Diagnosis and Management of Autoimmune Hepatitis

A Clinical Guide

Mark W. Russo
Editor

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ISBN 978-3-030-33627-1 ISBN 978-3-030-33628-8 (eBook)
https://doi.org/10.1007/978-3-030-33628-8

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

To Deborah, Nicholas, Samantha, and Ella for their love and support to allow me to pursue my career.

To Mom and Dad for providing me the opportunities and foundation to succeed.

Contents

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Chapter 1
Epidemiology and Burden of Disease

Mark W. Russo

Abbreviations

AIH	autoimmune hepatitis
Anti-LKM1	antibodies to liver kidney microsome type 1
Anti-SLA	antibodies to soluble liver antigen

Introduction

This chapter will focus on the epidemiology and burden of disease from autoimmune hepatitis. Autoimmune hepatitis is a chronic hepatitis characterized by interface hepatitis with plasma cell predominant infiltrate on liver biopsy, the presence of autoantibodies, elevated immunoglobulin G. It is a disease that predominantly afflicts middle-aged women but can affect either gender at any age. The incidence and prevalence of autoimmune hepatitis are highest in Scandinavian countries and native Alaskans.

Epidemiology and Burden of Disease

Autoimmune hepatitis is a chronic, progressive inflammatory disease of the liver that is relatively rare. The prevalence and incidence of autoimmune hepatitis are difficult to ascertain. Furthermore, estimates obtained prior to the availability of

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M. W. Russo (ed.), *Diagnosis and Management of Autoimmune Hepatitis*,
https://doi.org/10.1007/978-3-030-33628-8_1

serologic testing for hepatitis C and the development of diagnostic criteria by the Autoimmune Hepatitis Working Group may have been inaccurate. Patients with hepatitis C can have autoantibodies and histologic features compatible with autoimmune hepatitis and may have been misdiagnosed with autoimmune hepatitis prior to the development of assays for hepatitis C.

Type I autoimmune hepatitis characterized by the presence of ANA and anti-smooth muscle antibody accounts for 80% of AIH, while type 2 AIH characterized by the presence of antiliver/kidney microsomal antibodies or antiliver cytosol antibodies accounts for the remaining cases [1]. SLA antibodies are associated with a higher rate of liver failure, severe histology, and higher relapse rate [1].

Incidence and Prevalence in North America, Europe, and Asia

The prevalence of autoimmune hepatitis varies by country, gender, and race. There are high incidence and prevalence of AIH in Denmark, Norway, and New Zealand (Fig. 1.1). The prevalence and incidence of autoimmune hepatitis are 17 cases per 100,000 and 1.9 cases per 100,000, respectively, in Norway [2]. The highest prevalence in Scandinavia is in Denmark, 24 cases per 100,000 with an incidence of 1.68 per 100,000 [1, 3]. However, the highest prevalence for AIH is reported in Alaska, 42.9 per 100,000 [4] and more frequently present with acute icteric hepatitis [5]. In comparison, the prevalence of AIH in Spain is 11 cases per 100,000 [6]. In the United States, the annual incidence was reported as one per 200,000 [1]. The prevalence is reported to be lower in Asia: four per 100,000 in Singapore, three cases per 100,000 in China, seven cases per 100,000 in India, but this may be due to underreporting or access to healthcare and underdiagnosis [7, 8]. The prevalence and

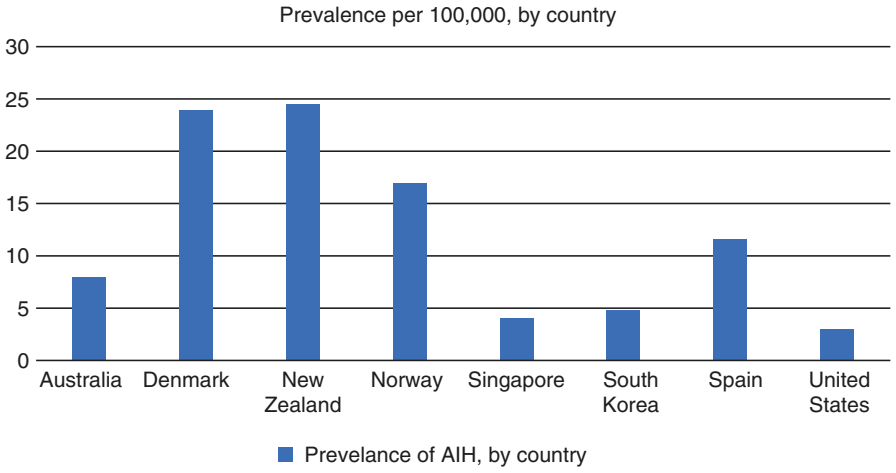

Fig. 1.1 Prevalence per 100,000, by country

incidence of AIH in South Korea are 4.82 per 100,000 and 1.07 per 100,000, respectively [9]. In women and men, the prevalence was 8.35 per 100,000 and 1.3 per 100,000, respectively.

The incidence of AIH is increasing in certain countries. In Valencia, Spain, the incidence has increased 28% over a 13-year period [6]. In Denmark, the incidence doubled over 18 years, and similar trends have been seen in Sweden and the Netherlands [3, 10].

In a meta-analysis of 22 studies, the worldwide annual incidence of autoimmune hepatitis was estimated to be 1.37 per 100,000 and the prevalence was estimated to be 17.44 per 100,000 [11]. The highest annual prevalence was seen in American population 22.8 per 100,000 compared to a prevalence of 19.4 in European population and 12.9 per 100,000 in Asian population. Incidence and prevalence were higher in the elderly and women.

Differences in incidence and prevalence of AIH by region suggest that genetic factors and environmental exposure play a role in the pathogenesis of AIH. Sanitation and differences in exposure to infectious agents may also explain differences in prevalence and incidence. The hygiene hypothesis proposes that the lack of exposure to foreign antigens during early childhood alters the composition of the gut microbiome and increases host immune response to foreign antigens later in life [12].

Risk Factors: Gender, Race, and Age

Women are more frequently afflicted with AIH compared to men, accounting for 75% of cases and typically present in fourth or fifth decade, but 20% are older than 60 years old at presentation. Women may present with fatigue, malaise, or amenorrhea, but a third of patients are asymptomatic and present for evaluation of abnormal liver tests. The female to male ratio is highest in Alaskan natives and Israelis, where 91% and 95% of AIH patients are women [13]. In Denmark, Sweden, and the United States 75–80% of individuals with AIH are female [14]. Men may present at a younger age and more frequently express HLA A1, BDR3 compared to women [15]. Relapse rates are higher in men, but men have better long-term survival compared to women [15]. Because women are more frequently afflicted with AIH than men, it is speculated that the effects of estrogens on cytokines, gene expression, and intestinal microbiome result in women being predisposed to developing AIH [16, 17]. Changes in gut microbiota may lead to increased intestinal permeability and exposure to bacterial antigens that precipitate autoimmune hepatitis.

A new diagnosis of AIH during pregnancy is rare, but women with AIH may relapse during pregnancy or occur after delivery. During pregnancy, disease activity or flares from AIH may be uncommon as a result of an increase in estrogen levels with a shift in cytokine profiles toward antiinflammatory effects [5]. North American women more frequently have HLA DRB1∗04 than men, which has been associated with autoimmune diseases [18].

Compared to Caucasians, African Americans are more likely to have cirrhosis at presentation, 38% and 57%, respectively, and present younger at diagnosis [19].

African Americans present with more severe disease and are more likely to present with liver failure, need for liver transplantation, and higher mortality, 24%, compared to 6% for Caucasians.

African Americans more frequently present with cirrhosis compared to White patients with autoimmune hepatitis, 57% compared to 38%, respectively, and present at a younger age [19]. Liver transplantation and mortality are higher in African American patients with AIH. Genetic polypmorphisms in drug metabolizing enzymes and the expression of different HLA haplotypes may explain some of the differences between White and African American patients. Patients of Hispanic ancestry usually present with more advanced disease with cirrhosis, and Asian patients are reported to have poor survival [20–22].

The risk of autoimmune hepatitis is higher in Black, Latino, and Asian/Pacific Islanders compared to White patients (Table 1.1). The risk of AIH was found to be 9–25-fold higher in these racial and ethnic groups, although there were no differences in ALT levels, total bilirubin levels, or liver fibrosis or cirrhosis at baseline. Japanese patients tend to present with mild disease and to response to ursodeoxycholic acid [5, 7, 22]. Hepatitis A has been implicated as a trigger for autoimmune hepatitis in South American children because HLADR1∗12 has been associated with AIH and protracted hepatitis A infection [23].

There is a bimodal peak in age when the onset of AIH occurs with the first in children in their teens and the second peak in the fourth to sixth decades [20]. The bimodal peak age for developing autoimmune hepatitis occurs between 10 and 30 years and 40 and 60 years old. Approximately, 20% of patients with autoimmune hepatitis are 60 years of age or older at presentation [24]. Similar to younger patients, 76% of elderly patients are female. Patients over the age of 60 years are more likely to be asymptomatic and have cirrhosis at presentation. The mode of onset can be insidious, and among 264 elderly patients with autoimmune hepatitis, 24.5% had cirrhosis without symptoms [24]. The HLA haplotypes most frequently identified are HLA DR3 and DR4, and HLA DR4 is more common in the elderly with AIH. HLA haplotype may influence the age of presentation with AIH, as well as treatment response. Biochemical parameters such as aminotransferases and gamma globulin are similar between elderly and younger patients. ANA and anti-smooth muscle antibody are seen at similar frequency in the elderly compared to younger patients. Older patients are more likely to have ascites on presentation, are as likely to respond to treatment compared to younger patients, but are less likely to relapse if treatment is withdrawn [25].

HLA haplotypes may explain the differences in age at the presentation of autoimmune hepatitis. Patients older than 60 years are more likely to have HLADRB1∗04 compared to younger patients. Individuals with AIH 30 years of age and younger more commonly have HLA DRB1∗03 [26]. Differences in response to antigenic

Table 1.1 Risk of autoimmune hepatitis by race and ethnicity

	OR [95% CI}
Black	9.6 [1.8–178]
Latino	25.0 [5.3–448]
Asian/Pacific Islander	10.8 [2.2–196]

From Lee B, autoimmunity 2018

stimuli based on HLA haplotype have been proposed as a reason for the phenotypic expression of AIH in younger versus older individuals [27].

Autoantibodies

The autoantibodies in AIH, ANA, smooth muscle antibodies, and liver kidney microsomal type 1 antibodies, soluble liver antigen antibodies, have diagnostic more than prognostic utility. In contrast to LKM1 and LK cytosol antibodies, ASMA and antiactin antibodies have been associated with biochemical and histologic activity, although this has not been consistently demonstrated [28, 29]. Anti-LKM1 characterize AIH in children and are antibodies to cytochrome monooxygenase 2D6. This form of AIH may be more severe and is infrequent in adults. It is important to recognize that serum autoantibodies may not initially be present and appear later in the clinical course [5].

There are differences among countries in the presence of these antibodies among countries. Anti-LKM1 is found in 12% of U.S. children with AIH and 38% of children in the U.K [30, 31]. Anti-LKM1 is found in only 1% of U.S. adults with AIH. Anti-SLA characterizes type 2 AIH and is found in 15% of patients in the U.S. [32]. Anti-SLA is an antibody against a ribonucleic acid protein complex. In comparison, anti-SLA is found in 7% of Japanese patients [32].

The presence of ANA and ASMA by itself does not indicate that the patient has autoimmune hepatitis. ANA or ASMA may be seen in up to 19.6% of patients with nonalcoholic fatty liver disease or alcoholic liver disease [33]. ANA is the most frequently positive marker present in 16.3% of NAFLD or ALD patients, and ASMA is present in 2.8% of this patient group [33]. Among patients with chronic hepatitis C serum, ANA or ASMA is found in 1.6–6% of individuals [34, 35].

Concurrent Nonhepatic Autoimmune Diseases

Twenty-four to 34% of patients with AIH have a concurrent autoimmune disease. Female gender and HLA DRB1∗04 and DRB4∗01 have been associated with concurrent autoimmune diseases [36]. Examples of concurrent autoimmune diseases include hypo- or hyperthyroidism, rheumatoid arthritis, and autoimmune hemolytic anemia (Table 1.2). The frequency of concurrent autoimmune diseases is similar between type I and type II AIH.

Table 1.2 Concurrent autoimmune conditions associated with autoimmune hepatitis

Thyroiditis
Graves' disease
Rheumatoid arthritis
Ulcerative colitis
Scleroderma
Autoimmune hemolytic anemia
Vitilogo
Raynaud's phenomena
Discoid lupus
Autoimmune polyglandular syndrome type 1

Burden of Disease

Early studies of untreated patients with AIH reported a 40% risk of death within 6 months of diagnosis [37]. More than 40% of patients with AIH develop cirrhosis, and among patients with cirrhosis, 54% develop esophageal varices [1]. End-stage liver disease from AIH is the indication for liver transplant in 5.9% of patients [38]. However, survival exceeds 90% with treatment with steroids and azathioprine [39, 40]. Over 10 years, 2% of patients undergo liver transplant [41].

Among hospitalizations for AIH, at least one complication from decompensated cirrhosis or hepatocellular carcinoma occurred in 36% of hospitalizations [42]. The most common complication from cirrhosis is ascites in hospitalized AIH patients. The average length of stay is 7 days. The mortality in AIH hospitalization is 4.2%, and Black race is associated with 2.8-fold higher risk of mortality compared to White patients [42]. Other predictors of mortality include female gender and cirrhosis. Mortality is highest among AIH patients with cirrhosis with a standardized mortality ratio of 1.9 compared to the general population, whereas the standardized mortality ratio was 1.2 among AIH patients without cirrhosis [41]. Among individuals with AIH who died, 43% of deaths were liver related. The most common non-liver-related cause of death in AIH patients are related to circulatory system diseases. In the United States, in hospitalized AIH patients, there is a decreased association between cardiovascular disease and AIH with 23–25% reduction of cardiovascular disease and coronary artery disease [43].

In the United States, the rate of hospitalization for AIH is 0.73 per 100,000, which is lower than the rate of 99 per 100,000 for hepatitis C. Similar hospitalization rates are seen in the United Kingdom and Spain [6]. Blacks and Latinos are hospitalized at a higher rate compared to White patients, 69% and 20%, respectively, while Asians and Pacific Islanders were hospitalized at a 64% lower rate [42]. Black race is associated with increased hospital mortality. In South Korea, the average annual cost per patient is $1174, and nationwide direct medical costs $4 million for AIH [9].

The cost of treatment varies based on the regimen chosen to maintain remission. Azathioprine is less expensive than cyclosporine, tacrolimus, and mycophenolate mofetil, which can be as much as ten times the cost of azathioprine [44]. However, the cost of treatment for AIH, even if lifetime, is far less than the cost of liver transplantation and association lifetime transplant-related medications.

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Chapter 2
The Pathogenesis of Autoimmune Liver Diseases

Alexander J. Kovalic and Herbert L. Bonkovsky

Abbreviations

AC	autoimmune cholangitis
ADCC	antibody-dependent cytotoxicity
AE	anion exchanger
AILD	autoimmune liver diseases
AIH	autoimmune hepatitis
AMA	antimitochondrial antibodies
ANA	antinuclear antibodies
(A)SMA	(anti-)smooth muscle antibodies
APC	antigen-presenting cells
ASGPR	asialoglycoprotein receptor
BEC	biliary epithelial cells (cholangiocytes)
CD	cluster of differentiation
(G)CDC	(glyco)chenodeoxycholic acid
CIRP	cold-inducible RNA-binding protein
CMV	cytomegalovirus
CTL	cytotoxic T lymphocytes
CYP	cytochrome P-450

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M. W. Russo (ed.), *Diagnosis and Management of Autoimmune Hepatitis*,
https://doi.org/10.1007/978-3-030-33628-8_2

CTL	cytotoxic T lymphocytes
CTLA-4	CTL antigen A-4
DAMPs	danger-associated molecular patterns
EBV	Epstein-Barr virus
GWAS	genome-wide association study
HAV	hepatitis A virus
HBV	hepatitis B virus
HCV	hepatitis C virus
HDV	hepatitis D virus
HEV	hepatitis E virus
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
HMGB	high-mobility group box
HSP	heat-shock protein
HSV	herpes simplex virus
IBD	inflammatory bowel disease
Ig	immunoglobulins
L	ligand
LKM	liver-kidney microsome
LSEC	liver sinusoidal endothelial cells
LPS	lipopolysaccharide
LT	liver transplantation
NLR	nucleotide binding and leucine-rich repeat
NLRP	NLR pyrin domain containing
NK (T)	natural killer (T) cells
NO	nitric oxide
OADC	organic acid dehydrogenase complexes
OS	overlap syndromes
PAMPs	pathogen-associated molecular patterns
pANCA	perinuclear antineutrophil cytoplasmic antibodies (now called pANNA)
pANNA	perinuclear antineutrophil nuclear antibodies
PBC	primary biliary cholangitis
PD	programmed death
PDC	pyruvate dehydrogenase complex
PSC	primary sclerosing cholangitis
SLA	soluble liver antigen
SLE	systemic lupus erythematosus
STAT	signal transducer and activator of translation
TGF	transforming growth factor
TGR5	Takeda G protein complex R-5
Th	T helper cells
TIPS	transvenous intrahepatic portosystemic shunt
TLR	Toll-like receptors
TNF	tumor necrosis factor
TRAIL	TNF-related apoptosis-inducing ligand
Treg	regulatory T cells

UC	ulcerative colitis
UDCA	ursodeoxycholic acid
UDP	uridine diphosphate
UGT	UDP-glucuronosyltransferase(s)
WAF	water-accommodating fraction

Overview

In this chapter, we first provide a general overview of the AILDs and the key factors involved in their pathogenesis, namely, inciting agents such as bacteria, fungi, viruses, and other infectious agents; xenobiotics and compounds from herbals and other sources; host factors, especially the host's panoply of immune responses and reactions; and other modulating environmental factors, such as nutrition, the microbiome, and the like.

We then describe aspects of pathogenesis important for classical AIH, PBC, and PSC and for overlap syndromes (OS). In Table 2.1, we compare some features of these autoimmune liver disorders with those typically associated with other autoimmune diseases, such as Hashimoto's thyroiditis, systemic lupus erythematosus, rheumatoid arthritis, and idiopathic inflammatory bowel disease (ulcerative colitis, Crohn disease).

Table 2.1 emphasizes that AILD shares many key factors with other AI diseases, including the presence of defined autoantigens and disease-specific autoantibodies, some of which, such as antimitochondrial antibodies (AMA), play a pathogenic rather than simply an epiphenomenological role in causing disease. There are important HLA and non-HLA genetic associations in all, and, with the exception of classical PBC, all are associated with idiopathic inflammatory bowel disease (ulcerative colitis and Crohn disease). Fortunately, with the notable exception of PSC, all respond, albeit variably and sometimes incompletely, to immunosuppressive and antiinflammatory therapy, as is described in greater detail in later chapters.

Table 2.1 Comparison of classic autoimmune diseases and AILD

Variable	Classic AI Dz	AIH	PBC	PSC	OS
Female predilection	Yes	Yes	Yes	No	Yes
Ages affected	Children & adults	Adults	C & A	C & A	C & A
Defined autoantigens	Yes	Yes[a]	Yes	Yes	Yes
Dz-specific auto-Abs	Yes	Yes	Yes	Yes	Yes
Host genetic factors contribute	Yes	Yes	Yes	Yes	Yes
Dz organ selective	Yes	Yes	Yes	Yes	Yes
Response to immunosuppression	Yes	Yes	Yes	No	Yes
Auto-Ag-specific animal models exist	Yes	Yes	Yes	No	Yes

Adapted from Hirschfield [226]

Abbreviations: *A* adults, *Abs* antibodies, *Ag* antigen, *AI(H)* autoimmune (hepatitis), *C* children, *Dz* disease(s), *OS* overlap syndromes, *PBC* primary biliary cholangitis, *PSC* primary sclerosing cholangitis

[a]Type 2 AIH only

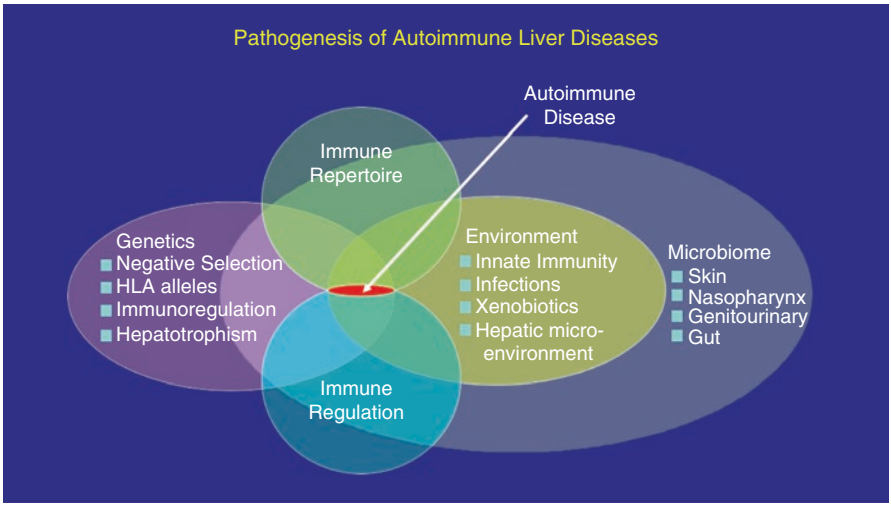

Fig. 2.1 Summary overview of interplay of factors that contribute to the pathogenesis of AILD. Major factors that lead to the development of AILD are summarized in this Venn diagram. They include host genetic factors, the host's immune repertoire and immune regulation, and several environmental factors, especially infectious agents, xenobiotics, and the microbiome. (Figure kindly provided by John Vierling, MD)

In Fig. 2.1, we emphasize the complex interplay of innate and acquired host factors and varied environmental factors, such as infections, exogenous chemicals, drugs, and xenobiotics, and components of the microbiome that, in a few persons among the billions with such exposures, leads to the development of AILD.

We now provide additional details about key factors in the pathogenesis of classical AIH, PBC, and PSC and of the OS.

Pathogenesis of Classic Autoimmune Hepatitis

AIH is an immune-mediated disease of the liver that, as for most autoimmune diseases, affects mainly women (female/male ratio ~ 4/1). It occurs across the world and in persons of all ages, although it especially affects young women and children and has a secondary increased incidence among women of middle age. Two major types of AIH have been described (Table 2.2). The more common type 1 occurs especially in women of all ages who usually have high titers of ANA, SMA, and other antibodies and notable hypergamma globulinemia, indicative of immune activation and B cell and plasma cell production of antibodies. None of these autoantibodies is unique to or diagnostic of AIH. ANA occurs not uncommonly in women with a family and personal history of autoimmune diathesis. SMA is somewhat more specific and accurate but still with overall accuracy not greater than 70%. High levels of Igs (>2 × ULN), especially IgG, are highly specific, albeit not sensitive.

