liver Transplantation

Liver transplantation (LT) is a treatment option for AIH and ASC patients with end-stage chronic liver disease, hepatic malignancy or intractable symptoms, as well as for AIH patients presenting with severe acute liver failure unresponsive to steroid treatment.

AIH accounts for 2–5% of paediatric LTs performed in Europe and the United States [76, 172]. The transplant rate for AIH is variable, ranging from 9 to 55%, the interval between presentation and transplantation being as short as days in case of fulminant onset to several years after diagnosis [30, 51, 53, 173]. These different transplant rates depend on several factors: expertise of the reporting centre (primarily transplant or hepatology unit), type of survey (single centre or population based), late referral/treatment, missed diagnosis of ASC and different ethnic background. The reported 5-year survival rate after LT for AIH is excellent, being 80–90% [174].

Sclerosing cholangitis accounts for 2–3% of LTs performed in paediatric-aged patients [175] (United Network for Organ Sharing (UNOS) Data Report—October 2009. http://www.unos.org/data/) only some of whom have ASC [140]. Overall, LT rate for sclerosing cholangitis ranges between 15% and 45%, and the interval between diagnosis and LT ranges from 6 to 12 years [53, 150–152, 176]. In the King's College Hospital prospective study, 4 out of 27 patients with ASC underwent LT during the 16-year study period [50], though it is likely that the rate of LT will increase when the long-term outcome and transition into adulthood data will be analysed [177].

10.5.1 Recurrence of AIH After Liver Transplant

Notwithstanding the good outcome of transplantation for AIH, the disease can recur in the allograft despite immunosuppression [178–182]. The reported recurrence rate is variable and depends on the criteria used for diagnosis, the immunosuppressive regimen, length of follow-up and performance of 'per protocol' biopsies. Mean time from LT to recurrence is 5 years [76, 183], and recurrence rate increases with the post-surgery interval, but it may occur as early as 35 days after LT [184]. The reported recurrence rates in children transplanted for AIH vary from 38 to 83% [51, 173, 185].

The recurrence of AIH after LT can be readily explained. The recipient's immune system is sensitized to species-specific antigens and has a pool of memory cells, which are restimulated and re-expanded when the target antigens, 'autoantigens', are presented to the recipient's immune system either by the recipient's APC repopulating the grafted liver or by the donor's APC sharing histocompatibility antigens with the recipient.

The diagnosis of recurrent AIH is based on the reappearance of clinical symptoms and signs, elevation of transaminases and IgG levels, autoantibodies and interface hepatitis, along with response to prednisolone and azathioprine [76, 186].

Features reported to be associated with recurrence of AIH after LT are possession of either HLA-DR3 or HLA-DR4 by the recipient [187, 188]; discontinuation of corticosteroids after LT [189–191]—therefore caution should be exercised in weaning patients off immunosuppression; and the severity of necroinflammatory activity in the native liver at the time of LT [184, 192]. Most transplant recipients with recurrent AIH respond to reintroduction or an increase in the dose of corticosteroids and azathioprine, which should be implemented as soon as the diagnosis is made. In the case of treatment failure, alternatives include addition of MMF in lieu of azathioprine to the standard therapeutic regimen, replacement of tacrolimus with cyclosporine [193] and replacement of calcineurin inhibitors with sirolimus.

Recurrent disease, particularly if not diagnosed and not treated promptly, may have serious consequences on graft function. In the first paediatric report, out of the five patients who developed recurrent AIH, three progressed to end-stage liver disease requiring re-transplantation [185]. In a series from Birmingham, UK, none of the patients with AIH-1 who developed recurrence progressed to graft failure, while 80% of patients originally transplanted for AIH-2 required re-transplantation [51]. Further support to the negative impact of disease recurrence on allograft survival comes from a United Network for Organ Sharing database; out of 174 children with AIH transplanted between 2002 and 2012, 19%

lost the graft due to recurrent disease [194]. Successful management of recurrent AIH relies greatly on its early diagnosis and prompt treatment. Because histologic evidence can precede clinical evidence of recurrence, it might be useful to include a follow-up liver biopsy in the protocol for the management of patients transplanted for AIH [183, 195].

10.5.2 Recurrence of Sclerosing Cholangitis After Liver Transplant

Recurrence of sclerosing cholangitis after paediatric LT has been reported in between 10% and 50% of recipients without distinction of the form of sclerosing cholangitis leading to transplantation [151, 152, 177, 196], the wide range depending on the length of follow-up, as the risk for recurrence increases over time.

The diagnosis of recurrent sclerosing cholangitis is suggested by histological and/or cholangiographic findings of bile duct disease. Indicative histological findings include fibrous cholangitis, fibro-obliterative lesions with or without ductopaenia, fibrosis or cirrhosis and/or interface hepatitis, whereas the cholangiography generally shows diffuse biliary stricturing [197]. Other causes of non-anastomotic biliary strictures in the graft should be carefully excluded, including ischemic biliary insults (e.g. as consequence of hepatic artery thrombosis), ABO incompatibility between donor and recipient, bacterial or fungal cholangitis and chronic ductopaenic rejection [198]. No consistent risk factors have been reported in association to the development of recurrent sclerosing cholangitis. Some paediatric studies point to an association between active IBD after LT and the development of recurrent disease [152, 177]. Similarly, a study in adult patients transplanted for PSC shows that persistent ulcerative colitis is associated with an increased risk of developing recurrent disease in the graft, whereas colectomy before or during LT conferred protection against the development of recurrent disease [199].