Table 2.2 Autoantibodies in AIH

AIH type	Auto antibodies	Auto antigens	Specificity for liver	Specificity for AIH
1	ANA	Multiple nuclear targets[a]	No	No
	SMA	F-actin	No	No
	pANCA (pANNA)	Beta tubulin, isotype 5	No	No
	ASGPR	ASGPR	Yes	No
	SLA/LP	SepSecS protein	No	Yes, prognostic
	HIP1R	Huntingtin-interacting protein-1-related protein	Yes	Yes
2	LKM-1	CYP2D6	No	No, also HCV
	LKM-3	UGT1A	No	No
	LC-1	FTCD	Yes	Yes
	ASGPR	ASGPR	Yes	No
	pANCA (pANNA)	Beta tubulin, isotype 5	No	No

Abbreviations: *ANA* anti-nuclear antibodies, *ASGPR* asialoglycoprotein receptor, *CYP2D6* cytochrome P-450 2D6, *FTCD* formiminotransferase cyclodeaminase, *HIP1* Huntingtin-interacting protein−1, *LC-1* liver cytosol type-1 antibody, *LKM-1* anti-liver/kidney microsomal antibody-1, *LKM-3* anti-liver/kidney microsomal antibody-3, *pANCA* perinuclear antineutrophil cytoplasmic antibody, *pANNA* perinuclear antineutrophil nuclear antibody, *SLA/LP* antisoluble liver antigen antibody, *SMA* antismooth muscle antibody, *SEPSECS t-RNA* selenocysteinyl-tRNA synthase
[a]Nuclear targets of ANA include centromere, chromatin, ss/ds DNA, histones (Engel et al. [1])

Recently, antibodies directed at Huntingtin-interacting protein1-related protein (HIP-1RP) have been proposed to be a more accurate marker for AIH based on studies thus far carried out only in Europe [1]. Anti-HIP-1 RP antibodies are not believed to be involved in the pathogenesis of AIH.

Type 2 AIH is chiefly a disease of young women, rarely encountered in the USA; it is more common in Europe and especially in Germany, and it is particularly associated with high titers of anti-LKM-1 antibodies, which are specifically directed against epitopes of cytochrome P-450 2D6. These antibodies appear to play a role in pathogenesis because CYP2D6 (and several other CYPs) have been found on the plasma membranes of hepatocytes and thus reasonably may be targets of ADCC (antibody-dependent cytotoxicity) [2].

Environmental Factors That Contribute to the Pathogenesis of AIH

Chemicals, drugs, xenobiotics—in most patients with AIH, the specific triggering factors that lead to the disease are unknown. However, in some, it is clear that exposure to drugs or chemicals plays the key role. This is usually after chronic exposure to such drugs as minocycline, nitrofurantoin, tienilic acid, oxyphenisatin, hydralazine, and alpha-methyl dihydroxyphenylacetate [3, 4].

Indeed, oxyphenisatin and tienilic acid have been withdrawn because of the high frequency with which they caused AIH. However, many more drugs, including statins [5–8], propylthiouracil, NSAIDs, pemoline, anti-TNF agents (infliximab, adalimumab, natalizumab) [9], and the newer immune checkpoint inhibitors, have also been found sometimes to trigger immune-mediated hepatitis. Some of these small molecule xenobiotics likely form reactive metabolites that form covalent linkages with CYPs and other cellular proteins, and these protein-Schlepper complexes serve as neoantigens that elicit first innate and then later adaptive immune responses. The checkpoint inhibitors include monoclonal antibodies directed at the programmed death domain-1 (PD-1) or its ligand PD-1 L or the immune checkpoint molecule cytotoxic T lymphocyte antigen A-4 (CTLA-4). A total of seven such monoclonal antibodies have been approved by the US FDA since 2011. They are ipilimumab, nivolumab, pembrolizumab, atezolizumab, durvalumab, and cemiplimab. These proteins have dramatic efficacy against a growing number of cancers, as well as slow the progression of Jacob Creutzfeldt disease [10]. By design, these monoclonal antibodies put the brakes on the brakes of the immune system, leading to upregulation of the host immune responses such that cytotoxic T cells will attack and kill cancer cells or control infectious pathogens. It is not surprising that such checkpoint inhibitors may sometimes lead to excess upregulation and systemic immune hyperactivation with dermatitis, colitis, acute hepatitis, thyroiditis, etc. [11, 12]. Indeed, there is a relatively narrow therapeutic window between insufficient immune activation, which leads to abrogation of the desired antitumor or antipathogen effects, and excess immune activation, which can lead to severe and sometimes fatal hepatitis, colitis, or other immune-mediated inflammatory disease.

Infectious agents, such as viruses, bacteria, mycobacteria, also are capable of eliciting intense and self-perpetuating immune responses in genetically susceptible hosts. Among the viruses implicated in AIH are the hepatitis A, B, C, D, and E viruses; EBV; CMV; HIV;VSV; and the measles virus [13–19]. Other pathogens, such as *Leishmania*, have occasionally been implicated, as well, in case reports [20]. It is speculated that such pathogens may occasionally elicit ongoing AIH as a result of molecular mimicry, the situation in which immune responses against epitopes on pathogen peptides cross-react with similar epitopes on host peptides.

Host genetic factors—several genetic polymorphisms have been found to confer increased susceptibility or resistance to AIH. Most of these are HLA alleles, which are known to be important in a panoply of immune-mediated diseases. Major ones for AIH are summarized in Table 2.3. Type 1 AIH has strong positive associations with three human leukocyte antigen (HLA) haplotypes in North America and Northern Europe, namely, DR3 and DR4, whereas DRB1∗1501 confers resistance. These class II HLA alleles are in strong linkage disequilibrium with HLA class I HLA-A, -Cw- and -B molecules with the extended haplotype HLA A∗0101-Cw∗0701-B∗0801-DRB∗0301-DQA1∗0501-DQ [21].

Upregulation of innate immunity—the current paradigm of immune-mediated cellular injury envisions the formation of damage-associated molecular patterns (DAMPS) produced by xenobiotics, for example, and/or pathogen-associated

Table 2.3 Genetic associations with AIH in selected geographic areas

Susceptibility alleles	Geographic areas
DRB1∗0301	North America and Europe
DRB1∗0401	North America and Europe
DRB1∗0404	China, Japan, Mexico (Mestizo)
DRB1∗0405	Argentina (adults), China, Japan
Resistance alleles	
DRB1∗1501	North America, South America, Europe, Japan
DRB1∗1301	South America
DRB1∗0301	South America

Adapted from Sahebjam and Vierling [21]

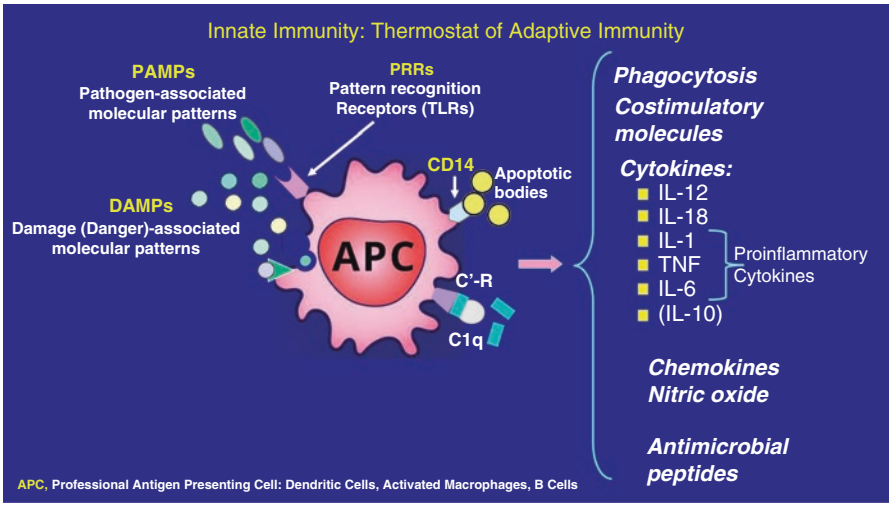

Fig. 2.2 Activation of the innate immune system by DAMPs and PAMPs is an important early step in the development of AILD. (Figure kindly provided by John Vierling, MD)

molecular patterns (PAMPS) produced by viruses, bacteria, and other pathogens (Fig. 2.2). As shown in Fig. 2.3, DAMPS and PAMPS bind to pattern recognition receptors (Toll-like receptors) on antigen-presenting cells (APC) in the liver, leading to the production of a host of cytokines, chemokines, and other factors, which call forth the innate immune response. DAMPS include HMGB1, histones, and CIRP from damaged cell nuclei; HSPs and lysosomal enzymes from the cytosol and small molecules, such as ATP; fragments of mitochondrial and nuclear DNA, RNA, ribonucleoproteins, and uric acid. PAMPs include LPS, peptioglycans, lipoteichoic acids, arabinomannans, glucans, unmethylated CpG, viral proteins, and some host proteins, such as HSP60 and fibronectin.

The DAMPs and PAMPs call forth hepatic inflammasome activity in multiple cell types of the liver, including hepatocytes, cholangiocytes, hepatic stellate cells, and immune cells. The increased inflammasomes recruit and stimulate natural

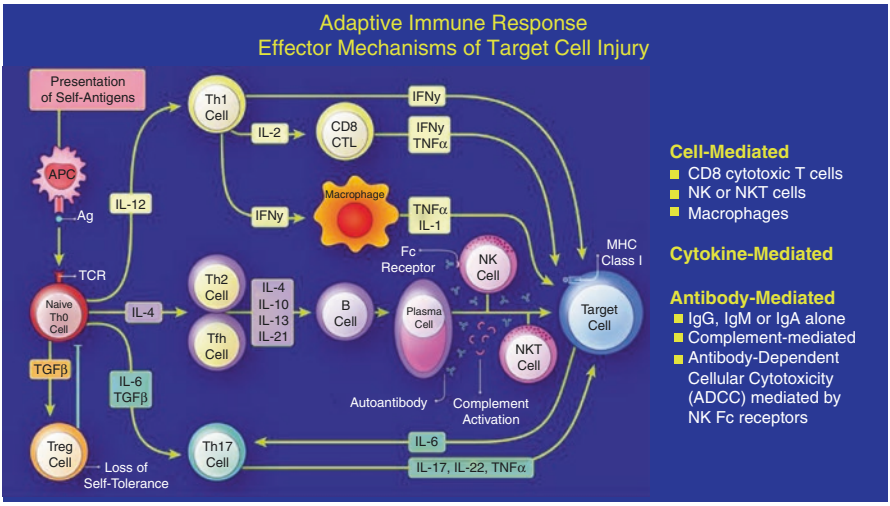

Fig. 2.3 Recruitment of the adaptive immune response, following the activation of the innate immune system, is essential for the development of AILD. (Figure kindly provided by John Vierling, MD)

killer (NK) and natural killer T cells (NKT) to attack and lyse injured hepatocytes. Thus, stimulation of the innate immune response is central to the pathogenesis of AIH.

The adaptive immune response—in addition, activation of the adaptive immune response leads to the full and exuberant pattern of T lymphocytes, B lymphocytes, and plasma cells, which are the hallmarks of full-blown AIH. The main pathways and mechanisms for the activation of these effectors of inflammation and lysis and clearance of damaged hepatocytes is summarized in Fig. 2.4. Worthy of emphasis are the large number of cytokines that are involved and the key roles of CD8+ cytotoxic T lymphocytes, which are the primary cells that attack hepatocytes, the B cells and plasma cells that chiefly produce the immunoglobulins that serve as markers of AIH, and the Th17 cells that enhance T-cell-mediated killing of hepatocytes. In addition, the downregulation of Treg cells leads to loss of self-tolerance.

Figure 2.5 provides an integrated summary working model of the pathogenesis of AIH, including the predisposition of susceptible hosts, mainly related to HLA type; the requisite environmental exposures, especially chemicals and infectious agents; the production of DAMPs and PAMPs, leading to hepatocyte activation via the innate immune system and then to activation of the adaptive T- and B-cell-mediated immune responses. If these are left unchecked, the end result is the development of hepatic fibrosis, the development of active cirrhosis or risk of decompensation of cirrhosis, and/or the development of hepatocellular carcinoma.

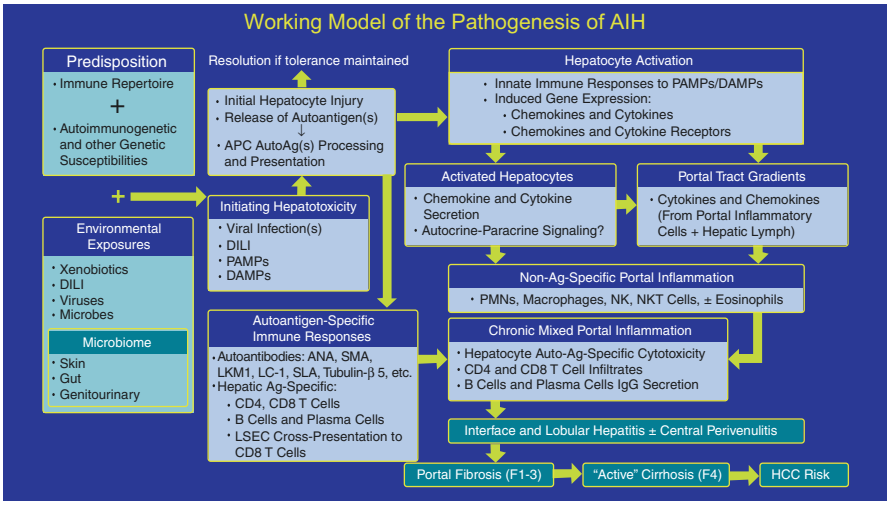

Fig. 2.4 Summary working model of the pathogenesis of AIH and its sequelae. The interplay of several factors, including environmental triggers, innate host immunity, autoantigens and autoantibodies, and the adaptive immune response is summarized. (Figure kindly provided by John Vierling, MD)

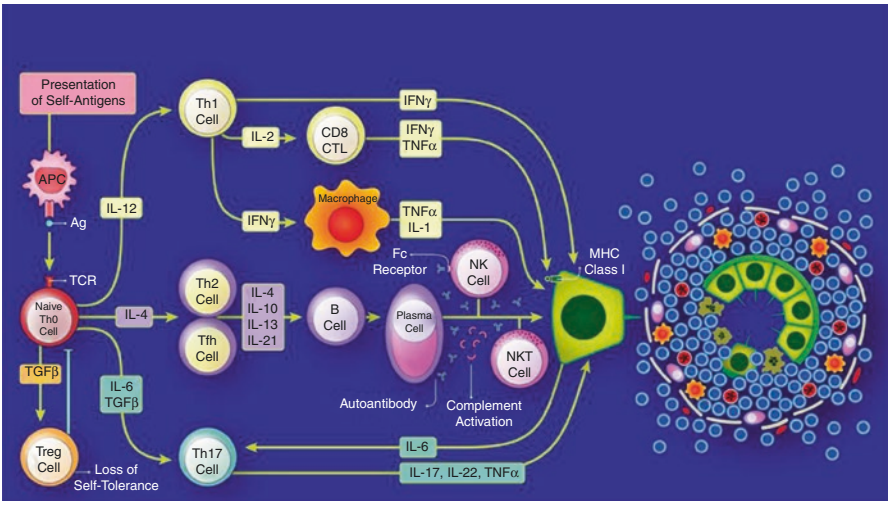

Fig. 2.5 Key factors and steps in the pathogenesis of primary biliary cholangitis. The self-antigens are different from those involved in other forms of AILD, but the effectors are similar. The main targets of the immune response are intrahepatic cholangiocytes. (Figure kindly provided by John Vierling, MD)

Pathogenesis of Primary Biliary Cholangitis, Primary Sclerosing Cholangitis, and Overlap Syndromes

Primary Biliary Cholangitis

Primary biliary cholangitis (PBC) is a form of autoimmune liver disease (AILD) primarily targeting interlobular bile ducts, which ultimately induces lymphocytic cholangitis, and my lead to progressive cholestasis, and biliary cirrhosis. PBC and AIH may present as an overlap syndrome with features of both diseases. As is the case for classical AIH, full-blown PBC develops as the end result of a multitude of pathophysiologic factors, mechanisms, and pathways, which have similarities but also important differences from those involved in the pathogenesis of AIH.

Cholangiocyte Physiology and Pathophysiology

Normal Functions of Healthy Cholangiocytes

Injury to cholangiocytes, also known as biliary epithelial cells (BECs), is a critical component in the pathophysiology for a number of hepatobiliary disorders. Healthy BECs are imperative for maintaining a normal intact biliary milieu by providing a primary barrier blocking the systemic entry of toxic compounds and gastrointestinal microbes in the biliary lumen. Healthy BECs secrete bicarbonate (HCO_3^-) into the biliary lumen in order to maintain an alkaline pH [22]. This is sometimes referred to as the "bicarbonate umbrella" because it serves to protect and prevent against the protonation of bile salts. Failure of this umbrella allows for the bile salts to undergo protonation, which facilitates their translocation across the apical membrane domain of the BECs and which, inside BECs, generate cholangiocyte cellular injury and apoptosis. Furthermore, BECs also serve an influential role as APCs and immunomodulators. Unsurprisingly, BECs are in close contact with innumerable microbes from the gastrointestinal tract, some of which return to the liver via enterohepatic circulation. BECs recognize and react to these microbes through the signaling of DAMPs and PAMPs, presenting this information as APCs, and, if necessary, the appropriate activation of immune cell-mediated cellular injury and apoptosis. The role of healthy BECs is critical, and it remains paramount that their activation and normal cellular function are counterbalanced by the maintenance of self-tolerance and avoidance of autoimmune destruction of native cholangiocytes.

Defective Biliary Bicarbonate Umbrella

As previously mentioned, the maintenance of bicarbonate secretion and alkaline pH in the biliary lumen serves as a major protective mechanism for BECs against bile salt-induced cellular injury and apoptosis. Aberrancies at any number of break-

points may precipitate PBC pathogenesis; however, perhaps, the best-supported hypothesis in current literature envisions a defect in the chloride/bicarbonate anion exchanger 2 (AE2) [23]. When this function is compromised, there is decreased luminal secretion of bicarbonate into the biliary lumen, which then leads to increased protonation and acidification of bile salts [24, 25]. These now uncharged, hydrophobic bile acids are cytotoxic and may now translocate across the apical BEC plasma membrane.

Intracellular detergent actions of these bile acids can exert manifold damaging effects. Several in vitro studies have illustrated interesting findings with respect to BECs in the presence of two major human bile acids, chenodeoxycholate (CDC) and its glycine conjugate, glycochenodeoxycholate (GCDC). One recent study with human BECs demonstrated that intracellular bile acid uptake into the BEC is a pH-dependent process [26]. A decrease of pH from 7.4 to 6.4 decreased cell viability >80% and increased apoptosis 10- and 30-fold at stable concentrations of bile acids CDC and GCDC, respectively, suggesting that acidification alone had no effect. This study also showed that AE2 knockdown led to a three- and twofold increase in apoptosis in the presence of CDC and GCDC, respectively [26]. Further in vitro studies within human cholangiocytes have corroborated these findings [27]. However, another study suggests an adverse positive feedback loop, namely, that the presence of hydrophobic bile acids suppresses AE2 expression in BECs and additionally induces bile duct inflammation in PBC [28]. Overall, it appears that a functioning bicarbonate umbrella, an intact apical glycocalyx, and sufficient AE2 expression are important factors in the protection against BEC injury and, most likely, PBC pathogenesis.

Once bicarbonate secretion is suppressed, primarily through defective AE2 function, there is an increased intracellular pH from the heightened concentration of bicarbonate within the cholangiocyte. This alkaline shift is tightly regulated and sensed by soluble adenylyl cyclase, an intracellular bicarbonate sensor. This enzyme has been shown to be increased in PBC, particularly in the setting of a defective bicarbonate umbrella [29]. Furthermore, it has been shown that this cyclase regulates bile-salt-induced cholangiocyte apoptosis [30].

Cholangiocyte Injury and Apoptosis

Up to this point of this putative pathogenic mechanism, BECs have experienced several modalities of cellular injury via impaired bicarbonate secretion and increased targeting for apoptosis by soluble adenylyl cyclase. Additionally, there are numerous other means of cholangiocyte injury and response, including interaction with gut microbes, DAMPs, and PAMPs, particularly in the setting of increased exposure to pathogens, which may occur via return through portal circulation or through their pathologic presence in the biliary tree. These effects may be synergistically compounded in the presence of circulating autoantibodies and immune dysregulation.

Nonetheless, there appears to be a familiar phenomenon with respect to apoptosis in PBC. Normally, cholangiocytes targeted for apoptosis undergo a glutathione-

mediated modification of mitochondrial PDC-E2 (E2 subunit of the pyruvate dehydrogenase complex). However, this mechanism appears to be lost in cholangiocytes in PBC [31]. This deviation of apoptosis leads to the formation of apoptotic blebs, also referred to as "apoptopes." These apoptopes are characterized by a common motif of the preserved lysine-lipoyl residue, which serves as the key antigen that calls forth antimitochondrial autoantibodies [32]. Not only does apoptope binding with circulating autoantibodies result in immune activation with subsequent cellular injury and inflammation, but the intrahepatic BECs will also undergo phagocytosis of the apoptotic blebs of neighboring BECs, thus precipitating a cycle of further cellular injury and inflammation [33]. Furthermore, some evidence suggests that the clearance of apoptotic blebs is limited in PBC. It has been demonstrated that macrophage-mediated phagocytosis of apoptotic cholangiocytes reverses biliary fibrosis [34], suggesting that the diminution of this process is integral to PBC pathogenesis.

Cholangiocyte Senescence

The concept of senescence, characterized by a permanent state of cell cycle arrest, has been theorized to serve as a conservative mechanism in order to remove exhausted cells and ensuing cellular damage from a local milieu. However, there is an increased number of senescent cells in PBC [35], in addition to increased expression of senescence-associated cell cycle regulators [36]. One possible mechanism explaining this manifestation is increased endoplasmic reticulum stress triggering increased BEC autophagy and cellular senescence in PBC [37]. However, the crucial component of senescence with respect to PBC pathogenesis is that these cholangiocytes will ultimately transition to a "secretory" phenotype, which will produce markedly increased amounts of IL-1, IL-6, CX3CL1, CXCL8, CCL2, growth factors, and matrix metalloproteases [38]. These proinflammatory cytokines establish a foundation for CD4+ and CD8+ T-cell recruitment, cholangiocyte cellular injury, inflammation, and cell death [31, 39]. The general pathway and key players (Fig. 2.6) resemble those already described for AIH but with the crucial difference that the principal target in PBC is intrahepatic cholangiocytes that have already been injured by failure of the bicarbonate umbrella mechanism.

Understanding putative pathophysiologic mechanisms among cholangiocytes is integral to the pathogenesis of PBC and PSC. Alternative aberrant factors at play, such as hepatic immune dysregulation, autoantibodies, and genetic predisposition, will not uncommonly affect these same common themes among cholangiocyte pathophysiology in PBC and other AILDs.

Environmental Factors

Several environmental factors exist, which have been postulated to active cholangiocytes and further contribute to PBC pathogenesis.

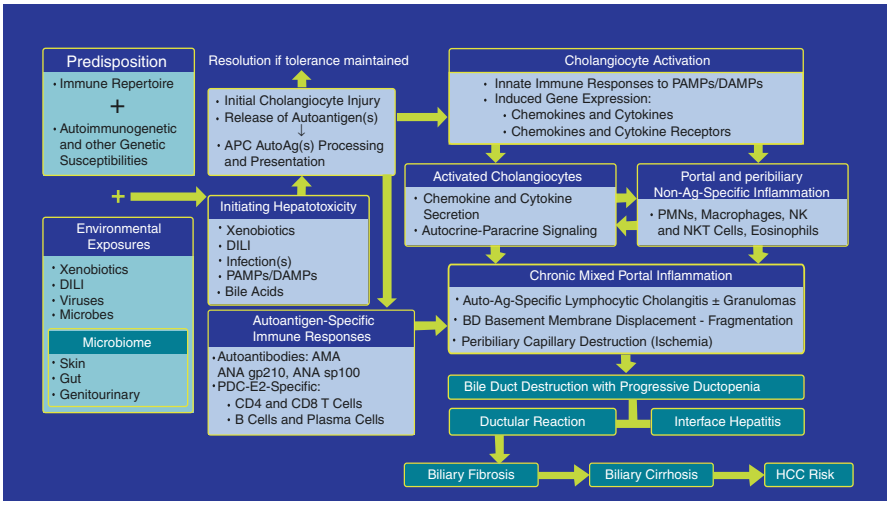

Fig. 2.6 Summary working model of the pathogenesis of PBC and its sequelae. The interplay of several factors, including environmental triggers, innate host immunity, autoantigens and autoantibodies, and the adaptive immune response is summarized. (Figure kindly provided by John Vierling, MD)

Molecular Mimicry

One overarching theme in the pathophysiology of AILD is the concept of molecular mimicry. In this mechanism, primed T and B cells cross-react against a similar antigen and/or epitope(s) that fosters loss of self-tolerance to a specific autoantibody, such as AMA PDC-E2, and subsequently elicits a vigorous immune response [40]. Common factors involved in molecular mimicry and the pathogenesis of PBC include microbial pathogens and xenobiotics.

Perhaps the best-supported facet of this theory is exemplified by the strong epidemiological relationship between recurrent UTIs and development of PBC. This is thought to be accounted for by molecular mimicry between epitopes of *Escherichia coli* bacteria and PDC-E2 autoantigen [41]. These findings have been corroborated in several studies showing the association between UTIs and PBC [42–45]. Furthermore, among women with a history of recurrent UTIs, a positive anti-sp100 antibody strongly correlated with AMA positivity in patients both with and without liver disease; additionally, 74% of PBC patients with prior UTI tested positive for anti-sp100 antibody in this study [46].

Other microbial pathogens have also been implicated as inciting factors for molecular mimicry. Chronic hepatitis C infection has been shown to impair APO2 ligand/TNF-related apoptosis-inducing ligand (TRAIL)-dependent dendritic cell cytotoxicity, thus potentiating increased risk of autoimmunity [47]. *Lactobacillus delbrueckii*, a probiotic commonly found in yogurt and other dairy products, has a confirmed IgG3 antibody cross-reactivity between both the SxGDL(IVL)AE motif

of *L. delbrueckii* galactosidase and human PDC-E2 [48]. Another bacterium, *Novosphingobium aromaticivorans*, which is a gram negative aerobe regularly found in water and soil, produces lipoylated proteins with the highest-known homology and cross-reactivity toward AMA anti-PDC-E2 to date [49].

Xenobiotics

Various xenobiotic agents have also been thought capable of eliciting molecular mimicry-induced pathogenesis. It has been shown in mouse models that xenobiotics are able to induce PBC through the activation of BEC apoptosis and the release of autoantigens [50]. One study described over ten xenobiotic compounds that stimulate robust IgM and IgG responses against the highly antigenic lipoylated PDC-E2 peptide. Among these, 2-octynoic acid appears to be the most potent and is found frequently in many cosmetics (perfumes, lipsticks, etc.) and food additives [51]. This biochemical-immunological association correlates with various other studies reporting an association between cosmetic use and PBC [43, 52–54]. Another study confirmed these findings with 2-octynoic acid and additionally revealed cross-reactivity of PDC-E2 autoantibodies with 6,8-bis(acetylthio)octanoic acid (Sac), which is a metabolite of acetaminophen [55]. Overall, the xenobiotics implicated in PBC to date may represent only the tip of a much larger iceberg that likely plays a role in the pathogenesis of PBC.

Smoking as a Risk Factor

Several studies have established a strong link between PBC and smoking history and/or tobacco use [44, 56–58]. Smoking even appears to worsen disease progression and liver fibrosis among patients with PBC [59, 60]. There are numerous compounds found in tobacco smoke, and it seems likely that, in the genetically susceptible host, one or more of these likewise lead to molecular mimicry with autoantigens.

Intestinal Dysbiosis

As a progressive cholestatic disease, PBC has been characterized by elevated serum and fecal bile acids, and disease severity closely correlates to intestinal dysbiosis [61]. Ursodeoxycholic acid (UDCA) remains one of the therapeutic mainstays for PBC, and it has been shown to partially ameliorate the dysbiosis seen in gut microbiota. Not only has the transformation toward intestinal dysbiosis become a biomarker for disease, but the reversal and correction of this anomaly may serve as a viable therapeutic target for therapy of PBC.

Hepatic Immune Dysregulation

While pathologic mechanisms among the cellular functions of cholangiocytes are certainly at play, the pathogenesis of PBC also requires a genetically susceptible host (Fig. 2.1). This is thought to be the reason that, among the many persons with a history of *E. coli* UTI and other exposure to bacteria, xenobiotics, tobacco smoke, octynic acid, etc., only a relatively small number develops PBC. Thus, immune activation and dysregulation within the liver are of central importance as well. Immune disturbances occur at multiple levels, including activation of cholangiocytes with subsequent autocrine and paracrine signaling, aberrancies in innate immunity, adaptive immunity, cytokine production, and autocrine-paracrine signaling.

Autocrine and Paracrine Signaling from Activated Cholangiocytes

Whether it be from toxic exposure to bile acids themselves or direct targeting by cytotoxic immune activation, in PBC there is an underlying insult to the BECs leading to the release of proinflammatory mediators TNF-α, IL-6, IL-8, TGF-β2, nitric oxide (NO), and various growth factors upon cholangiocyte activation [62]. These mediators act in an autocrine and paracrine fashion to provide considerable crosstalk with inflammatory cells, lymphocytes, hepatocytes, hepatic stellate cells (HSC), stem cells, endothelial cells, and subepithelial myofibroblasts [63]. This autocrine and paracrine signaling ultimately instigates the recruitment and activation of innate and adaptive immune cells, cholangiocyte proliferation, senescence, the modulation of apoptosis, local inflammation, and fibrosis.

Dysregulation of Innate Immunity

As mentioned previously, there are several mechanisms of cholangiocyte immune activation, particularly after interaction with PAMPs and lipopolysaccharide (LPS) endotoxins from the biliary lumen itself or derived from the portal blood returned from the gut. BECs interact with and regulate the binding of these PAMPs/endotoxins to Toll-like receptors (TLRs) via their intrinsic properties as APCs [64]. LPS specifically binds to TLR4 and activates NF-kB via the IL-1 signaling cascade [65]. With respect to PBC, TLR-mediated pathways lead to natural killer (NK) cell activation (upregulation of the innate immune response), resulting in increased cytotoxic cholangiocyte cell death [66]. Additionally, the activation of NF-kB and subsequent biliary injury results in increased cytokine release, in particular IL-8 and CX3CL1 [67]. Perhaps the largest role in PBC pathogenesis, however, lies within the IL-12 pathway. After TLR activation and IRF5 phosphorylation, the APCs produce large amounts of IL-12, which subsequently activates NF-kB and STAT4 pathways via transcription factor IRF8 in the costimulated CD4+ T cell [68].

This perpetuates an increased release of TNFa and IFN-γ, which not only catalyze a polarization for Th1 cell formation but also produce positive feedback on the APC, thus amplifying this entire process [69]. Overall, the underlying activation of cholangiocytes and resultant innate immune response establishes an auspicious milieu for PBC pathophysiology, characterized by ongoing inflammation and further immune activation.

One study analyzed the role of NK cells in detail, particularly with respect to the NK:BEC ratio, and yielded some interesting results [70]. In the presence of a high NK:BEC ratio, similar to early findings of PBC, there was increased direct NK-cell-mediated cytotoxicity toward BECs. However, with a low NK:BEC ratio, analogous to findings seen later in PBC progression, there was a lack of BEC lysis but with a corresponding increase in IFN-γ. This induced an increased expression of MHC class I and II molecules on the surfaces of BECs, which play a crucial role for autoreactive CD4+ T-cell-mediated cytotoxicity upon future exposure to autologous BECs. These findings support the role of IFN-γ in the chronic cytopathic response via autoantigen-specific T cells with respect to the progression of PBC.

Aberrancies of Adaptive Immunity

There clearly is a strong influence of immune activation through cholangiocyte pathophysiology, loss of immune tolerance, and reactivity toward circulating autoantibodies. A potent innate immune response, as detailed above, sets the stage for a robust adaptive immune response. The presence of autoantigens is the primary inciting factor for this adaptive immune response, directly resulting in the recruitment and activation of CD4+ and CD8+ T cells [71, 72]. This is further supported by the continuous autoantigen-specific B-cell activation observed in PBC [73]. These specific B-cell populations are dependent on the autoantibodies present, which are further detailed in the next section below.

The initial innate immune response, secretion of proinflammatory cytokines, and strong presence of IFN-γ help bolster a Th1-dominant adaptive immune response. The increased activity of Th1 cells serve to upregulate TLR expression, which leads to increased vulnerability to PAMPs and further augments the innate immune response [74]. This also constructs a platform for increased apoptosis of BECs, as recognized through increased expression of Fas, FasL, TRAIL, perforin, WAF1, and granzyme B in PBC [75, 76]. This response is even further sustained and enhanced after the formation of apoptotic blebs, which can again be the targets of plasma and B cells, circulating autoantibodies, and sources of subsequent cell injury and inflammation.

There appear to be irregularities in other subpopulations of the adaptive immune response in the pathophysiology of PBC. Th17 (T helper type 17) cells are increased in PBC, which overall augment further cholangiocyte injury and the progression of fibrosis [77]. Meanwhile, Treg (T regulatory) cells, which normally function to dampen excessive immune responses, modulate APCs, and secrete anti-inflammatory cytokines, are found to be decreased among patients with PBC [77, 78]. Finally,

there are specific populations known as Tfh (follicular helper T cells) and Tfr (follicular regulatory T cells) that have been shown to be skewed in PBC. Recent studies have illustrated an increase in Tfh and a decrease in Tfr cells, which together bolster the B-cell response, augment the production of specific autoantibodies, and improve B-cell memory [79–81]. Another study has illustrated the concomitant increase in both Tfh cells and CD38+ plasma cells in patients with PBC [82]. Overall, the shift in these specific T-cell subpopulations amplifies the effects of pathologic immune responses that fuel further PBC disease progression.

Histologic Hallmarks of PBC

These pathologic mechanisms of hepatic immune dysregulation correspond to the hallmark histologic findings of PBC characterized by lymphocytic cholangitis of interlobular bile ducts, mediated by CD4+ and CD8+ T-cell infiltration in addition to plasma and B-cell-induced IgM production [83]. The destruction of peribiliary capillary plexuses and the displacement of BEC basement membrane by inflammatory cells can also be seen in PBC [84]. These attributes coincide with cholangiocyte pathophysiology of altered protective barrier to bile acids, impaired bicarbonate umbrella, and defective cholangiocyte transportation of secretory IgA (sIgA). Overall, this sets the stage for classic histological findings of PBC disease progression from lymphocytic cholangitis, ductular reaction and progressive ductopenia, interface hepatitis, biliary fibrosis, and ultimately biliary cirrhosis (for more details, see the Chap. 4).

Autoantibodies

As mentioned previously, the presence of circulating autoantibodies appears to be a central factor in PBC pathogenesis. Potentially invoked by molecular mimicry toward viruses, xenobiotics, and/or bacteria or to the specific intracellular targets themselves, it appears that the production of autoantibodies by plasma cells is integral to the formation of apoptotic blebs, activation of immune dysregulation, and triggering of cellular injury, inflammation, and apoptosis [85]. A list of autoantibodies relevant to PBC is provided in Table 2.4.

The autoantibody with the most robust evidence for a pathogenic role remains the antimitochondrial antibody (AMA), and specifically targeting the E2 subunit of PDC. Other antimitochondrial antibody targets exist; all appear to be part of the 2-OADC family of complexes positioned on the inner mitochondrial membrane with a preserved lipoyl-lysine residue [32]. Other categories of autoantibodies are also often present, including the novel autoantibodies of antihexokinase-1 and anti-KLHL12 (Kelch-like 12 peptide) [86]. But while these various autoantibodies can yield similar pathophysiologic and clinical effects, future studies are awaited in order to delineate the propensity of disease activity toward BECs lining small- and medium-caliber bile ducts [87] as opposed to alternative targets [88].

Table 2.4 Autoantibodies and their respective targets with relevance to PBC pathogenesis

Autoantibody and target	Reported frequencies [189]	Selected references
Antimitochondrial antibody		
2-OADC/BCOADC-E2	Up to 95%	Leung et al. [190], Masuda et al. [191]
E3BP	Up to 95%	Dubel et al. [192], Palmer et al. [193]
OGDC-E2	Up to 95%	Koike et al. [194]
PDC-E2	Up to 95%	Shuai et al. [195]
Antinuclear antibody, nuclear rim pattern		
gp-210	10–40%	Wang et al. [102]
p62	10–40%	Baur et al. [196]
Antinuclear antibody, multiple nuclear dots		
PML	20–40%	Szostecki et al. [197], Zuchner et al. [197], Mytilinaiou et al. [198]
sp100	20–40%	Wang et al. [102]
sp140	20–40%	
Antihexokinase-1 antibody		
HK1	16–45%	Norman et al. [86]
Anti-Kelch-like 12 peptide antibody		
KLHL12	16–40%	Norman et al. [86]
Anticentromere antibody		
CENP-B	9–30%	Parveen et al. [199]
Anti-GWB antibodies		
GRASP-1	2–28%	Stinton et al. [200]
GW182	2–28%	Stinton et al. [200]
GW2	2–28%	Stinton et al. [200]
RAP55	2–28%	Stinton et al. [200]

2-OADC 2-oxo-acid dehydrogenase complex, *BCOADC* branched-chain 2-oxoacid dehydrogenase complex, *CENP* centromere protein, *E2* E2 subunit of BOADC, 2-OADC, OGDC, PDC, *E3BP* E3-binding protein of pyruvate dehydrogenase complex, *gp210* glycoprotein 210, *HK1* hexokinase-1, *GRASP-1* glutamate receptor interacting protein-associated protein-1, *GWB* G (glycine) W (tryptophan)-containing bodies, *KLHL12* Kelch-like 12 peptide, *OGDC* oxoglutarate dehydrogenase complex, *PDC* pyruvate dehydrogenase complex, *PML* promyelocytic leukemia, *RAP55* RNA-associated protein 55, *sp100* nuclear body speckled 100 kDa, *sp140* nuclear body speckled 140 kDa

Genetic and Epigenetic Influences

Genetic Factors

To date, there have been a wide variety of studies characterizing the genetic influences on PBC pathogenesis. Genetic variants causing a malfunctioning of their respective downstream protein products along any number of intracellular pathways can potentially contribute to this pathophysiology. An index of primary genome-wide association studies (GWAS), HLA associations, and candidate gene studies implicated in this pathophysiology are listed in Table 2.5. While there has been an explosion of GWAS in recent years, several limitations exist. GWAS studies do not

Table 2.5 Genetic associations linked with PBC pathogenesis

GWAS or genetic association studies			
Study	**Study type**	**Genes associated with increased PBC risk**	**Cohort characteristics**
Im et al. [97]	GWAS	TNFSF8, TNFSF15, UMAD1	Japanese
Paziewska et al. [101]	GWAS	ABCB11, ALLC, CACUL1, CCR6, COL11A2, DPPA2, HLA-DQA1, HLA-DQB2, IL12RB2, IL24, IKZF3, IQCK, MIR1284, MIR1295A, MIR3120, MIR4681, MIPOL1, MITF, POLR2G, PVRL3-AS1, RBM47, SMKR1, SNX19, SPIB, TBC1D19, TRAF2, TRAM1L1	Polish
Qiu et al. [90]	GWAS	ARID3A, CD28/CTLA4/ICOS, CD58, CD80, IL16, IL21, IL12A, IL21R, NF-κB, ORMDL3, RPL3/SYNGR1, STAT1/STAT4	Chinese
Kawashima et al. [98]	GWAS	POU2AF1, PRKCB, TNFSF15	Japanese; included data from prior GWAS
Cordell et al. [91]	GWAS	CCL20, C5orf30, DGKQ, IL1RL1/2, IL12B, OLIG3, TNFAIP3	North American, Italian, North European
Liu et al. [93]	GWAS	CD80, CLCC16A, CRHR1, CXCR5, DENND1B, DDX6, IKZF3, IL7R, IL12A, IL12RB2, IRF5/8, LTBR, MANBA, MAPT, NFKB1, RAD51B, SH2B3, SOCS1, STAT1/4, SYNGR1, TBR, TNFRSF1A, TNFSF11, TNP03, TYK2	United Kingdom; included 1838 PBC and 2356 control patients from prior GWAS
Nakamura et al. [99]	GWAS	HLA, TNFSF15, POU2AF1	Japanese
Juran et al. [92]	GWAS/ immunochip	IL12RB2, IRF5, TNFSF11	European
Mells et al. [100]	GWAS	CD80, CLEC16A, CXCR5, DENND1B, ELMO1, IL7R, IRF8, MAP3K7IP1, NFKB1, PLCL2, RAD51L1, RPS6KA4, STAT1/4, TNFAIP2, TNFRSF1A	Northern European
Liu et al. [94]	GWAS	IKZF3, IRF5-TNPO3, SPIB	Italian, North American
Hirschfield et al. [95]	GWAS	IKZF3, IRF5-TNPO3, MMEL1	North American, Caucasian; included 494 PBC and 1502 controls from prior GWAS
Hirschfield et al. [96]	GWAS	HLA, IL12A, IL12RB2	North American, Caucasian
HLA and candidate gene studies			
Study	**Study type**	**Gene**	**Cohort characteristics**
Hitomi et al. [201]	Candidate gene study	POGLUT1	Japanese

(continued)

Table 2.5 (continued)

Nishida et al. [202]	Candidate gene study	CTSZ, NELFCD	Japanese
Hitomi et al. [203]	Candidate gene study	NFKB1, MANBA	Japanese
Yasunami et al. [204]	HLA	HLA-DQB1∗06:04, HLA-DQB1∗03:01	Japanese
Hitomi et al. [205]	Candidate gene study	GSDMB, ORMDL3	Japanese
Li et al. [206]	Candidate gene study	HELZ2, IL12A	Chinese
Tang et al. [207]	Candidate gene study	CLDN14	Chinese
Zhao et al. [208]	HLA	HLA-DRB1∗08:03-DQB1∗06:01	Chinese, Japanese
Juran et al. [92]	HLA	HLA-DQA1∗04:01-DQB1∗04:02-DRB1∗08:01-B∗39:05, HLA-DRB1∗04:04-DQB1∗03:02	American, United Kingdom, Italian
Invernizzi et al. [209]	HLA	HLA-DRB1∗08, HLA-DRB1∗14-DPB1∗03:01	Italian
Umemura et al. [210]	HLA	HLA-DRB1∗08:03-DQB1∗06:01, HLA-DRB1∗04:05-DQB1∗04:01	Japanese
Liu et al. [93]	HLA/GWAS	HLA-DQA1∗04:01-DQB1∗04:02-DRB1∗08:01-B∗39:05, HLA-DRB1∗04:04-DQB1∗03:02	American, United Kingdom, Italian
Tanaka et al. [211]	Candidate gene study	FCRL3	Japanese and Italian
Aiba et al. [212]	Candidate gene study	CTLA4, SLC4A2	Japanese
Juran et al. [213]	Candidate gene study	SLC4A2	American/ European, Caucasian
Poupon et al. [214]	Candidate gene study	TNFα, CTLA-4, SLC4A2/AE2	French, Caucasian
Prieto et al. [215]	Candidate gene study	AE2	Spanish

completely capture variation seen across different nationalities and ethnicities. Furthermore, some elements of disease phenotype are not fully accounted for by GWAS findings. The actual genes that have functional polymorphisms that are important to disease pathogenesis are not uncovered by GWAS associations. Finally, results of GWAS may not detail underlying epigenetic modifications that may be involved in pathogenesis.

In summation, several overarching trends have emerged with three generalized pathways replicated among many genetic studies [89]: IL-12 production and antigen presentation (IL12RB, IL-12A, IRF5, NF-kB, TNFAIP3) [90–96], generation of IFN-γ and T-cell activation (IL12R, NF-kB, SOCS1, STAT4 and TNFAIP3, TNFSF15, TYK2) [90, 93, 97–100], B-cell recruitment and stimulation of immunoglobulins (ARID3A, IKZF3, POU2AF1, PRKCB, SPIB) [93–95, 98, 99, 101], and

HLA associations. However, there appears to be a lack of one or more select genetic components solely responsible for PBC pathophysiology. Much disparity exists among studies, and these differences are compounded when comparing genetic studies analyzing study populations of vastly disparate genetic backgrounds.

A GWAS performed by Wang et al. recently demonstrated that, as opposed to gp210, the sp100 autoantibody was found to have significant association with HLA alleles [102]. This interaction was primarily driven by *HLA DRB1∗03:01*, but including *DRB1∗03:01*, *DRB1∗15:01*, *DRB1∗01*, and *DPB1∗03:01* alleles accounted for almost the entire association of sp100 autoantibodies with HLA. In the setting of strongly associated autoantibodies, this unique study perhaps has opened an avenue for more in-depth analysis regarding the exact mechanisms for how autoimmunity is conferred in PBC.

Epigenetic Influences

The role of epigenetic influences represents a rather newer insight into PBC pathogenesis. Rather than variants or mutations within genes themselves, differences in expression and modification of genetic information, as described by these epigenetic factors, generate similar downstream effects within this disease process. There have been a wide range of documented epigenetic changes seen in PBC, including alterations within DNA methylation, histone modification, and noncoding RNAs. For example, hypermethylation and inactivation along the X chromosome have been described among PBC subjects, which may help to account for the striking female predilection for this disease [103–105].

Perhaps the most robust evidence for epigenetic influences in PBC pathogenesis comes from profiles of micro-RNAs (miRNAs). Several miRNAs were implicated in initial GWAS studies [101]. However, it has been demonstrated that miR-506 upregulation directly promotes decreased expression of the Cl^-/HCO_3^- exchanger [106]. This ultimately induces loss of the aforementioned bicarbonate umbrella, which is so central in pathophysiology. These findings have recently been reproduced by illustrating that miR-506 promotes PBC features with respect to cholangiocyte and immune activation [107].

Figure 2.7 provides a summary overview of our current understanding of the pathogenesis and progression of PBC.

Primary Sclerosing Cholangitis

Primary sclerosing cholangitis (PSC) is characterized as a fibrous obliterative cholangitis of both intrahepatic and extrahepatic bile ducts, eventually resulting in concentric bile duct fibrosis, stenosis, and destruction. There does not appear to be a single, validated pathophysiological mechanism at play; however, there are multiple theories currently proposed, which we now discuss.