There is no established treatment for recurrent sclerosing cholangitis after paediatric LT. If dominant strictures are present, they should be dilated by interventional cholangiographic means whenever possible [200].

Ursodeoxycholic acid treatment has been advocated in the setting of transplanted adult PSC patients because it seems to improve biochemical indices of liver disease, but it remains unknown whether it has an impact on outcomes [200].

While in adults the impact of recurrence of sclerosing cholangitis on graft survival is controversial, in paediatrics recurrent disease, particularly in the context of ASC, is associated with seriously compromised graft survival: in the King's College Hospital prospective study, two-thirds of patients who experienced recurrent disease eventually required re-transplantation [177].

10.5.3 De Novo Autoimmune Hepatitis After Liver Transplant

De novo AIH after LT affects patients transplanted for disorders other than autoimmune liver disease. While non-specific development of autoantibodies over time after LT is common, affecting over 70% of recipients [178, 201], the prevalence of de novo AIH in children ranges from 2 to 6% [179, 180, 202–206]. The condition was first reported in a paediatric cohort, affecting 4% of children transplanted in a single centre for various non-autoimmune conditions [202]. The patients developed a form of graft dysfunction with features identical to those of classical AIH, namely, high transaminase levels, hypergammaglobulinemia, positivity for autoantibodies—ANA, SMA and typical and atypical anti-LKM-1 (i.e. staining renal tubules only)—and histological features of chronic hepatitis with portal/periportal inflammation and centrilobular necrosis. Other causes of post-LT graft dysfunction, like rejection, infection and hepatic artery thrombosis, were excluded. Patients with de novo AIH did not respond to conventional antirejection treatment but only to the classical treatment of AIH. None of the children had undergone transplantation for autoimmune conditions, and all had serum concentration of calcineurin inhibitor within therapeutic antirejection levels at the time of de novo AIH diagnosis. Since the original observation, several other groups have reported the occurrence of de novo AIH after both paediatric and adult LT. De novo AIH has been described also as a complication in living-donor LT [207]. In the largest study published to date in children, describing 41 (5.2%) patients—out of 788 LTs performed in a single centre—who developed de novo AIH, rejection and steroid dependence were identified as factors predisposing to this complication [206]. In adults, it has been suggested that a histological pattern of centrilobular injury characterized by necroinflammatory activity with plasma cell infiltration might predict the development of this condition [208]. In a paediatric series, the most common early histological feature of de novo AIH was lobular hepatitis, often without interface necroinflammatory activity or prominent plasma cell infiltrates [209].

Awareness that treatment with prednisolone alone or in combination with azathioprine or MMF is successful in de novo AIH has led to excellent graft and patient survival [210]. Akin to the treatment for classical AIH, children should be given a starting dose of 1–2 mg/kg/day of predniso(lo)ne, without exceeding a daily dose of 60 mg, in combination with azathioprine (1–2 mg/kg/day); the steroids should then be tapered over 4–8 weeks, to reach a maintenance dose of 5–10 mg/day. In the absence of response, azathioprine should be replaced by MMF [210]. The importance of maintenance therapy with steroids in de novo AIH was shown in a study comparing treatment with and without steroids: whereas all steroid-untreated patients developed

cirrhosis and either died or required re-transplantation, none of the steroid-treated patients had progressive disease [211].

Akin to autoimmune liver disease outside the context of transplantation, the pathogenesis of post-LT de novo AIH remains to be defined. There are several possible explanations, which are not mutually exclusive. In addition to the release of autoantigens from damaged tissue, one possible mechanism is molecular mimicry, in which exposure to viruses that share amino acid sequences with autoantigens leads to cross-reactive immunity [212]. Viral infections, which are frequent after LT, may also lead to autoimmunity through other mechanisms, including polyclonal stimulation, enhancement and induction of membrane expression of MHC class I and II antigens and/or interference with immunoregulatory cells. Another possible mechanism documented in experimental animals is linked to the use of calcineurin inhibitors, which predispose to autoimmunity and autoimmune disease, possibly by interfering with the maturation of T lymphocytes and the function of T-regs, with consequent emergence and activation of auto-aggressive T-cell clones. Another proposed mechanism stems from observation that patients with de novo AIH often have an antibody directed to glutathione-S-transferase T1 (GSTT1) [213]. Since the gene encoding this protein is defective in a fifth of Caucasoid individuals and the encoded enzyme was absent in patients experiencing de novo AIH, the authors speculated that graft dysfunction resulted from the recognition as foreign of GSTT1 acquired with the graft. However, we have been unable to confirm this observation, having investigated reactivity against GSTT1 sequentially on 60 occasions in 20 patients with post-transplantation de novo AIH.

In murine models of heart allograft, heart transplantation from an allogeneic donor results not only in rejection but also in the production of antibodies and CD4 T cells directed against cardiac myosin in the recipient [214]. The relative importance of autoantigenic and allogeneic stimuli in the development of de novo AIH after liver transplantation remains to be elucidated.

10.6 Conclusions

Autoimmunity is an important cause of liver disease in childhood. The prognosis with immunosuppressive treatment is excellent, with symptom-free long-term survival in the majority of patients with AIH and in some 50% of those with ASC. However, a failure to diagnose and promptly treat these conditions has severe consequences, including progression to cirrhosis, end-stage liver disease, transplantation or death. During the past 40 years, several pathogenic aspects of liver autoimmunity have been elucidated, including predisposing genetic factors and disease-specific humoral and cellular immune responses. Research tasks for the future include further elucidation of the pathogenesis, and the establishment of novel treatments aimed at specifically arresting liver auto-aggression or, ideally, at reinstating tolerance to liver antigens.

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