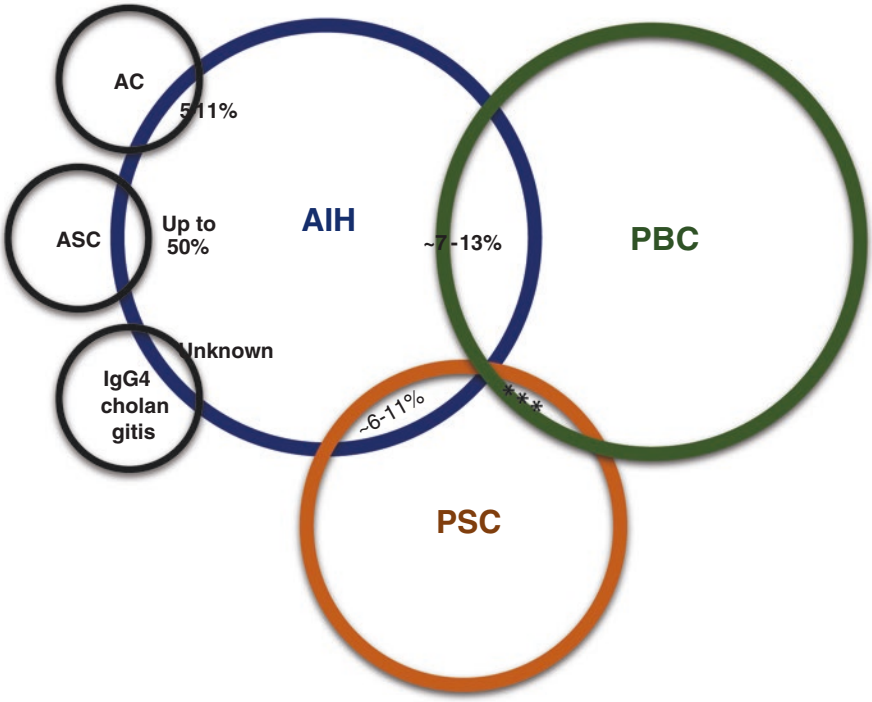

Fig. 2.7 General overview of overlap syndromes and their approximate frequencies. The sizes of the circles approximate the differing frequencies of the severe AILDs and their overlap syndromes (OS). Overlap syndromes (OS) of AIH with PBC and its variants have been observed in up to 13% of cases, while AIH and overlap with PSC variants appears in ~ 11% [168]. Less well-defined overlap syndromes of AIH with AC and ASC have been characterized by incidence rates as high as 11% and ~50%, respectively, while the AIH-IgG4 cholangitis OS remains relatively unknown. A PBC-PSC OS has been reported; however, this appears to be a rare finding, and the diagnosis remains controversial.∗∗∗ Abbreviations: AC – autoimmune cholangitis, AIH – autoimmune hepatitis, ASC – autoimmune sclerosing cholangitis, PBC – primary biliary cholangitis, PSC – primary sclerosing cholangitis

Cholangiocyte Pathophysiology

Of paramount importance is the strong association of PSC with inflammatory bowel disease (PSC-IBD), especially with UC (ulcerative colitis), which occurs in over 75% of patients with PSC [108]. This association suggests unique considerations that may not be at play in other autoimmune liver diseases. However, portions of analogous framework and motifs described in PBC carry over to some of these proposed theories regarding the pathogenesis of PSC.

Proposed Mechanisms for Cholangiocyte Injury and Activation

Alterations in the bicarbonate umbrella have also been implicated in PSC, similarly to PBC, in that protonated bile salts are then able to translocate across the apical BEC membrane and mediate direct cellular injury [109]. This alone would be able to spark a persistent immune response from cholangiocyte activation. However, the linkage to IBD also suggests the *leaky gut hypothesis*, namely, that damaged gastrointestinal epithelium and increased bacterial gut translocation into the portal circulation fuel a sustained inflammatory response within the biliary tree. A study in PSC patients demonstrated strikingly elevated levels of LPS-binding protein and soluble CD14 (sCD14), markers of bacterial translocation, in compared to healthy controls [110]. Also of note, this same study indicated a direct association with increased hepatobiliary cancer and shorter liver transplantation-free survival among PSC patients with increased LPS-binding protein and sCD14, emphasizing the clinical significance of the gut-biliary axis in this disease process [110]. The increased delivery of endotoxins, microbes, and PAMPs activate inflammatory signals from cholangiocytes, such as through the Toll-like receptors, which precipitate proinflammatory mediators to incite innate and adaptive immune responses. Furthermore, LPS molecules induce cholangiocyte senescence, which may ultimately convert to a secretory phenotype whereby an N-Ras-mediated production of proinflammatory cytokines occurs [111]. This inundation of cytokines and chemokines, including IL-6, IL-8, and CCL2, serves as a harbinger for the cellular injury, inflammation, and magnified immune response to come.

Of particular interest, the Takeda G protein-coupled receptor 5 (TGR5) plays a significant role in PSC pathophysiology. Normally triggered through interaction with bile salts, the activation of TGR5 leads to cholangiocyte proliferation, antiapoptosis, and the cystic fibrosis transmembrane regulator-mediated secretion of chloride into the biliary lumen, which subsequently is utilized by AE2/SLC4A2 in order to maintain the bicarbonate umbrella [112]. However, TGR5 is downregulated in BECs of PSC patients based on immunofluorescence staining and microscopy [113], which may explain the loss of bicarbonate umbrella, amplified inflammation cascade, and increased cholangiocyte cell death seen with this disease.

Environmental Factors

While several environmental factors have been implicated in the activation of cholangiocytes and precipitation of PSC pathogenesis, there is a paucity of data implicating specific targets of molecular mimicry and xenobiotics in the pathogenesis of PSC.

Protective Effect of Smoking

Contrary to its contributory role in PBC, smoking appears to have a protective effect against the formation of PSC [93–96]. While this association with smoking may potentially mitigate the development of PSC, smoking continues to increase the risk of cholangiocarcinoma among those with PSC [114–116].

Intestinal Dysbiosis

The absence of appropriate intestinal microbiota exacerbates biliary disease progression in a multidrug resistance 2 knockout (Mdr2−/−) PSC mouse model [117]. Recent analysis performed in a similar murine Mdr2−/− PSC model generated intriguing results [118]. Compared to wild type controls, Mdr2−/− mice displayed dysbiosis of gut microbiota in addition to pronounced NLRP3 inflammasome activation within the gut-liver axis. This directly led to increased hepatic NLRP3-mediated innate immune response via intestinal barrier dysfunction and increased bacterial translocation. Furthermore, the transfer of Mdr2−/− microbiota into healthy wild type controls induced significant liver injury. This damage was reversed upon treatment with the pan-caspase inhibitor IDN-7314, which depressed inflammasome activity, reduced liver injury, reversed the disordered serum bile acid profile, and improved cholestasis-associated microbiota signature. There is currently a lack of definitive data regarding the potential role of gut dysbiosis among human subjects with PSC; however, intestinal dysbiosis has been described in PSC human subjects independent of the presence of IBD [119]. Clearly, there is much yet to be learned regarding the role of intestinal dysbiosis in PSC pathophysiology.

Immune Dysregulation in PSC

Several aspects of the immune response are unique to the pathophysiology of PSC. It has been well established that PSC is characterized by a predominance of T cells within hepatobiliary tissues [120]. There are several explanations for the innate immune response and ensuing T-cell recruitment and activation. These elements include the toxic effects of bile acid, the *leaky gut hypothesis*, increased cholangiocyte senescence, the release of proinflammatory mediators, in addition to the involvement of circulating autoantibodies, as detailed below.

However, with respect to the concomitant presence of IBD, proposed mechanisms regarding T-cell recruitment and activation include shared antigens and gut activation. The first hypothesis entails exposure of a similar shared antigenic T-cell trigger at both the gut and biliary level [121, 122]. The second hypothesis involves T-cell activation in the gut with subsequent homing to the hepatobiliary tree [123]. After T-cell priming in the gut, while also under the auspicious cytokine influences

already described, an upregulation of portal venous endothelium adhesion molecules, particularly MAdCAM-1 and CCL25, helps promote the adherence and diapedesis of T cells into the hepatobiliary milieu [124, 125]. Similarly, vascular adhesion protein-1 has recently been shown to have increased expression in hepatic endothelium among PSC patients as compared to controls, which enables increased uptake of gut-tropic lymphocytes into the liver [126]. Further immuno-histochemical analysis of liver biopsies from PSC patients reveals the presence of nonactivated memory T cells at sites of portal inflammation, a majority of which also are positive for gut-homing integrin alpha4beta7 expression [127]. Autocrine and paracrine signaling of a host of potent cytokines and chemokines, such as CCL21, have also been shown to recruit additional cytotoxic immune cells, including CCR7+ NK cells, further subsidizing the cellular injury and inflammation accompanied in PSC pathophysiology [128].

Similar to PBC, activation of the IL-2 pathway is important in PSC pathogenesis. Once cholangiocytes present antigenic material to the T lymphocyte via the costimulation of T-cell receptor and CD28, IL-2 is secreted and leads to B-cell proliferation, CD4+ T-cell activation, further IL-2 production, NK cell stimulation, and the development of Tregs. However, when there is a defect in IL-2 or one of its corresponding subunits, there is a lack of effective Treg formation, which fosters increase in autoimmune disorders [68]. These findings correspond with some of the findings in GWAS studies, as detailed in the section below.

Histologically, CD4+ and CD8+ T cells are recruited to and are involved in portal inflammation while demonstrating a paucity of peribiliary infiltration. This appears to match the classic histologic findings of an intact, yet thickened, basement membrane, oftentimes with the periductal capillaries pushed aside due to the concentric, onion-skin fibrosis and obliterative cholangitis seen in PSC [84, 129].

Autoantibodies

The aforementioned presence of circulating autoantibodies remains an essential fixture in PSC pathogenesis. Table 2.7 lists the major autoantibodies associated with this disease process. While no disease-specific antibody exists, the presence of perinuclear antineutrophil nuclear antibodies (pANNA), also referred to as perinuclear antineutrophil cytoplasmic antibodies (pANCA) in previous literature, appear to be tightly linked with this pathophysiology in addition to displaying cross-reactivity toward the bacterial protein, FtsZ [130]. This is not surprising because PSC had been linked to an antibody common to both biliary and colon epithelium in a study over 20 years ago [131]. These findings undoubtedly are relevant to the close linkage to IBD, UC in particular. Other antinuclear antibodies (ANA) and novel antibodies, such as antiglycoprotein antibodies, have been associated with PSC; however, there is lack of specificity for this disease (Table 2.6). More novel, specific identification of circulating autoantibodies remains an undertaking for future studies and will ultimately lead to more accurate diagnosis of PSC. In general, it remains

Table 2.6 Autoantibodies in PSC

Autoantibody and target		Reported frequencies [189]	Selected references
Anti-perinuclear antineutrophil nuclear antibody (pANNA)			
	Beta-tubulin isotype 5	26–94%	Terjung et al. [130]
Antinuclear antibody			
	dsDNA	8–77%	Terziroli et al. [189]
	SSA/B	8–77%	Terziroli et al. [189]
	RNP	8–77%	Terziroli et al. [189]
	SCL70	8–77%	Terziroli et al. [189]
	ssDNA	8–77%	Terziroli et al. [189]
Antiglycoprotein antibody			
	GP2	47–71%	Jendrek et al. [216]
Antismooth muscle antibody			
	SMA	0–83%	Terziroli et al. [189]

dsDNA double-stranded DNA, *GP2* glycoprotein 2, *GWAS* genome-wide association study, *pANNA* perinuclear antineutrophil nuclear antibody, *RNP* ribonucleoprotein, *SCL70* scleroderma 70, *SSA/B* Sjögren's syndrome type A/B, *SMA* smooth muscle antibody, *ssDNA* single-stranded DNA

less clear in PSC that pANNA or other autoantibodies are causative in pathogenesis, compared with the central role of antibodies to PDC-E2 in PBC (vide supra).

Considerations in PSC-IBD

The interwoven nature of PSC and IBD pathophysiology have already been discussed, most notably with respect to the *leaky gut hypothesis* and homing of gut-primed T cells. However, this interplay raises several clinical issues that delve even deeper into this pathogenesis and that are unique to this AIH compared to PBC.

IBD Disease and Its Effect on PSC

Initially, there was concern that the presence of one autoimmune disease may portend a worse prognosis for another, and this correlation has been questioned several times over the past few decades. In a 1996 longitudinal Swedish study, patients with PSC-IBD did not harbor a significant difference in prognosis as compared to patients with only PSC [132]. However, 30% of the PSC-IBD patients had already undergone proctocolectomy at the time of this study. A subsequent, retrospective Israeli study compared 141 PSC-IBD and PSC patients and found no significant difference in cirrhosis, transplant-free survival, and mortality [133]. A retrospective Belgian study analyzed differences in the numbers of liver transplantations, liver-related deaths, and survival without transplantation among patients with large duct PSC and one of the following: ulcerative colitis (UC), Crohn's disease (CD), and no IBD

[134]. Overall, patients with PSC-CD achieved better outcomes, while patients with PSC-UC and PSC without IBD yielded statistically similar outcomes. In conclusion, there does not appear to be robust evidence supporting worse prognosis or outcomes in PSC-IBD as compared to PSC alone.

Status of PSC-IBD After Colectomy

PSC is less frequently associated with CD and more tightly linked to UC, the penultimate treatment for which is proctocolectomy. It stands to reason that the organ removal of symptomatic IBD involvement would improve patients' overall symptoms. Also, this would theoretically eliminate the impetus and location for initial activation of T cells, thus potentially reducing lymphocyte homing and immune response in the hepatobiliary system. Early studies, however, failed to show an improvement in histology on liver biopsy or overall survival among patients with PSC-IBD who had undergone proctocolectomy [135, 136]. Conversely, more recent data have suggested a reduced rate of PSC recurrence if patients had colectomy either before or during initial liver transplantation for PSC [137]. A 2018 systematic review reported similar findings of a protective effect of either pre- or peri-LT colectomy on preventing PSC recurrence [138]. Another systemic review/meta-analysis failed to demonstrate the benefit of colectomy in the progression of the PSC disease process, with particular respect to the requirement of TIPS or LT [139]. Of note, these final two systematic reviews did not include any large-scale randomized, controlled trials in their analysis. There does not appear to be convincing evidence at this time to support proctocolectomy as a treatment option for PSC. This also may bring into question the theory of gut-primed T-cell homing to the hepatobiliary system as the sole, or even primary, source of immune response in PSC pathogenesis.

Malignancy Risk

PSC has long been associated with an increased malignancy risk, especially corresponding to colorectal cancer and cholangiocarcinoma [140, 141]. However, it remains difficult to delineate the key inciting factors responsible for this malignancy risk. The increased risk of colorectal cancer among IBD patients, primarily driven through association with UC, has been well known for some time [142]. Patients with PSC-IBD harbor an even greater risk of colon cancer as compared to patients with IBD alone; meanwhile, the incidence of such cancers in PSC patients without IBD remains relatively low [143–145]. This increased risk does not appear to correct or improve after LT, even among PSC-IBD patients [146, 147]. Overall, the patients with PSC-IBD have a reported colorectal risk approximately threefold higher than risk for development of cholangiocarcinoma [143]; however, the diagnosis of the latter generally portends a lower life expectancy [148]. Notwithstanding, the role of IBD in the risk of cholangiocarcinoma among PSC has varied among current literature. One study reported that IBD did not correlate with cholangiocar-

cinoma risk among PSC patients [149]. A retrospective study concluded that the duration of IBD is associated with increased cholangiocarcinoma risk among PSC-IBD patients; however, this risk was not abrogated among patients who had undergone colectomy [150]. The mechanism of the pathogenesis culminating increased risk of malignancy remains poorly understood. There does not appear to be a standalone culprit, and the inner workings of both PSC and IBD appear to be at play. Several etiologies have been proposed, including long-standing inflammatory mediators, such as IL-6, leading to heightened malignancy risk via MCL-1 upregulation and AKT signaling cascade [151]. WNT-mediated signaling cascade also appears to contribute to increased risk of cholangiocarcinoma development [152]. Continued exposure to bile acids in chronic cholestasis and biliary cirrhosis can also elicit a boosted malignancy risk through the activation of sphingosine 1-phosphate receptor 2 and downstream effects of ERK1/2 and the AKT signaling cascade [153–155]. Overall, the increased malignancy risk in PSC patients as compared to other AILDs remains relatively unexplained.

PSC Recurrence Post Liver Transplantation

Historically, there has been an elevated risk of PSC recurrence post-LT, with some studies reporting a recurrence rate ranging from 9% to 47% [156], while another study describes rates as high as 37% [157]. The reasons for these relatively high rates is not well established and, frankly, are convoluted given the decreased efficacy of UDCA and immunosuppressive agents as compared to response among other AILDs. Then, too, immune rejection of the transplanted liver produces changes in the biliary tree that resemble those of PSC. More studies and RCTs in the future ideally will focus on this area as developing methods for early diagnosis and effective treatment of recurrent PSC are important unmet needs.

Genetic and Epigenetic Influences

Genetic Factors

There have been a multitude of genetic components suspected to contribute to PSC pathogenesis (Table 2.7). To date, no single genetic variation has emerged as central or essential to PSC development. However, there appears to be a high overall predilection toward genes governing immune activation (e.g., HLA and NF-kB) and antigen presentation (e.g., IL-2 and T-cell receptor) [158]. Two repeating motifs have been observed. First, the IL-2 signaling pathway is important for PSC pathogenesis and has been linked via several genetic studies, as mentioned above [159, 160]. The second theme involves the fucosyl transferase 2 pathway. This protein has been demonstrated to maintain bicarbonate-mediated protection from bile acid cellular

Table 2.7 Genetic associations relevant to PSC pathogenesis

GWAS or genetic association studies			
Study	**Study type**	**Gene**	**Cohort characteristics**
Alberts et al. [217]	Genetic association analysis	RSPO3	American, European, Canadian
Paziewska et al. [101]	GWAS	ADGRB3, ALLC, CACUL1, COL11A2, CLNK, CTAGE4, DNAJC13, EPB41L4A, HLA-DQA1, MIR4278, MSH5-SAPCD1, PGBD1, PRRC2A, PSORS1C3, SKIV2L, SPAG7, TRAF2, TRIM40, USP17L10, WWC1	Polish
Ji et al. [218]	GWAS	CCDC88B, CLEC16A, FOXP1, UBASH3A	European, American, African, Chinese, Japanese; specifically in PSC patients with IBD
Liu et al. [159]	GWAS	ATXN2, BACH2, CD28, CD226, FUT2, HDAC7, HLA, IL2, IL2RA, IL21, MMEL1-TNFRSF14, MST1, PRKD2, PSMG1, SIK2, SH2B3, STRN4	European and North American
Ellinghaus et al. [219]	GWAS	GPR35, TCF4	German and Scandinavian; in both PSC and UC patients
Srivastava et al. [160]	GWAS	IL2RA, MST1	United Kingdom, Caucasian
Folseraas et al. [220]	GWAS	FUT2, MMEL1-TNFRSF14	German, Scandinavian, European
Melum et al. [221]	GWAS	BCL2L11, HLA, MST1	German and Scandinavian
HLA and candidate gene studies			
Study	**Study type**	**Gene**	**Cohort characteristics**
Paziewska et al. [101]	HLA/GWAS	HLA-DQA1	Polish
Henriksen et al. [222]	HLA	HLA-DRB1∗13:01-DQA1∗01:03-DQB1∗06:03	Non-European and Scandinavian
Liu et al. [159]	HLA/GWAS	HLA-B∗08:01, HLA-DQA1∗01:03, HLA-DQA1∗01:01	European and North American
Melum et al. [221]	HLA/GWAS	HLA-B∗08, HLA-DRB1∗03	German and Scandinavian
Karlsen et al. [223]	HLA/GWAS	HLA-B∗08, HLA-DRB1∗03, HLA-DRB1∗04. HLA-DRB1∗07, HLA-DRB1∗1301	German and Scandinavian
Bowlus et al. [224]	HLA	HLA-B∗08:01	North American
Donaldson et al. [225]	HLA	HLA-DRB1∗03:01-DQA1∗05:01-DQB1∗02:01, HLA-DRB1∗13:01-DQA1∗01:03- DQB1∗06:03, HLA-DRB1∗15:01-DQA1∗01:02-DQB1∗06:02, HLA-DRB1∗01:01-DQA1∗01:01	North American and European

Abbreviations: *GWAS* genome-wide association study, *HLA* human leukocyte antigen

injury, and thus polymorphisms to such genetic components may potentially lead to a breakdown of the bicarbonate umbrella and portend increased risk for PSC [161].

Epigenetic Influences

Compared with AIH or PBC, the breadth and depth of genetic studies in PSC are limited, and this holds true also regarding studies characterizing epigenetic mechanisms in PSC pathogenesis. No published evidence to date has established the role of one or more epigenetic mechanisms with this pathophysiology; however, etiologies involving DNA methylation, histone modification, and activity of noncoding RNAs, in addition to interactions with IBD and the gut microbiome, have been proposed as potential theories [162].

Overlap Syndromes

In addition to typical AIH, PBC, and PSC, there remain phenotypes that share features consistent with one or more categories of these AILDs. These are called "overlap syndromes." Overlap syndromes most frequently describe AIH in addition to salient phenotypic elements of PBC, PSC, or autoimmune cholangitis (AC). A PBC-PSC overlap syndrome has also been described but is exceedingly rare, with only a handful of cases reported in primary literature [163–167]. Table 2.8 summarizes the characteristics among these overlap syndromes [168].

To date, there is no integrating mechanism that accounts for the pathogenesis of these overlap syndromes. Of the limited data that exist, the overwhelming majority of evidence specifically details the PBC-AIH overlap syndrome. There are currently two primary schools of thought. Either two simultaneous disease processes concurrently exist or each overlap syndrome actually represents the atypical manifestations of disease along a much wider pathological spectrum.

Addressing the former, recent evidence has demonstrated the shared findings of circulating autoantibodies of AIH and PBC, such as AMA and dsDNA [169], anti-p53 [170], and anti-SLA [171]. In particular, the autoantigenic lipoyl domain appears to be conserved among overlap syndromes, including PBC [172]. Moreover, there has been evidence to suggest a common HLA fingerprint [173, 174]. To date, no purported epigenetic mechanisms have been established [175].

However, alternative evidence suggests that these overlap syndromes are merely varying presentations of disease along a wide spectrum of pathology. Ample evidence has shown that there can be wide variability both in the histologic [176] and radiologic [177] manifestations of individual AILDs. While literature supports intrahepatic predominance of IgG or IgM favoring diagnoses of AIH and PBC, respectively [178], immunohistologic characterization of AIH-PBC overlap syndrome does not appear to favor one dominant immunoglobulin in particular [179]. In fact, patients with AIH-PBC overlap syndrome have varying degrees of cholangitis,

Table 2.8 Selected features of overlap syndromes in autoimmune liver diseases

Type	Diagnostic criteria	Typical characteristics	Frequency of occurrence in AILD
AIH-PBC	*AIH group (2 out of 3)*: ALT ≥5× ULN IgG ≥ 2× ULN or SMA+ interface hepatitis *PBC group (2 out of 3)*: AP ≥ 2× ULN -OR- GGT ≥ 5× ULN AMA+ Florid duct lesions	AIH predominant phenotype IAIHG score for AIH AP ≥ 2× ULN AMA+ Bile duct destruction/loss	7–13%
AIH-PSC	Predominant AIH AMA negative Bile duct injury/loss Biliary sclerosis	IAIHG score for AIH +/− IBD	6–11%
AIH-AC	Predominant AIH AMA negative Bile duct injury or loss Normal cholangiography	IAIHG score for AIH Most likely mixed syndrome, including AMA negative PBC and small-duct PSC	5–11%
AIH-AMA negative PBC	Predominant AIH AMA negative Bile duct injury or loss Normal cholangiography	IAIHG score for AIH	5–8%
AIH-small duct PSC	Predominant AIH AMA negative Bile duct injury or loss Normal cholangiography	Poor response to UDCA	~3%
AIH-ASC	Predominant AIH AMA negative Bile duct injury/loss Biliary sclerosis	Responds to corticosteroids Can occur in pediatric population	~50%
AIH-IgG4 cholangitis	>5 IgG4+ plasma cells per HPF on liver biopsy Abnormal biliary cholangiogram	Responds to corticosteroids Variable serum IgG4 level	Unknown
PBC-PSC	None present	Histologic findings consistent with both PBC and PSC	Rare

Adapted from Czaja [168]

AC autoimmune cholangitis, *AIH* autoimmune hepatitis, *ALT* serum alanine aminotransferase, *AMA* antimitochondrial antibody, *AP* serum alkaline phosphatase, *ASC* autoimmune sclerosing cholangitis, *GGT* serum gamma glutamyl transpeptidase, *HPF* high power field, *IAIHG* International Autoimmune Hepatitis Group, *IBD* inflammatory bowel disease, *PBC* primary biliary cholangitis, *PSC* primary sclerosing cholangitis, *SMA* smooth muscle antibody, *UDCA* ursodeoxycholic acid, *ULN* upper limit of normal

ductopenia, and fibrosis based on the presence or absence of AMA [180]. Another recent study demonstrated significant association of anti-dsDNA-Crithidia antibodies with AIH-PBC overlap syndrome as compared to patients with PBC alone, suggesting the utilization of this antibody as a distinguishing diagnostic factor [181].

To convolute the matter yet further, there appears to be an unexplained, variable response to immunosuppression, as well as UDCA, in the treatment of overlap syndromes [182, 183].

Clinical evaluation of overlap syndrome is also bridled by the constraints of current diagnostic criteria, which may be challenging given the lack of uniformity among these guidelines. For example, the incidence of overlap syndrome can vary widely depending on the utilization of Paris [184] versus original IAIHG criteria [185]. This continues to be a moving target as there have been revised and modified criteria released as new aspects of pathophysiology unfold [186, 187].

Given the dearth of evidence characterizing the overlap syndromes, there has been a recent call for future studies to delineate the disparities within the pathophysiology and diagnostic evaluation of these diseases. Some of the crucial elements for proposed future research include more sharply defined diagnostic criteria, improvements in serologic biomarkers, assessment of the utility of routine liver biopsy versus MRI, and assessment of nonalcoholic fatty liver disease among controls [188].

Acknowledgements We acknowledge with thanks John M. Vierling, MD for making available to us figures and tables that are featured in this chapter. Supported by awards from NIH/NIDDK U01 DK 065201, U54 DK 083909, and R01 DK 119913 (to HLB).

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Chapter 3
Clinical Presentation and Diagnosis

Sean R. Rudnick

Introduction

Given the heterogeneity of presentations, prompt and accurate diagnosis can be challenging. The initial suspicion of AIH as a diagnosis is based on recognizing one of the typical phenotypes, exclusion of other causes of liver disease, and compatible laboratory findings (including presence of auto antibodies and hypergammaglobulinemia). The diagnosis is largely dependent upon typical or compatible liver histology. Accurate diagnosis is paramount, as empiric therapy exposes patients to unnecessary risks or adverse events associated with immunosuppressive therapy.

Classification of AIH

Classification of AIH based on the pattern of auto antibodies is of clinical value in discerning between two relatively distinct disease phenotypes which differ in severity and response to therapy.

Type 1

Type 1 AIH has a female to male predominance of 4:1 and may present at any time throughout the patient's life. The main auto antibodies identifed include antinuclear antibody (ANA), antismooth muscle antibody (ASMA), and anti F-actin antibody.

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M. W. Russo (ed.), *Diagnosis and Management of Autoimmune Hepatitis*,
https://doi.org/10.1007/978-3-030-33628-8_3

Less common auto antibodies include anti-soluble liver antigen/liver pancreas (SLA/LP) and atypical p-anti-neutrophil cytoplasmic antibodies (p-ANCA). SLA/LP auto-antibodies are the most specific in Type 1 AIH, but are only present in 10–30% of case [1, 2]. It is important to recognize up to 20% of patients test negative for all autoantibodies. The spectrum of disease ranges from asymptomatic to ALF and can include active cirrhosis. Type 1 AIH generally responds well to immunosuppressive therapy, and withdrawal of therapy can be successful in a minority of patients. However most patients will require long-term immunosuppressive therapy.

Type 2 AIH

In comparison, type 2 AIH has a more robust female to male ratio (10:1). Presentation is during childhood to young adulthood, and though there is global prevalence, there are geographic predilections and adult prevalence is more common in Europe compared to the United States. Signature auto antibodies include anti-liver kidney microsomal 1 (LKM1) and anti-liver cytosol 1 (LC1). As opposed to type 1, type 2 has a lower response rate to immunosuppressive therapy compared to type I AIH, resulting in treatment failure more frequently. Nearly all patients with type 2 AIH will require long-term immunosuppressive therapy.

Clinical Presentations

Acute AIH

Even within the acute setting, AIH spans a diverse spectrum of clinical presentations and can include acute hepatitis, acute severe autoimmune hepatitis (AS-AIH) without ALF, acute severe AIH with ALF, and acute on chronic liver disease [3]. Although standardized definitions do not exist, previous publications have defined AS-AIH as an acute presentation ($\leq$26 weeks) with an INR $\geq$ 1.5 without histologic evidence of cirrhosis [4]. Additional proposed definitions of acute presentations of AIH are based on the presence or absence of coagulopathy and/or encephalopathy [3]. Further complicating characterization is autoimmune drug-induced liver injury (DILI), including AIH with superimposed DILI, DILI-induced AIH, and immune-mediated DILI.

Acute Liver Failure

Acute liver failure (ALF) is defined as the development of coagulopathy and hepatic encephalopathy in patients without chronic underlying liver disease [5]. Accurately identifying AIH as the cause of ALF is challenging because histologic findings on liver biosy may overlap in patients with acute and chronic AIH. This fact highlights

that a proportion of patients presenting with "acute" AIH will have histologic evidence suggesting pre-existing liver disease, and hence would not meet the strictest definitions of ALF [6]. However, the fulminant presentation of AIH (coagulopathy and encephalopathy often requiring liver transplant) is important to recognize because there are implications for liver transplantation.

In contrast to the diagnosis of chronic AIH which relies heavily upon patterns of autoimmune markers and elevations in immunoglobulin levels, autoantibody positivity and elevation in gamma globulin may not be present in the setting of acute AIH. Furthermore, seronegativity for autoantibodies can be a feature of more severe presentations. Up to 25–39% of patients with acute onset AIH have normal levels of IgG, and 9–17% will not have circulating autoantibodies [7–9].

It is also important to note that autoimmune markers may be mildly positive in a variety of etiologies of ALF, and caution should be taken in their interpretation. The index of suspicion should remain high in patients presenting with ALF especially in the presence of other autoimmune disorders. The diagnosis in this setting will ultimately require histology in the overwhelming majority of cases.

Detailed review of the histopathology of AIH can be found in Chap. 4. Histologic findings are nonspecific and may overlap with other etiologies including viral hepatitis and DILI. Importantly, the classic histologic features of AIH including interface hepatitis, portal inflammation (plasma cell predominant) and hepatocyte rosettes are not the typical pattern seen in the acute presentation. There is evidence to suggest that central lobular confluent necrosis without portal involvement and plasma cell enrichment are consistent findings in the acute setting.

Chronic Liver Disease

Contrary to its severe/fulminant presentation, AIH can have an insidious presentation and behave similarly to other chronic liver diseases. Even chronic AIH demonstrates heterogeneity including periods of spontaneous remission interspersed with acute flares (similar to HBV) or a smoldering disease course (such as NAFLD). This presents a diagnostic challenge as subclinical disease can often precede symptoms, or follow after initial presentation.

Up to a third of asymptomatic patients will come to a diagnosis of AIH during evaluation of unexplained elevation of serum aminotransferase levels. These findings are based on epidemiologic studies of AIH presentation in different nations and age groups [10–14].

The goal of AIH treatment is to obtain complete biochemical and histologic resolution in order to prevent progression of fibrosis. The side effects of treatment must be weighed against the risk of subclinical disease progression or evolution to symptomatic disease. This is especially pertinent given the 10-year survival in untreated patients with mild disease ranges from 67% to 90%. It is important to note however that despite therapy, progression to end-stage liver disease remains possible [15, 16].

Cirrhosis

If chronic AIH is left untreated, fibrosis progression to cirrhosis with its accompanying portal hypertensive complications and risk of hepatocellular carcinoma can occur. Approximately one third of adult patients have already developed advanced disease/cirrhosis by the time of initial diagnosis [10, 11, 14]. Patients with autoimmune hepatitis and cirrhosis have significantly higher mortality compared to patients with autoimmune hepatitis without cirrhosis (whose mortality is similar to the general population) [17]. Patients with advanced fibrosis/cirrhosis and evidence of disease activity on biopsy warrant therapy as this is a negative prognostic predictor [14, 18]. Treatment can lead to regression/stabilization of fibrosis [19]. When histology lacks evidence of disease activity (so-called "burned out" cirrhosis), treatment should not be pursued as there is no clear benefit, and treatment is accompanied by increased likelihood of treatment related side effects [14].

Diagnosis and Clinical Presentation

Autoimmune hepatitis affects both sexes. Although the exact ratio of female: male patients affected with AIH varies by study (approximately 3–4:1), AIH is characterized by a strong female preponderance the mechanism of which is not completely understood [20].

Patients can present with one or more nonspecific symptoms such as, but not limited to fatigue, malaise, right upper quadrant pain, lethargy, anorexia, weight loss, nausea, pruritus, fluctuating jaundice, and polyarthralgias involving small joints in the absence of diagnosed arthritis. Patients with AIH may have concurrent autoimmune diseases including thyroiditis, ulcerative colitis, type 1 diabetes, rheumatoid arthritis, and celiac disease [21, 22].

The clinical presentation of AIH varies widely according to ethnicity and the diagnosis can be more challenging in non-white populations. Much of the data regarding these differences stem from a heterogeneous group of studies and limit application in clinical practice.

In Europe, Black patients present at a younger age, have higher IgG levels, have higher rates of SLE, and have greater risk of liver transplant/liver related mortality suggesting a more aggressive disease in this subpopulation [23]. In the United States, African-American patients are more likely to have cirrhosis, treatment failure, and higher mortality compared to white American patients [24]. In a study comparing clinical outcomes between Asians, Caucasians, and Hispanics in the United States, Hispanics have the highest prevalence of cirrhosis while Asians had a poor overall survival [25].

It is important to note that although AIH can present at any age, age does influence presenting symptoms, survival, and possibly treatment response. Older studies have suggested higher rates of cirrhosis at diagnosis in patients older than 60 years

of age, fewer symptoms, and a more treatment responsive disease course [14, 26]. More recent data suggest that response to treatment is not influenced by age, but that survival decreases with older age at diagnosis [27].

Auto-Antibodies

Autoantibodies are one of the hallmarks of AIH and indeed their presence and titers are included in both diagnostic scoring systems. If tested correctly, over 95% of AIH patients will demonstrate some degree of serologic reactivity [28]. Despite the critical importance of autoantibodies in the diagnosis of AIH, the appropriate ordering/interpretation of these studies remains a barrier to accurate diagnosis.

There are differences in the frequency of certain autoantibodies in type 1 versus type 2 AIH, their sensitivity/specificity, and the methods by which they are obtained. Relevant considerations include the method used to measure antibodies. Indirect immunofluorescence (IIF) is considered the gold standard for the testing of liver autoantibodies including ANA, ASMA, anti-LKM and anti-LC1 antibodies [29]. Solid-phase assays (i.e. ELISA) are commercially available and widely used despite lack of standardization. Aside for its use in detecting anti-SLA/LP (which is not detectable by IIF), these tests should not be used in first-line screening for AIH [29].

Anti-nuclear antibody (ANA) Although ANA is a marker of type 1 AIH, it is not disease specific. It is found in a number of autoimmune, infectious, and malignant diseases. It may also be present at low titers in healthy adults with prevalence increasing with age [30, 31]. ANA may also be present in other liver diseases including viral hepatitis, alcohol-related liver disease, and nonalcoholic fatty liver disease [32–34]. Titers $\geq$1:40 are considered significant in adults (1:20 in children). The majority of patients with type 1 AIH demonstrate a homogeneous immunofluorescence (IIF) pattern, though speckled or nucleolar patterns can be seen and are still compatible with the diagnosis of AIH [35, 36]. ANA positivity with nuclear multiple dots or rim-like membranous IIF pattern is highly specific for PBC. Approximately one-third of patients with PBC will have positive ANA [37].

Anti-smooth-muscle antibody (ASMA) Up to 65% of patients with type 1 AIH will have positive ASMA, and its presence may be associated with more severe disease course [38, 39]. It is important to note that technical laboratory considerations may influence the results and interpretation of ASMA. Solid-phase assays (i.e. ELISA) can miss up to 20% of cases, making confirmation with immunofluorescence necessary [40]. There are multiple staining patterns for ASMA on immunofluorescence, some of which may be more specific to autoimmune hepatitis [28].

Anti-liver-kidney microsome (anti-LKM) The presence of anti-LKM antibodies is considered the hallmark of type 2 AIH, and may be found alone or in combination with anti-LC1. In approximately 10% of cases, anti-LKM may be the only positive

autoimmune marker in type 2 AIH [41, 42]. Of note, anti-LKM can be positive in up to 11% of patients with HCV [36]. In the appropriate clinical (adolescent or young adult) and geographic setting (i.e. rarely in North America), the presence of anti-LKM antibody makes the diagnosis of type 2 autoimmune hepatitis likely.

Anti-liver cytosol type 1 antibodies (anti-LC1) Anti-LC1 antibodies are found in approximately one third of patients with type 2 AIH and can be the only serologic marker [43]. However, anti-LC1 antibodies are not entirely disease specific as they can be seen rarely in type 1 AIH, autoimmune sclerosing cholangitis, and HCV infection (though less frequently than anti-LKM) [44, 45]. In the absence of HCV or evidence of sclerosing cholangitis, the presence of anti-LC1 antibodies alone or in combination with anti-LKM strongly supports a diagnosis of type 2 AIH [40].

Anti-soluble liver antigen/liver-pancreas antibodies (anti-SLA/LP) and antineutrophil cytoplasmic antibodies (ANCA) Anti-SLA/LP is a disease specific antibody (not seen in healthy patients or other diseases) which is not detected by immunofluorescence. Although it is the most specific autoantibody for type 1 AIH, it is found in only 10–30% of cases, and is rarely the only positive autoantibody [1, 28]. Presence of anti-SLA/LP may portend a more severe disease course [46]. Atypical pANCA is frequently present (50–96%) in type I AIH, but rarely occurs as the only positive autoantibody [2, 47].

Practical Clinical Considerations

In patients with compatible clinical presentations and suggestive autoantibody patterns in whom alternative etiologies of liver disease have been excluded, liver biopsy should be performed to confirm diagnosis and to stage fibrosis in preparation for treatment considerations.

The presence of positive ANA and ASMA (and elevated immunoglobulins) supports a diagnosis of AIH. Clinical scenarios remain in which AIH is the underlying diagnosis despite these auto-antibodies being negative. If conventional tests are negative, additional testing for SLA/LP, LC1, LKM, and p-ANCA should be considered, and scoring systems should be utilized.

It remains important to be cognizant that the acute/fulminant presentation of AIH may often present without positive autoimmune markers. In this scenario histology is requisite for appropriate diagnosis.

Histology

In the absence of contraindications, liver biopsy is required for a definitive diagnosis of AIH [48, 49]. The classic histologic diagnosis is based on interface hepatitis, dense plasma cell rich portal inflammation, and hepatocyte rosette formation [50].

Even in this "classic" presentation variability can occur. Plasma cells are expected at the interface and throughout the lobule; however a shortage of plasma cells in the inflammatory infiltrate does not preclude the diagnosis [51–53]. Furthermore, interface hepatitis is not specific to AIH, and can be seen in patients with DILI or viral hepatitis. It must be stressed that no pathognomonic histologic features of AIH exist. Furthermore, the limitations of biopsy, especially the possibility of sampling error must be considered.

The histologic appearance of acute AIH can differ greatly from that described in "classic" AIH. As opposed to findings concentrated in the interface and portal areas, acute AIH preferentially affects the centrilobular zone [54]. As massive necrosis can be observed in the acute setting, discriminating between post-necrotic reticulin collapse and the presence of fibrosis is especially challenging. This distinction is of clinical relevance, as certain therapies are not recommended in patients with cirrhosis. Over time, biopsies may demonstrate a transition from the centrilobular zone to the more classic appearing interface hepatitis, suggesting that the perivenular pattern of injury may be an early histological manifestation [55].

Further complicating histologic diagnosis is the observation that lesions such as granuloma, cholangitis, steatosis, or steatohepatitis can be seen in AIH. However, if these features dominate the histologic landscape, the likelihood of autoimmune hepatitis is reduced. Worth special mention, a variant of lymphocytic bile ducts injury in patients lacking clinical, serologic, and immunologic features of PBC will respond to corticosteroids similarly to patients with classic AIH and highlights the truly myriad presentations of AIH [56, 57].

Scoring Systems

The Simplified Score diagnostic criteria of the International Autoimmune Hepatitis group (IAIHG) are a user-friendly tool that can be utilized in every day clinical practice. They are a streamlined version of the original comprehensive scoring system (the "Revised Criteria") which was published by IAIHG in 1999. Remember there is no gold standard for the diagnosis of AIH, and utilization of any diagnostic score is meant to augment clinical judgment, not replace it.

The Revised Criteria includes autoimmune markers/titers, gender, presence/absence of drug exposures, viral infection, alcohol intake, liver histology, history of other autoimmune diseases, HLA, and response to treatment [58]. The initial intent of these criteria was for clinical trials, though the scoring system has been validated in clinical use [59–61]. Criticisms of the Revised Criteria include its complexity, and the failure to consistently distinguish AIH from cholestatic syndromes.

The Simplified Score was proposed in 2008 by the IAIHG [49]. This system utilizes four parameters from the comprehensive system including presence/titer of autoantibodies, IgG concentration, histology, and absence of viral hepatitis (Table 3.1). Scores < 6 do not meet criteria for diagnosis of AIH, while a score ≥ 7 is required for definite AIH. Though more user-friendly and useful in excluding AIH

Table 3.1 Simplified Diagnostic Criteria of the IAIHG [adapted from [49]]

Feature/parameter	Value	Score
ANA or ASMA +	≥1:40	+1
	≥1:80	+2
or		
Anti-LKM +	≥1:40	+2
or		
Anti SLA/LP +	Any titer	+2
IgG or y-globulin	> ULN	+1
	>1.1× ULN	+2
Liver histology	Typical of AIH	+2
	Compatible w/AIH	+1
	Atypical	0
Viral hepatitis	Present	0
	Absent	+2

Definite AIH: ≥ 7
Probable AIH: = 6

Table 3.2 Performance Characteristics of AIH Diagnostic Criteria

	Revised [58]	Simplified [49]
Sensitivity	100	95
Specificity	73	90
Accuracy	82	92

Refs. [61, 62, 65, 66]

in patients with other conditions and concurrent immune features, this comes at a cost of potentially excluding atypical cases [61–64].

The Simplified Score is more specific and accurate but lacks sensitivity (Table 3.2). Furthermore, patients presenting with acute or fulminant onset of AIH are likely to be missed by both scoring systems given variability of circulating IgG and auto antibody levels. In this clinical scenario the Revised Criteria and the Simplified Score supported the diagnosis of AIH in only 40%, and 24% of cases respectively, and highlights the limitations of these scoring systems and the need for histologic examination [62].

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Chapter 4
The Pathology of Autoimmune Hepatitis

W. Carl Jacobs and William A. Ahrens

Introduction

Autoimmune hepatitis (AIH) was first described by Jan Waldenström in 1950 as a severe form of chronic active hepatitis involving women [21]. It is characterized as an unresolving form of inflammatory liver injury in the setting of autoantibodies and hypergammaglobulinemia. The disease affects women disproportionately at roughly a 4:1 ratio in comparison to men [5]. For the decades that followed Waldenström's initial characterization of the disease, there remained significant ambiguity for diagnostic criteria of the disorder known then as "autoimmune chronic active hepatitis" [12]. In the early 1990s, the International Autoimmune Hepatitis Group (IAHG) was ultimately formed in the wake of this uncertainty, in the attempt to provide expert consensus and clarity for the disease that still remains elusive in many ways today [1].

As with other autoimmune diseases, autoimmune hepatitis may follow a relapsing and remitting disease course and as such may present acutely with significant lymphoplasmacytic inflammatory activity and no signs of chronicity. Alternatively, AIH may present as an acute flare superimposed on chronic injury and hepatic fibrosis, or as cirrhosis with non-specific inflammatory activity. There is significant overlap between autoimmune hepatitis and other pathologic entities involving the liver, and as a result the diagnosis by definition requires clinical and pathologic correlation to rule out other processes.

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M. W. Russo (ed.), *Diagnosis and Management of Autoimmune Hepatitis*,
https://doi.org/10.1007/978-3-030-33628-8_4

Autoimmune Hepatitis Types

Autoimmune hepatitis occurs in all age groups, but most patients are young or middle aged. Approximately 20% of adults with AIH present after the age of 60 [6, 7, 16].

Type 1 autoimmune hepatitis is by far the most common form of AIH, especially in the adult population. It classically involves the autoantibodies anti-nuclear antibody (ANA) and anti-smooth muscle antibody (SMA). Perinuclear anti neutrophil cytoplasmic antibodies (p-ANCA) are also found in 50–96% of patients with type 1 AIH [3]. Of note, ANA and SMA are often mildly elevated in immune-mediated drug injury as well, therefore low level positivity is considered non-specific and only mildly supportive of a diagnosis of autoimmune hepatitis. Similarly, IgG may be elevated in immune-mediated drug injury as well as in the setting of active infection. Specificity for autoimmune hepatitis increases with higher titers.

Autoimmune Hepatitis Scoring Systems

A number of scoring systems have been proposed to aid in the diagnosis of autoimmune hepatitis. A unifying theme of all systems is that histology is a cornerstone for diagnosis and the inclusion of histologic and clinical features typical of both autoimmune hepatitis and other entities that may mimic or overlap with the disease process are present. The diagnosis, in short, must be made by demonstrating clinical and histologic features typical of autoimmune hepatitis, as well as excluding clinical and histologic features typical of other entities.

The Revised International Autoimmune Hepatitis Group modified scoring system, established in 1999, is a sensitive and specific scoring system to assess the likelihood of autoimmune hepatitis using both histologic and clinical parameters. In 2009, the IAHG introduced a simplified scoring system for the diagnosis of autoimmune hepatitis [11]. This simplified scoring system applies 1–2 points for autoantibodies at certain levels of titers, 1–2 points for elevated IgG level, 0–2 points for histology atypical for, compatible with, or typical for autoimmune hepatitis, respectively, and finally 2 points for the absence of viral hepatitis for a maximum score of 8. A score of 6 is defined as probable autoimmune hepatitis, and a score greater than or equal to 7 is defined as definite autoimmune hepatitis [11].

To be considered typical for autoimmune hepatitis under the 2009 IAHG simplified scoring system, a biopsy must demonstrate three features:

1. Interface hepatitis (lymphoplasmacytic infiltrates extending into the lobule)
2. Emperipolesis (active penetration of a hepatocyte by a lymphocyte or plasma cell)
3. Hepatic Rosette formation (not specifically defined by the IAHG).

Figures 4.1, 4.2, 4.3 and 4.4 show histologic features compatible with AIH. A biopsy is considered “compatible with” AIH under this system if it shows some but

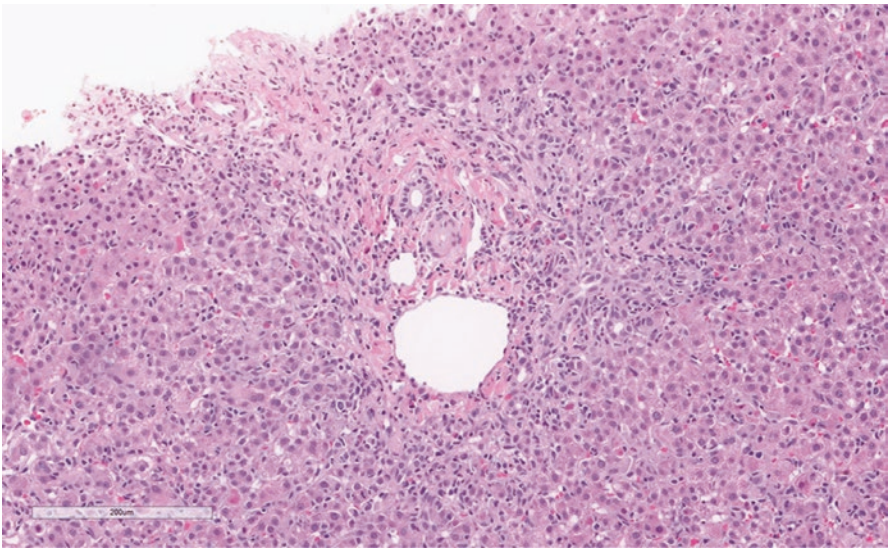

Fig. 4.1 Medium power image showing autoimmune hepatitis with portal, periportal and lobular activity

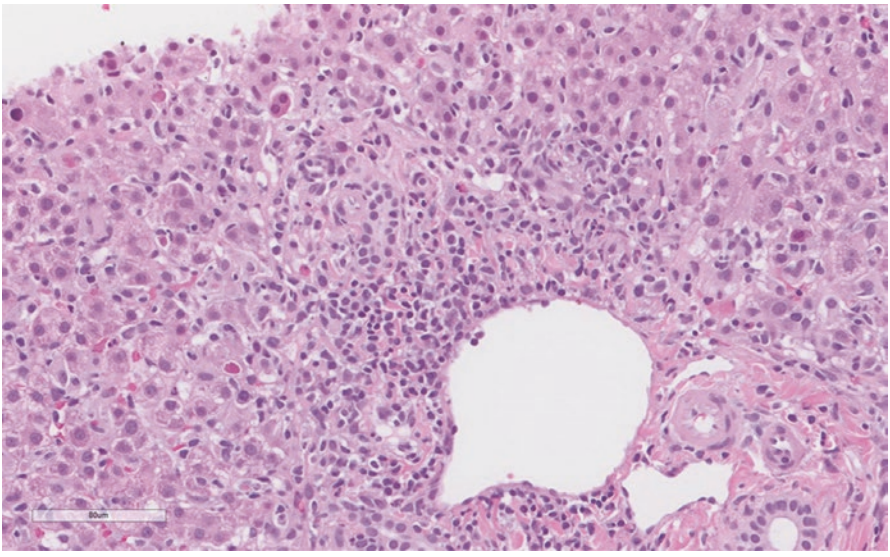

Fig. 4.2 Higher power image showing interface activity – periportal lymphoplasmacytic inflammation with apoptotic hepatocytes or "piecemeal necrosis"

not all three features. Histologic patterns "atypical for" autoimmune hepatitis was defined as biopsies showing signs of another discrete diagnosis such as steatohepatitis. Effectively, a definitive diagnosis of autoimmune hepatitis by this simplified scoring system requires some level of autoantibodies, elevated IgG, histologic support, and the exclusion of viral hepatitis.

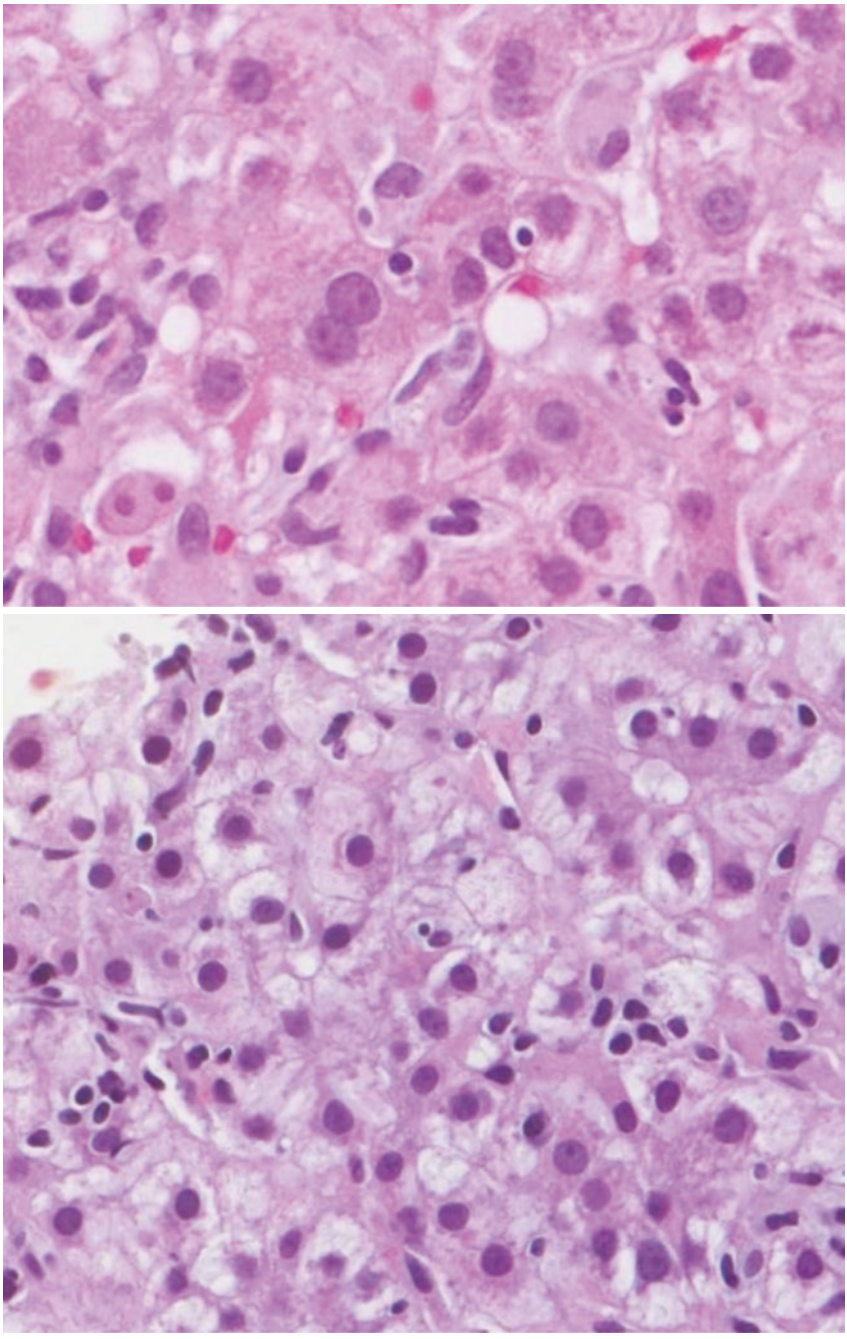

Fig. 4.3 Emperipolesis (active penetration of a hepatocyte by a lymphocyte or plasma cell) –In these photos lymphocytes with pericellular clearing are seen entirely within the confines of a hepatocyte

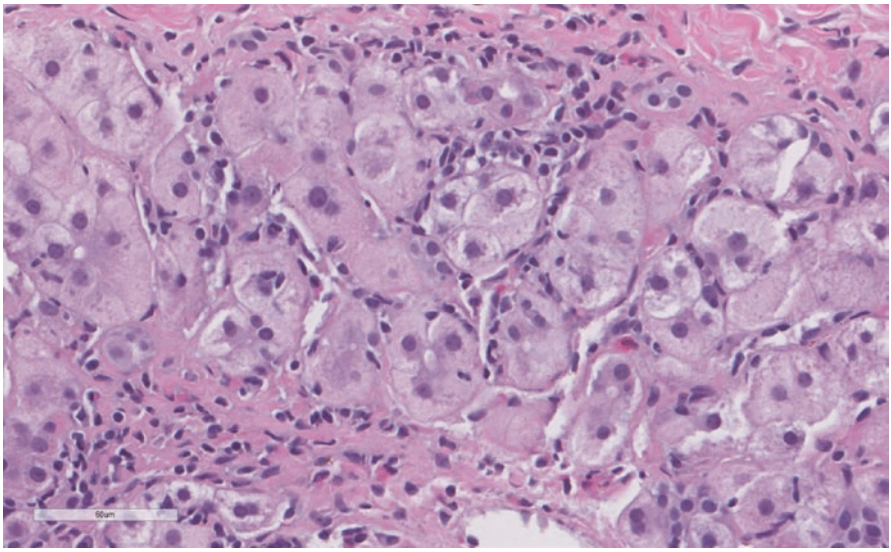

Fig. 4.4 Hepatocyte rosette formation in a case of active autoimmune hepatitis: clusters of hepatocytes centered around a central space

Newly Discovered Problems with Classic Autoimmune Hepatitis Histology

There are problems with the histologic criteria proposed by the IAHG. On the one hand, there seems to be significant interobserver variability in the recognition and interpretation of what constitutes emperipolesis and hepatic rosette formation. Like the IAHG, many studies have not clearly defined criteria for identifying rosettes (most strictly defined in a couple studies as an arrangement of hepatocytes around a central luminal space). Similarly, emperipolesis suffers from a lack of specific defined diagnostic criteria in many studies and therefore the incidence of these features varies drastically from study to study [2]. In addition, other entities such as drug injury and infection have been shown to demonstrate emperipolesis and hepatic rosette formation, therefore both the sensitivity and specificity of these features have been called into question.

Studies have shown wide ranging numbers with respect to classic histologic features of autoimmune hepatitis. Rosettes have been reported in 29–75% of autoimmune hepatitis, 11% of primary biliary cholangitis, 2–41% of drug induced liver injury, 23% of chronic viral hepatitis, and 4% of Wilson's disease [2].

In one study, emperipolesis was identified in 65% of acute autoimmune hepatitis cases and in 77% of non-autoimmune acute hepatitis cases. Rosettes were identified in 33% of acute autoimmune hepatitis cases and in 38% of non-autoimmune acute hepatitis cases. Both emperipolesis and rosettes were identified in only 26% of autoimmune hepatitis, yet found in 31% of non-autoimmune hepatitis cases [2].

Interface activity and necrosis were also found to be non-specific histologic features. Interface activity was present in 80% of autoimmune hepatitis cases and 77% of non-autoimmune hepatitis cases. Confluent necrosis was noted in 40% of autoimmune hepatitis cases, and in 69% of non-autoimmune hepatitis cases. Significantly, numerous plasma cells were much more commonly found in autoimmune hepatitis cases (75%) in comparison to non-autoimmune hepatitis cases (8%) [2].

Acknowledging the limitations with specificity of "classic" histologic features of autoimmune hepatitis, interface hepatitis, emperipolesis and rosette formation may be indicative of severity of hepatitis rather than specific to a given etiology. Many older studies that found these features specific to autoimmune hepatitis failed to control for stage of fibrosis or grade of inflammation [10].

Several recent studies have mentioned the presence of Kupffer cells with "hyaline droplets" [20] or "hyaline globules" [10] which appear to be more common in autoimmune hepatitis than other entities although they have been seen in other processes as well, including viral hepatitis C (Fig. 4.5) [4]. This feature is thus being considered in newer scoring systems. In addition, some authors have proposed scoring systems that include periportal positivity for copper and CK7 stains as markers of chronic biliary disease, which are not typically found in cases of isolated autoimmune hepatitis [2].

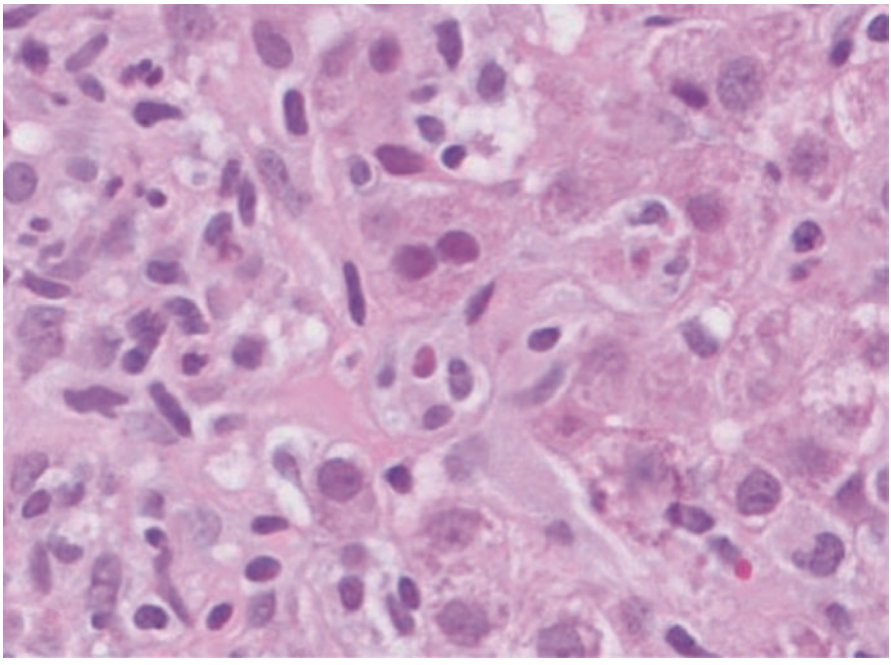

Fig. 4.5 Kupffer cell with hyaline globule – these are readily seen on PAS and PAS-D stains, although this one is also easily seen on routine H&E stain. They are often seen in autoimmune hepatitis and may be related to the immune regulatory functions of Kupffer cells [4]

Acute, Acute on Chronic, and Chronic Presentation of Autoimmune Hepatitits

Autoimmune hepatitis can present at any age and any population, but it is more likely to present acutely in children and young adults [9, 17] and often presents with chronic changes/fibrosis in an older patient population.

In the acute setting, autoimmune hepatitis may present classically with a striking portal, periportal, and lobular lymphoplasmacytic infiltrate, with interface activity including apoptotic hepatocytes at the portal-periportal interface or limiting plate (historically termed 'piecemeal necrosis'). Other common features include hepatocyte rosette formation, emperipolesis, lobular eosinophils (in contrast to portal eosinophils which some studies have shown may be more common in drug injury [19]), and zone 3 damage with pericentral necrosis and plasma cell central venulitis. Portal and lobular ceroid-laden macrophages are often seen and will be highlighted by PAS stains with and without diastase. They signify 'resolving/ongoing' liver injury, representing the liver's attempt to clean up and remove damaged cells. In more severe acute presentations, parenchymal drop out is common (Figs. 4.6 and 4.7). This will sometimes present as bridging parenchymal collapse that can result in a nodular appearance that can be confused for cirrhosis both radiologically and histologically (Figs. 4.8, 4.9 and 4.10). Extreme cases may present as fulminant hepatitis with extensive panlobular necrosis, necessitating emergent liver transplant.

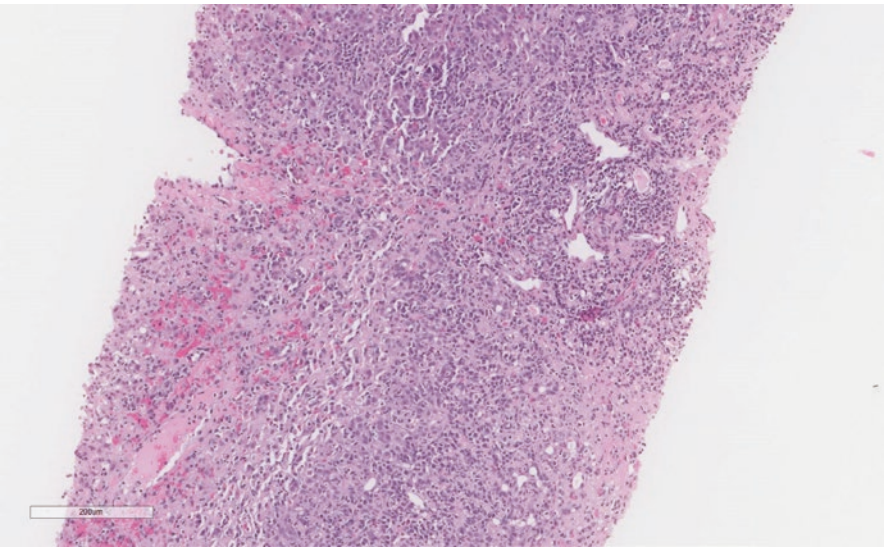

Fig. 4.6 Acute autoimmune hepatitis with bridging parenchymal collapse, numerous plasma cells, and central venulitis

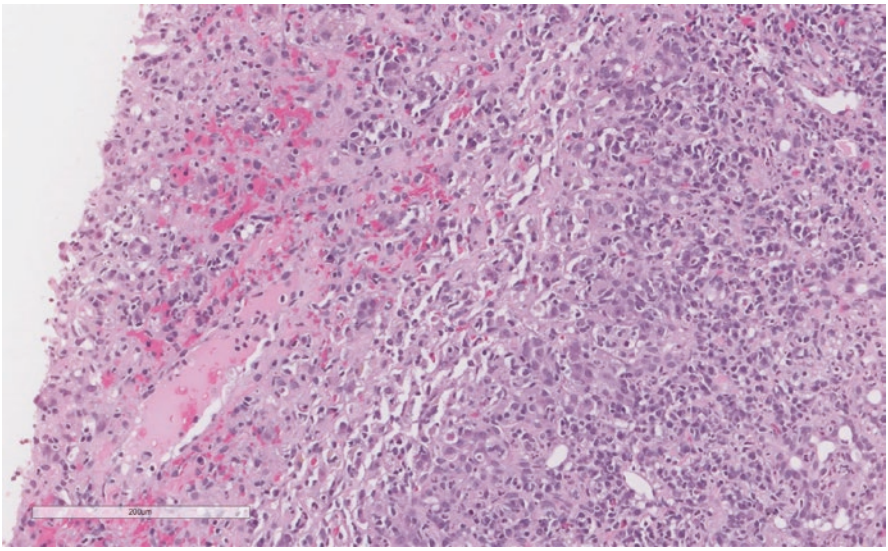

Fig. 4.7 Higher power image showing central venulitis with pericentral necrosis and bridging parenchymal collapse. Numerous plasma cells are seen admixed with lymphocytes, eosinophils and neutrophils in this case

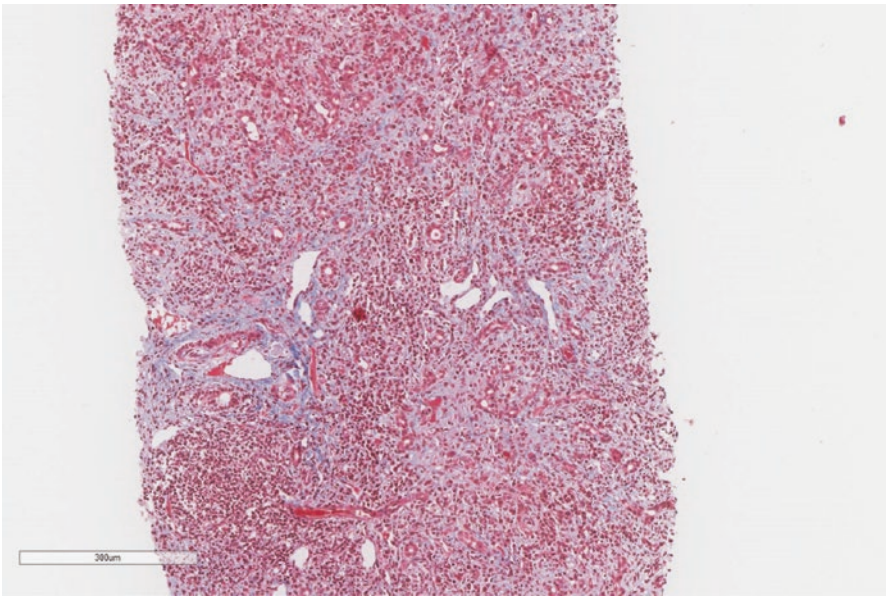

Fig. 4.8 Trichrome stain showing a portal tracts (dark blue) as well as panlobular parenchymal collapse (pale blue) associated with diffuses bile ductular proliferation. A bile ductular reaction is typical to all cases of significant acute hepatitis with parenchymal loss. Bridging parenchymal collapse can be mistaken for cirrhosis both radiologically and histologically

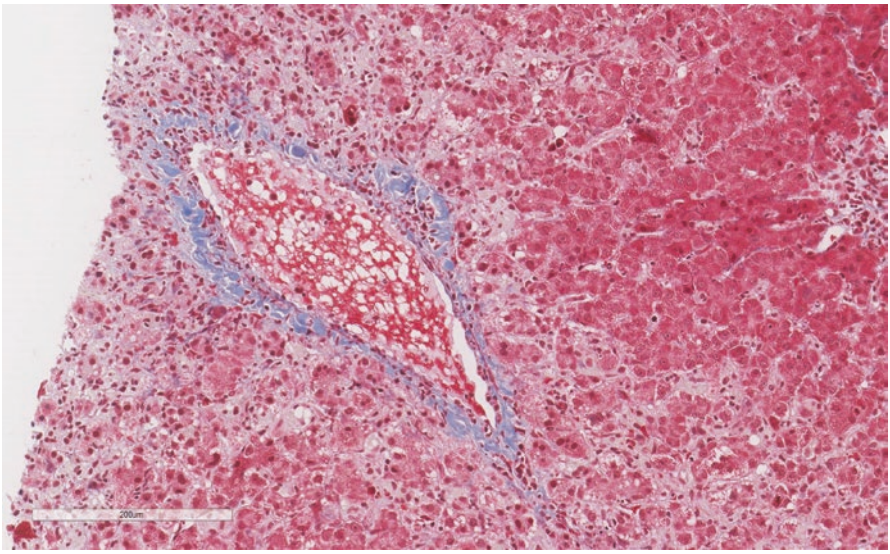

Fig. 4.9 High power image of a central vein of a largely intact central vein (dark blue), with pericentral hepatocyte loss/drop out (pale blue) associated with lymphoplasmacytic inflammation and admixed ceroid laden macrophages (Trichrome stain)

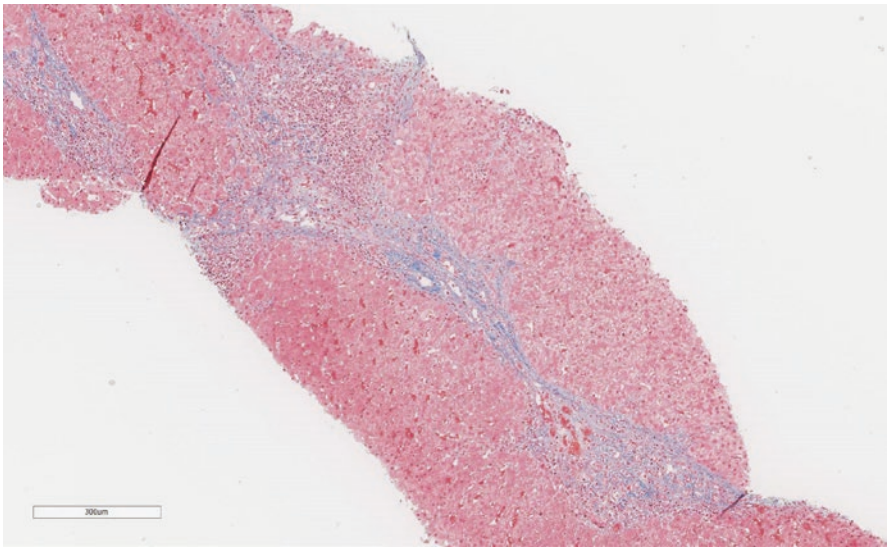

Fig. 4.10 At least bridging fibrosis is shown here in a case of acute on chronic hepatitis. Staging of fibrosis can be difficult in the setting of acute on chronic liver injury and often must be deferred if there is significant parenchymal collapse, but in this case the trichrome stain is definitive. Reticulin stain may be a helpful adjunct stain to distinguish collapse from fibrosis

Reticulin stain and trichrome stain are both helpful in distinguishing true fibrosis from parenchymal collapse in the setting of significant acute hepatitis. Mature collagen/fibrosis will stain darkly with trichrome stain whereas parenchymal collapse will look paler (in comparison to the native portal tracts which act as a positive control for fibrous tissue in the setting of acute hepatitis where there is no true fibrosis). Reticulin stain is typically pale gray in portal tracts and areas of fibrosis but will stain the reticulin fibers of the hepatic plate darkly. In the setting of parenchymal collapse, reticulin will highlight areas of lost hepatocytes/collapsed plates as the dark black reticulin fibers are typically retained.

If a liver biopsy is obtained after steroid therapy has been initiated, the diagnosis may be difficult or impossible to histologically confirm. Immunosuppression can lead to rapid resolution of the lymphoplasmacytic inflammatory infiltrate, leaving empty appearing, previously expanded portal tracts, reduced lobular activity, and non-specific portal and lobular ceroid-laden macrophages cleaning up the previous damage.

Patients presenting with an acute flare superimposed on chronic autoimmune hepatitis will show some degree of hepatic fibrosis and varying degrees of lymphoplasmacytic inflammation (Figs. 4.11 and 4.12). Patients with advanced fibrosis or cirrhosis may demonstrate very limited inflammatory activity and therefore show a non-specific histologic picture of end stage liver disease.

In the setting of cirrhosis and limited inflammation, sometimes the best a pathologist can do is to exclude features typical for other specific entities such as primary biliary cholangitis and primary sclerosing cholangitis which will both show an irregular/biliary pattern of fibrosis, and may show ductopenia which is not typical

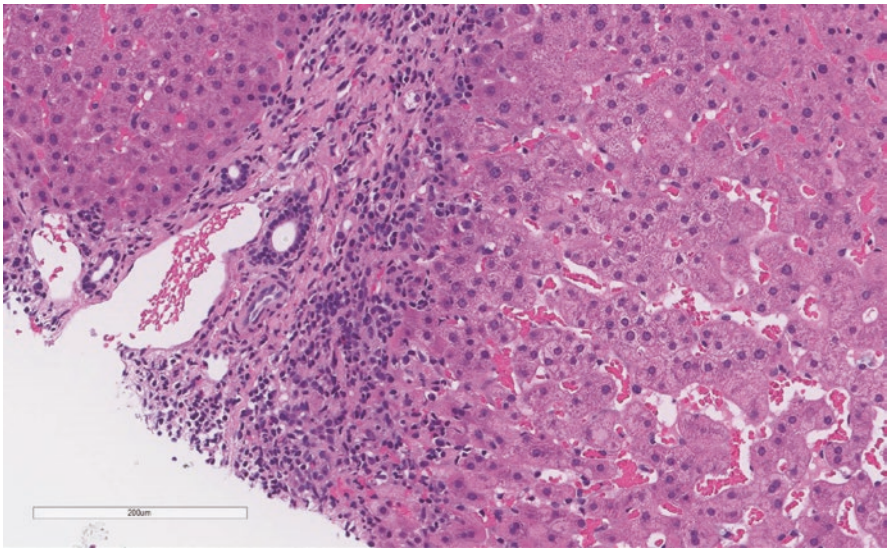

Fig. 4.11 Significant ongoing portal/periportal lymphoplasmacytic activity is seen in this case of acute on chronic autoimmune hepatitis. The trichrome stain shows at least bridging fibrosis (see previous photo)

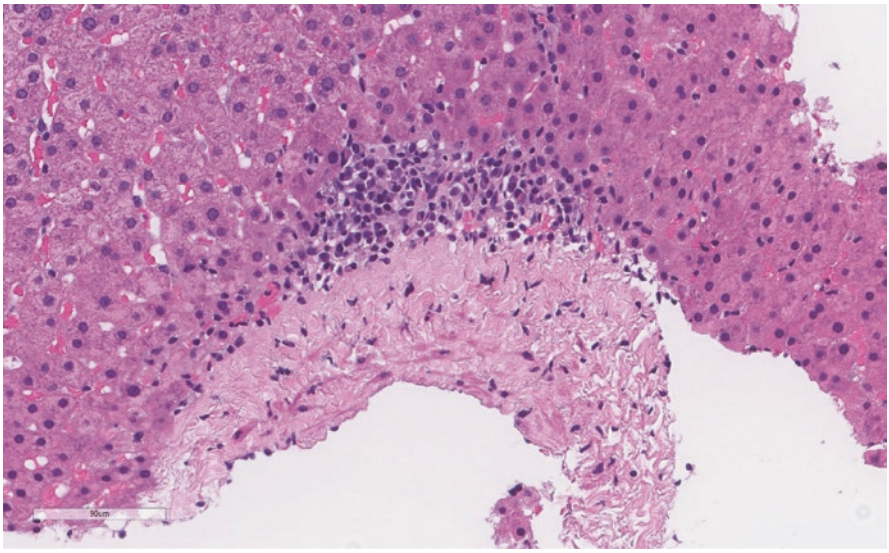

Fig. 4.12 A striking plasma cell dominant population is seen adjacent to a larger central vein in this case of acute on chronic autoimmune hepatitis

for autoimmune hepatitis. The biopsy may also show the residual nodular scars highlighting the areas of lost interlobular native bile ducts pathognomonic for primary sclerosing cholangitis. Remote toxic/metabolic liver injury may still show residual intracytoplasmic hyaline and sinusoidal fibrosis, even in the absence of steatosis.

Differentiating Autoimmune Hepatitis from Infection and Drug Induced Liver Injury

The differential diagnosis of acute autoimmune hepatitis includes infection as well as immune-mediated drug injury. Acute viral hepatitis, especially acute hepatitis B or acute hepatitis C infection, will often show prominent plasma cells. Acute viral hepatitis is less likely than autoimmune hepatitis to demonstrate interface activity. Since interface activity is seen in at least some cases of acute viral hepatitis as well, distinguishing between these entities ultimately requires clinical and serologic correlation (Fig. 4.13). Epstein Barr virus (EBV) infection may also show some overlapping features with autoimmune hepatitis including the presence of plasma cells, though typically EBV hepatitis will show a more prominent sinusoidal lymphocytic infiltrate. In situ hybridization for EBV (EBER) can confirm or exclude this entity.

Women are more likely than men to present with autoimmune hepatitis and are also more likely to present with drug induced liver injury that shows an autoimmune-like histology including frequent plasma cell infiltration, hepatocyte rosette formation and lobular disarray. Men, in contrast, are more likely to show cholestasis in

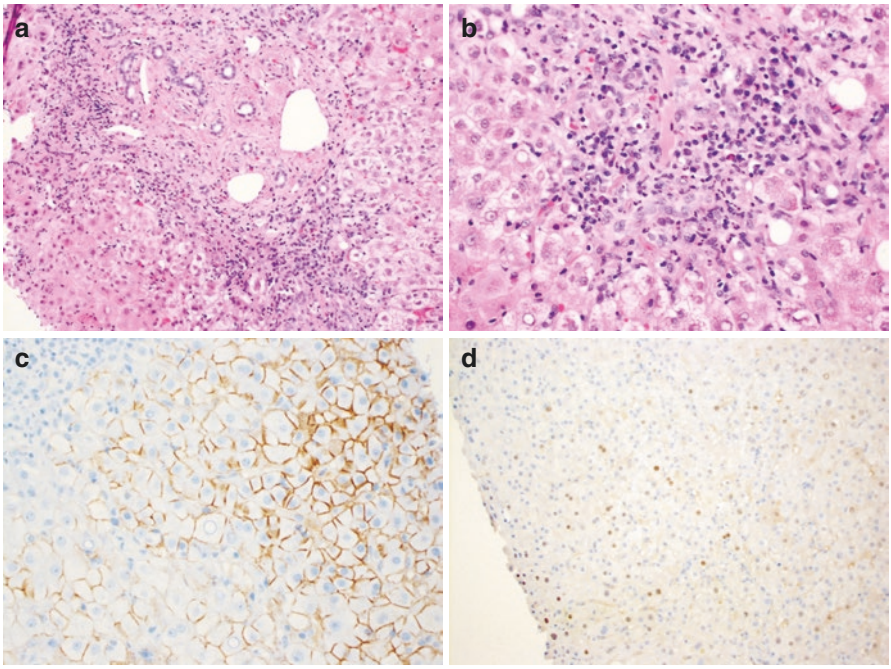

Fig. 4.13 Forty-nine year old female with 2 week history of jaundice. History of doxycycline therapy 1 week ago. Remote history of cholecystectomy with current biliary stones. Autoimmune and viral serologies still pending at the time of biopsy. (**a**) Histologic sections show a portal and lobular hepatitis with interface activity and patchy prominent plasma cells. There is also a bile ductular proliferation. (**b**) Mixed lobular inflammation with conspicuous plasma cells. (**c**) Immunostains for HBsAg (**c**) and HBcAg (**d**) were both positive, confirming the diagnosis. The overall findings are compatible with acute viral hepatitis B, with the biliary proliferation representing either a component of the acute hepatitis or biliary obstruction from cholelithiasis

drug induced liver injury [15]. Autoimmune serologies were later confirmed to be negative. Drug induced liver injury may present with a mixed histologic pattern of injury and inflammation, whereas autoimmune hepatitis will often, but not always, have a striking plasma cell predominant infiltrate. Other features classically considered specific to autoimmune hepatitis including hepatocyte rosette formation, intra-acinar eosinophils, plasma cell rich central venulitis with pericentral hemorrhagic necrosis, and emperipolesis may be less helpful than previously believed in making a diagnosis of autoimmune hepatitis. may be a helpful feature to favor autoimmune hepatitis over immune-mediated drug injury, as drug injury will not typically present with fibrosis [8], except in cases of chronic injury from drug or supplement use. Features that may favor drug induced liver injury include canalicular and hepatocellular cholestasis, prominent neutrophils, and intra-acinar lymphocytes [19].

Ultimately, plasma cell rich liver biopsies in patients who have been exposed to potential offending drugs or supplements will often require clinical correlation over a period of months to arrive at a definitive diagnosis. Immune mediated drug injury

may take months to resolve after the offending agent has been removed. These patients respond to steroid therapy as well, and the only way to definitively confirm immune mediated drug injury may be to taper the immunosuppression therapy and follow up liver enzymes over time to assess for prolonged resolution. Autoimmune hepatitis (idiopathic or drug induced AIH) are more likely to relapse after cessation of immunosuppression, whereas immune mediated drug injury will not, provided that the offending agent has been successfully identified.

Autoimmune Hepatitis with PBC and PSC Overlap

Patients presenting with autoimmune hepatitis not uncommonly present with an overlap syndrome. In the setting of advanced fibrosis, periportal copper and CK7 positive cholangiocytes may be seen in isolated autoimmune hepatitis and are non-specific (Figs. 4.14 and 4.15). However, these findings should not be seen in less advanced cases of autoimmune hepatitis and thus, if present, they should prompt consideration for a chronic biliary process such as primary biliary cholangitis or primary sclerosing cholangitis.

Autoimmune hepatitis/Primary biliary cholangitis (PBC) overlap syndrome can often be diagnosed with certainty based on histologic and clinical findings. For any

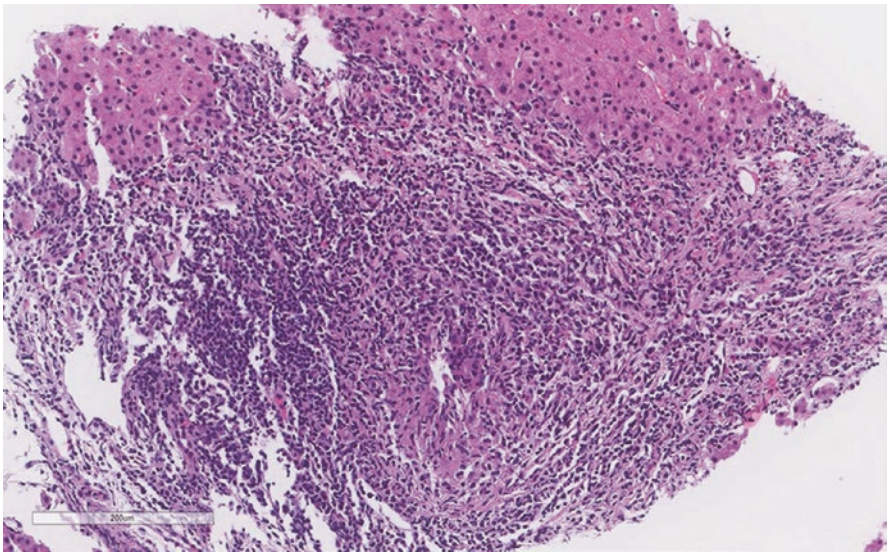

Fig. 4.14 A single portal granuloma associated with localized duct destruction is seen in this case of primary biliary cholangitis-autoimmune hepatitis overlap. A striking plasma cell population is seen in this portal tract, which may be seen in PBC alone, however the case also showed prominent interface and lobular lymphoplasmacytic activity which is not typically seen in PBC. Serologic workup revealed elevated ANA and anti-smooth muscle antibody, as well as elevated IgG and IgM, supporting the diagnosis

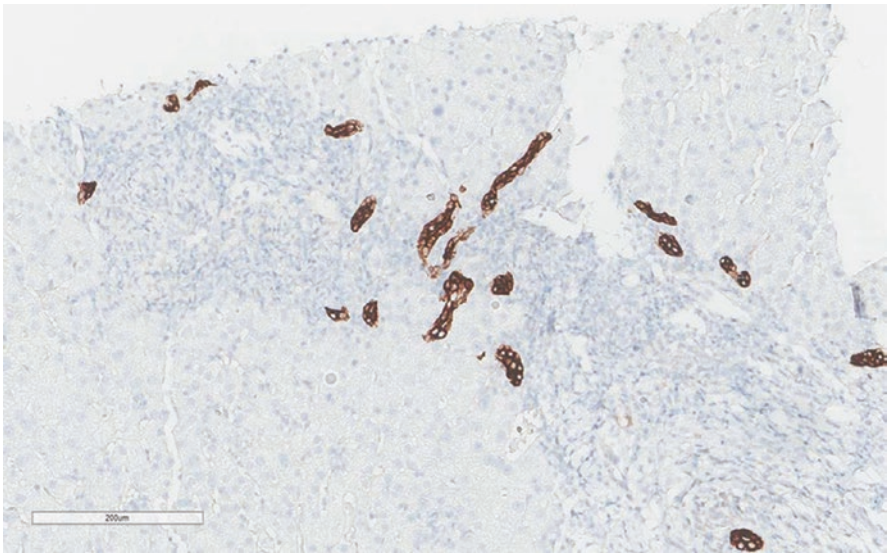

Fig. 4.15 Immunostain for CK7 in this case of PBC-AIH overlap highlights a biliary proliferation (which is not typically seen in cases of AIH, except in the setting of parenchymal collapse and hepatic regeneration). Notably, the CK7 stain also shows marked reduction in canals of Hering. The typical portal tract will demonstrate 3–16 canals of Hering [14], which appear on immunostains for CK7 or CK19 as isolated cholangiocytes or short strings of cells arrayed around the portal tract at the limiting plate. Only very rare canals were identified in this case (1–2 canals of Hering are seen in this image). Canals of Hering are an early marker for PBC, although the finding is not entirely specific and loss has been documented in drug injury (specifically methotrexate) as well [18]. In this particular case, the finding is supportive of the diagnosis of PBC-AIH overlap

patient with positive AMA, a component of PBC should be considered. PBC will often have prominent plasma cells in portal tracts, so even a striking portal plasma cell infiltrate does not necessarily denote a component of autoimmune hepatitis. However, lobular activity should not be prominent in PBC alone, therefore classic features of PBC combined with interface and lobular activity with scattered apoptotic hepatocytes (ideally including prominent lobular plasma cells) should prompt consideration for a component of AIH. Autoimmune hepatitis may show some subtle bile duct damage, but will not typically show the classic florid duct lesions or granulomatous duct destruction typical in PBC. In addition, evaluation of canals of Hering using CK7 or CK19 might be helpful, as these are often reduced or lost in PBC. Loss of canals of Hering and CK7 positive cholangiocytes may be the central histologic findings of so-called “minimal change PBC”, a diagnosis which must be made in conjunction with clinical/serologic findings [13].

Autoimmune hepatitis/primary sclerosing cholangitis overlap requires similar diagnostic rationale. Significant lobular activity is not typical in PSC. Autoimmune hepatitis will not typically demonstrate peribiliary concentric fibrosis (Figs. 4.16, 4.17 and 4.18). Native ductopenia, another clue pointing towards a chronic biliary process, is not typically seen in autoimmune hepatitis. Portal nodular scarring in areas of lost bile ducts is virtually diagnostic for a component of PSC.

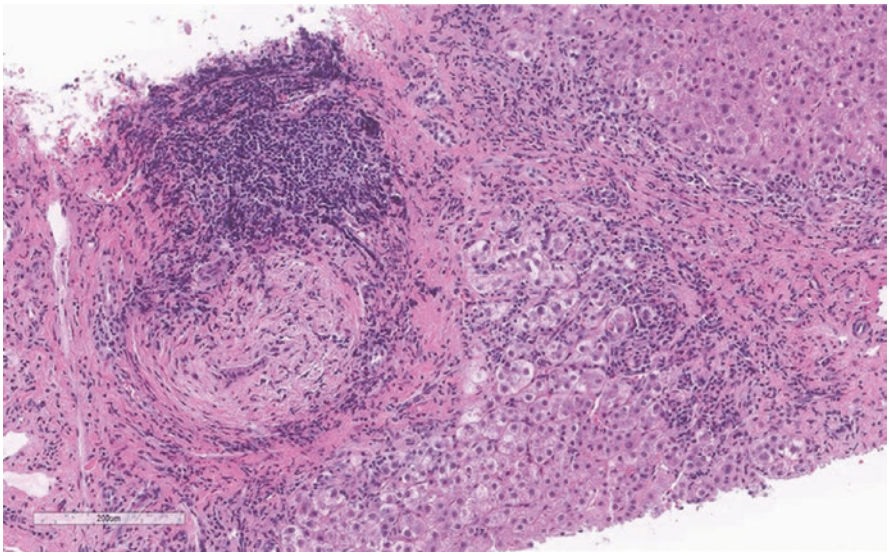

Fig. 4.16 Autoimmune hepatitis-primary sclerosing cholangitis overlap. On the left peribiliary concentric fibrosis is seen surrounding what is left of an interlobular bile duct (this is nearly a complete nodular scar). On the right periportal plasma cell rich inflammation is seen with hepatocyte rosette formation. This biopsy is from a pediatric patient with ulcerative colitis and elevated ANA and anti-smooth muscle antibody

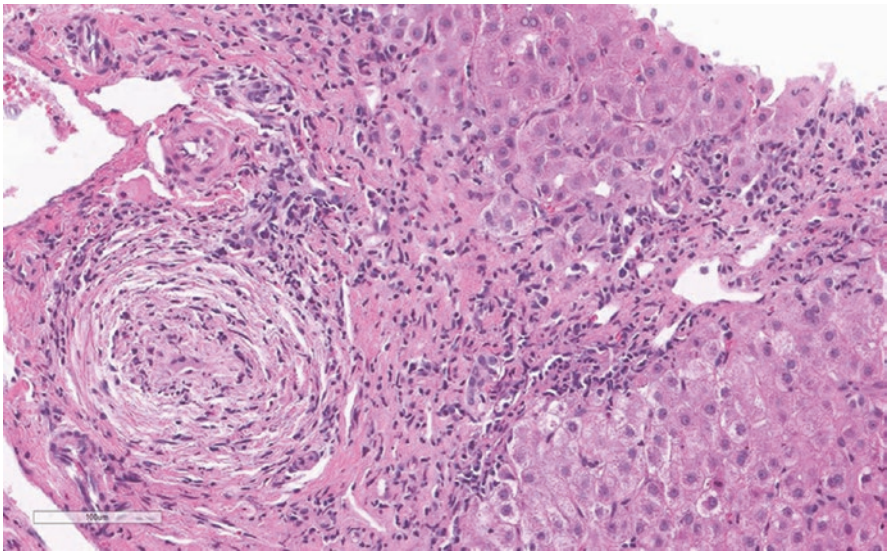

Fig. 4.17 Autoimmune hepatitis-primary sclerosing cholangitis overlap. On the left peribiliary concentric fibrosis is seen surrounding what is left of an interlobular bile duct (this is nearly a complete nodular scar). On the right periportal plasma cell rich inflammation is seen with hepatocyte rosette formation

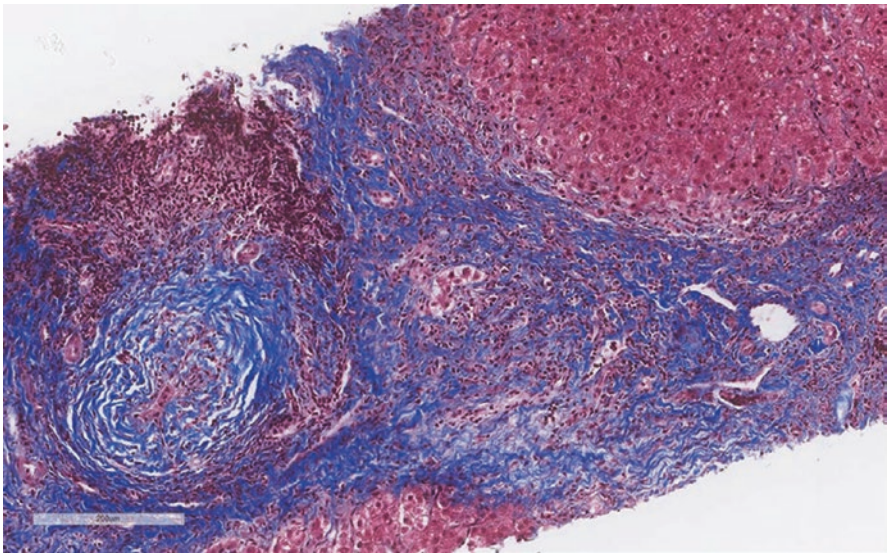

Fig. 4.18 Trichrome stain highlights the concentric periductal fibrosis and advanced fibrosis in this case of autoimmune hepatitis-primary sclerosing cholangitis overlap

IgG4 mediated disease is another consideration for patients with prominent plasma cells and bile duct damage. Neither autoimmune hepatitis, nor primary sclerosing cholangitis should present with many IgG4 positive plasma cells therefore immunostain for IgG and IgG4 can lead to the correct diagnosis in these cases.

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Chapter 5
Treating the Adult Patient: First Line Therapy

Andrew S. deLemos

Introduction

After establishing a diagnosis of autoimmune hepatitis (AIH) and prior to deciding on optimal management, it is advisable to classify the degree of chronicity and as well as disease activity to help inform treatment decisions. The histologic features of AIH, including the fibrosis stage and inflammatory activity as assessed by histologic activity index (HAI) are therefore crucial in directing treatment (Fig. 5.1). Moreover, the clinical presentation, which can vary from acute and fulminant to insidious with features of decompensated cirrhosis, also helps guide the approach to

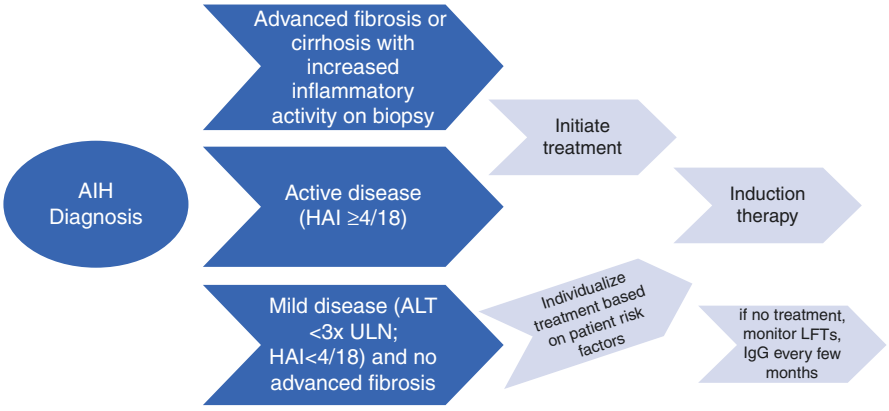

Fig. 5.1 Therapeutic strategy for initiating corticosteroids based on clinical presentation and histologic activity index (HAI)

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M. W. Russo (ed.), *Diagnosis and Management of Autoimmune Hepatitis*,
https://doi.org/10.1007/978-3-030-33628-8_5

therapy. After deciding that treatment is necessary, the goal should be to induce a clinical and biochemical remission and then maintain a remission while minimizing toxicities related to immunomodulation.

Treatment of Mild AIH

The decision to treat patients with mild AIH requires perhaps the most nuance. Mild AIH as categorized by the European Association for the Study of the Liver (EASL) and is defined by an ALT < 3× ULN; HAI < 4/18; and without advanced fibrosis and the decision to pursue therapy should be individualized [1]. This recommendation considers older natural history studies of AIH demonstrating a 10-year survival of 67–90% without therapy as well as a retrospective analysis of 31 patients who presented asymptomatically with AIH, half of whom eventually received therapy [2–4]. This uncontrolled study demonstrated a similar 10-year survival in treated (83%) vs. untreated (80%) patients. In mild AIH, the provider should consider the risks associated with steroid exposure including age and presence of co-morbid conditions such as diabetes. Patient preference is also an important consideration since a watchful waiting strategy still necessitates close monitoring and an appreciation that flares can occur. Since disease activity can vary substantially, and the natural history of mild AIH can be unpredictable, offering patients treatment immediately or following a period of chronic low level aminotransferase elevations is justifiable. When deciding on a monitoring approach, patients should have ALT and IgG checked every 3 months. The development of symptoms or an ALT ≥ 3× ULN should trigger a reevaluation and initiation of therapy.

Induction Therapy

Corticosteroids remain the standard of care for inducing remission, while the addition of azathioprine during induction is viewed as a preferred strategy to mitigate the risks of excess steroid exposure. Steroids were initial shown to confer a survival benefit in the 1970s by Cook et al., and this finding was confirmed by a randomized controlled trial from the Mayo Clinic [5, 6]. This study compared 60 mg/day of prednisone tapered to 20 mg/day by week 4 vs. 100 mg of azathioprine monotherapy vs. 30 mg/day of prednisone tapered to 10 mg/day maintenance with azathioprine 50 mg/day vs. placebo. Prednisone monotherapy and combination therapy were equally effective with 75% of patients achieving a histologic remission after 18 months of therapy. A meta-analysis of eleven randomized controlled trials in AIH found no survival benefit between induction therapy with prednisone vs. prednisone and azathioprine in treatment naïve patients (RR = 0.98; 95% CI 0.65–1.47) and a significant benefit compared to no treatment at all (6–7% vs. 41% mortality in placebo-treated patients) [7]. Combination therapy, however, reduced the risk of

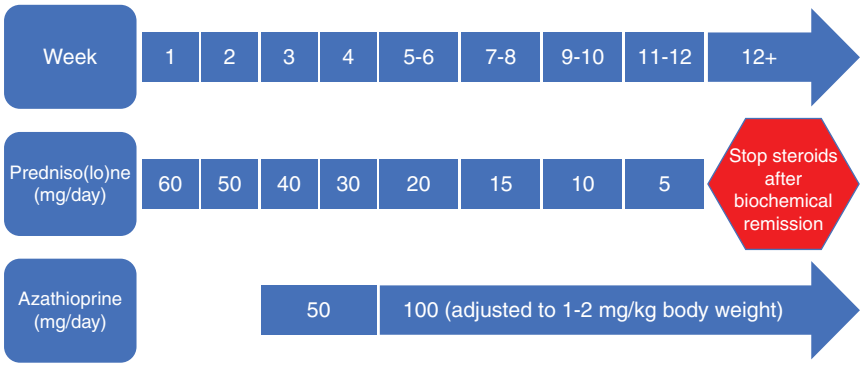

Fig. 5.2 Treatment algorithm for predniso(lo)ne taper and azathioprine treatment for induction of remission for autoimmune hepatitis (AIH)

steroid-related side effects (10% vs. 44%), while azathioprine monotherapy for induction was associated with increased mortality (30%) [7].

The initial dose and formulation of corticosteroid should be tailored to specific patient factors. A dose of predniso(lo)ne of 0.5–1.0 mg/kg is a typical starting dose, but patients rarely require more than 60 mg/day and a lack of response to this dose should prompt investigation of alternative diagnoses or question compliance. The predniso(lo)ne starting dose and taper can follow a conventional course (Fig. 5.2). Alternatively, a personalized approach which considers disease severity with the risks of prolonged high dose steroids in patients with pre-existing conditions such as diabetes, obesity, osteoporosis, hypertension, and psychiatric disease is stylistically superior. Within 2 weeks of steroid initiation, aminotransferases should decline. The pace of symptom and biochemical response can steer the taper, but after completing the fourth week of therapy, most patients can be reduced to 20 mg of prednisone.

The addition of azathioprine at 50 mg/day should occur between weeks 2 and 4. Avoiding azathioprine initiation at the outset removes the possibility of confusing side effects of prednisone with azathioprine. EASL also recommends withholding azathioprine until the bilirubin falls below 6 mg/dl (100 μmol/L) [1]. The dose of azathioprine can then be increased gradually to 1–2 mg/kg/day with further tapering of steroids over the next 8 weeks, such that by month 3 the prednisone dose is reduced to 5–10 mg/day. A more aggressive approach with high dose predniso(lo)ne at 1 mg/kg of body weight tapered over 3 months to 5–10 mg/day with immediate treatment with 1–1.5 mg/kg/day of azathioprine resulted in a complete remission at 6 months in 77% of noncirrhotic patients with AIH. Whether this strategy translates to better long-term outcomes remains unproven [8]. The final doses of combination therapy to induce a remission should ultimately be tailored to the individual patient, but the goal by the end of induction is to achieve normalization of ALT and IgG. IgG normalization during induction has been shown to correlate with resolution of inflammatory activity and is used in conjunction with aminotransferases to define a biochemical remission [9]. During this phase, clinic visits with patients should be frequent, such as monthly during the first 3 months, to monitor for clinical

benefit and safety, while a laboratory assessment every 2 weeks for the first month and monthly thereafter during induction is prudent. When the response to combination induction therapy is inadequate and assuming confidence in the diagnosis of AIH, a short trial period of increased predniso(lo)ne and azathioprine can be considered prior to seeking alternative therapy.

Maintenance Treatment

Azathioprine is the treatment of choice to maintain remission, and patients who can tolerate it without side effects or toxicities should be given the opportunity to stop steroid therapy completely. The success of azathioprine monotherapy in maintaining a remission was first demonstrated in a randomized controlled trial by Stellon et al. comparing 2 mg/kg of azathioprine with combination therapy (1 mg/kg AZA and prednisolone) [10]. At 1-year there was no difference in liver function tests or histology between the two groups. A subsequent study followed 72 patients who had steroids withdrawn after at least one year of a complete remission on 5–15 mg/day of prednisolone with 1 mg/kg/day of azathioprine. 60 patients (83%) remained in remission on 2 mg/kg azathioprine monotherapy with a median follow-up of 67 months (range 12–128) [11]. 48 liver biopsies were performed in follow-up in 42 patients and 45 showed inactive or minimal disease. Both studies supported the case for complete steroid withdrawal by showing a significant reduction in steroid-related side effects. When azathioprine is well-tolerated but other considerations such as cytopenias or concerns about long-term oncologic or teratogenic risks are considered, a strategy of lower dose of AZA with the lowest of dose predniso(lo)ne to maintain normal serum transaminases is sensible. A clinic visit with laboratory assessment should take place every 3 months during the first year of maintenance therapy and then at least biannually during the subsequent years of treatment.

Maintaining a biochemical remission during maintenance therapy is the standard of care in AIH due to its association with improved clinical outcomes. The introduction of vibration controlled transient elastography (VCTE) offers an additional modality to follow AIH patients over the course of treatment. Use of VCTE early after diagnosis of AIH was shown to correlate with histologic inflammatory grading as opposed to fibrosis [12]. However, a long-term follow-up study of patients with AIH with serial VCTE exams at intervals of at least 12 months found a statistically significant association between a biochemical remission and regression of liver stiffness (LS) [13]. In 125 AIH patients of whom 69% were in a complete biochemical remission, LS improved on average by 7.5%/year. 31% of patients not in remission had an increase in mean LS by 1.7%/year ($p < 0.001$). Remarkably, patients with stage 4 fibrosis at baseline had the largest decrease in LS: −11.7%/year. The VCTE data reinforces the benefit of a biochemical remission and may serve as an adjunctive data point in following patients with AIH over time.

Budesonide as an Alternative First-Line Agent

Budesonide may be an initial therapeutic option in acute uncomplicated AIH particularly for patients at highest risk for corticosteroid toxicities. Budesonide undergoes extensive (90%) first-pass metabolism in the liver thereby limiting systemic glucocorticoid exposure [14]. A randomized controlled trial comparing budesonide 3 mg TID or BID to prednisone 40 mg/day tapered to 10 mg/day both in combination with 1–2 mg/kg/day of azathioprine demonstrated a benefit for the budesonide arm [15]. At 6 months, a complete response, defined as normalization of ALT and AST without steroid-related side effects occurred in 48% of patients given budesonide vs. 18.4% of those given prednisone ($p < 0.001$). The trial, however, has been critiqued for allowing response guided therapy in the budesonide arm but not in the prednisone arm which likely introduced bias into the study. Apart from Manns et al., the experience with budesonide in combination with azathioprine as a first-line treatment of choice is limited [16]. EASL currently advises its use in combination with azathioprine as an alternative induction agent for patients anticipated to have complications due to predniso(lo)ne treatment [1].

Long-term data for budesonide use in maintaining remission are lacking, but can be inferred by a recent retrospective analysis [17]. 60 patients with AIH initially treated with prednisolone (mean time 47 mos) were switched to budesonide either for steroid side effects or dependency. 12 months after switching to budensonide therapy, 70% of patients maintained a biochemical response. After a mean follow-up of 31 months, long-term remission was observed in 40–45% of patients. 25% of patients had to be switched back to prednisolone therapy due to insufficient response or side effects. 15 patients with osteopenia at baseline were evaluated by DEXA scans and after a median treatment duration of 24 months with budesonide, 6 patients had improved bone mineral density scores and 8 remained stable. In summary, budesonide therapy in combination with azathioprine may be a practical option for early stage AIH both for induction and remission. Like predniso(lo)ne, the budesonide taper during induction and maintenance should be individualized with a goal to reduce the dose while maintaining a complete response.

Importantly, budesonide is contraindicated for patients with cirrhosis and/or portosystemic shunting due to impaired first pass metabolism and risk of systemic effects from increased drug levels [18, 19]. Additionally, extrahepatic autoimmune manifestations could theoretically be exacerbated or unmasked by using budesonide in contrast to predniso(lo)ne. Lastly, in the U.S., some insurance drug formularies have switched to cover Uceris® which is budesonide, but with a polymer film that breaks down at or above a pH of 7.0 for use in ulcerative colitis. Using this formulation in lieu of standard budesonide for AIH would be expected to negatively impact efficacy. Access to budesonide may be limited because it can be costly.

Acute Severe Autoimmune Hepatitis

Patients presenting with AIH and features of acute liver injury defined by jaundice, coagulopathy and elevated aminotransferases with signs of effective portal hypertension such as ascites, deserve special attention given ther risk of progression to acute liver failure. These patients should be considered for prompt referral to a tertiary care facility for evaluation for liver transplantation. High doses of intravenous corticosteroids (≥1 mg/kg), preferably after confirmatory transjugular liver biopsy or alternatively with high pre-test probability for AIH, is a recommended strategy. Treatment for a total of 5–7 days without response, as defined by improvement in bilirubin and MELD-Na, should prompt discontinuation of steroids and assessment for liver transplantation [1]. Corticosteroids were not shown to benefit patients presenting with AIH and acute liver failure from the Acute Liver Failure Study Group (ALFSG) in the U.S. [20], a cohort in France [21], or in the U.K. [22], but a recent retrospective series from Germany did report a surprisingly high (91%) spontaneous survival rate [23]. Since patients can present on a spectrum between acute liver injury and ALF and the infectious complications of steroids can be profound, the decision to start therapy is best determined at a liver transplant center whenever possible. One approach is empiric therapy for SBP and antifungal prophylaxis for AIH-ALF patients treated with high dose steroids.

Treatment Duration and Withdrawal

Patients with AIH may be carefully considered for discontinuation of therapy, though only after completing a few important milestones are achieved. The importance of treating AIH to achieve transaminase and IgG normalization cannot be understated since failure to attain a biochemical remission is associated with relapse after withdrawal of therapy, progression to cirrhosis, and adverse outcomes [24–28]. Persistent transaminase elevation despite first-line therapy reinforces the need to fine-tune current therapy or to evaluate second-line therapeutic options. Fortunately, most patients can achieve and maintain a remission with first-line therapy. Following 2 years of successful maintenance of remission, and a minimum of three total years of treatment, select patients can be evaluated for treatment withdrawal [1] (Fig. 5.3). Several factors influence the choice to stop treatment. Age at diagnosis is an important variable. A young patient may benefit from treatment withdrawal to avoid the risks inherent with lifelong therapy. On the other hand, an older patient presenting with AIH and advanced fibrosis or cirrhosis may not tolerate recrudescence of AIH after stopping therapy, and continuing therapy if well-tolerated is preferable. Certainly, side effects of therapy are a key consideration informing the decision to attempt withdrawing therapy. A liver biopsy, while not required prior to stopping therapy, is recommended, particularly for patients who present with severe acute AIH and who may not tolerate repeat induction therapy

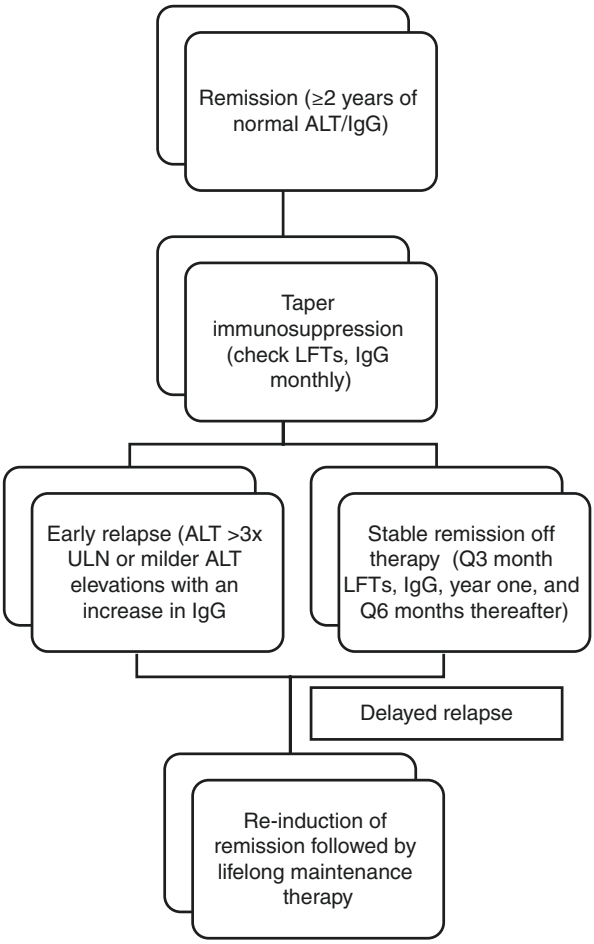

Fig. 5.3 Guidance for laboratory monitoring during withdrawal of maintenance therapy for autoimmune hepatitis (AIH)

with a subsequent flare [1]. Treatment withdrawal in patients with persistent inflammatory activity on biopsy (HAI > 3) should not be attempted since a relapse is essentially guaranteed to occur.

Relapse occurs in 50–90% of cases after drug withdrawal and is defined by an ALT > 3× ULN or milder ALT elevations with an increase in IgG [29, 30]. Most relapses occur within 12 months of stopping therapy but can occur years after stopping treatment. In a Dutch retrospective cohort, 59% of patients recurred within a year of stopping, a number which increased to 81% by 3 years [31]. Combination therapy, concomitant autoimmune disease and younger age at time of treatment withdrawal were associated with increased risk of relapse. Another study examined characteristics of 28 patients who had treatment successfully stopped [32]. After a median duration of treatment of 48.5 months (range 35–179), 15 patients (54%) remained in long-term remission after a median follow-up of 28 months (range 17–57 months). Patients who stayed in remission all had an ALT less than half the

ULN and an IgG level not higher than 1200 mg/dl when drug therapy was discontinued. After discussing the relative risks and benefits of withdrawing therapy and the critical importance of close monitoring for relapse, patients can be tapered slowly off therapy over a few months. Following transaminases and IgG during withdrawal is prudent and can help avert a significant flare, since drug therapy can be reintroduced promptly with a relapse. Checking transaminases and IgG every 3 months for the first year after drug discontinuation and then biannually thereafter is advisable. Relapse during or following treatment withdrawal typically does not require a step-up in therapy to second-line agents but may require resumption of corticosteroids and a treatment algorithm like induction therapy. Once a relapse occurs, treatment should be continued indefinitely.

Therapeutic Drug Monitoring

Azathioprine is a pro-drug of 6-mercaptopurine (6-MP) an intermediate metabolite which subsequently undergoes competing routes of metabolism by thiopurine methyltransferase (TPMT), xanthine oxidase (XO), and hypoxanthine phosphoribosyltransferase (HPRT). TPMT converts 6-MP to the inactive metabolite 6-methylmercaptopurine (6-MMP). Polymorphisms in the TPMT gene that result in reduced enzymatic activity of the TPMT protein, may to some degree predict toxicity of azathioprine by increasing 6-MP metabolism through HPRT to 6-thiogaunine (6-TGN) metabolites [33]. 6-TGNs underlie the anti-inflammatory properties of azathioprine but can also cause myelosuppression [34]. 11% of the population are heterozygous for polymorphisms causing intermediate or low TPMT activity, most commonly the 3A allele. 1 in 300 patients will be homozygous for a mutation associated with negligible TPMT activity and are at high risk for bone marrow suppression from azathioprine [35]. TPMT genotype and enzymatic activity tests are commercially available. Either test is recommended to determine the starting dose of azathioprine, since the difference between intermediate and normal enzymatic activity can result in a threefold difference in initial target dose to achieve therapeutic 6-TGN concentrations [36]. For example, an intermediate metabolizer should begin therapy with 25 mg of azathioprine and the target dose during induction and maintenance therapy may end up being less than 1 mg/kg/day. While TPMT testing provides reassurance, it does not obviate the need to monitor for toxicities on therapy, since studies are equivocal in demonstrating an ability of TPMT testing to reliably predict toxicity, particularly since patients with normal TPMT testing can still develop cytopenias on treatment [37, 38].

In addition to bone marrow toxicity, azathioprine can cause several types of hepatoxicity including an acute hepatocellular or predominantly cholestatic injury within the first year of therapy. Additionally, a chronic injury associated with nodular regenerative hyperplasia and sinusoidal obstructive syndrome is also well described. Monitoring the CBC and LFTs frequently during induction therapy for AIH and with any dose increases in AZA is critical to assess the safety of drug

Table 5.1 Azathioprine therapy-related toxicities

Azathioprine related toxicities	Comments
Hematologic	
Leukopenia	Myelosuppression can occur irrespective of TPMT activity, CBC monitoring required, macrocytosis also common
Thrombocytopenia	
Anemia	
Oncologic	
Lymphoma	Hepatosplenic T-cell lymphoma (young men)
Nonmelanoma skin cancer	Surveillance recommended
Gastrointestinal	Consider dividing dose or administering after meals
Nausea, vomiting, anorexia	Up to 23% of patients, typically after starting therapy
Pancreatitis	Associated with HLA-class II variants
Diarrhea	Rare, may occur with fever, skin rash and be associated with hypersensitivity reaction
Hepatic	
Acute hepatocellular	Linked to high levels of 6-MMP (>5000), resolves with discontinuation
Acute cholestatic	Presentation with jaundice and fatigue, rarely chronic, but can evolve to with vanishing bile duct syndrome
Non-cirrhotic portal hypertension	Nodular regenerative hyperplasia, sinusoidal obstructive syndrome, peliosis hepatitis
Neuromuscular	
Malaise, myalgias	

therapy. Patients on AZA can develop side effects including nausea, vomiting, and diarrhea which are not uncommon and can impact compliance. Pancreatitis with arthralgias, fevers, and a skin rash is an infrequent complication of therapy leading to discontinuation. When azathioprine therapy cannot be tolerated as a first-line agent (Table 5.1), a second line agent should be considered Interestingly, patients from one study who were intolerant of AZA did well after switching to 6-MP [39]. Alternatively, for mild AIH cases, low-dose steroid monotherapy is an option.

Azathioprine intolerance is not infrequent, and since adherence to therapy can be an issue, 6-TGN metabolite testing is a valuable resource. Undetectable 6-TGN levels are consistent with lack of adherence to therapy or less likely increased metabolism. For patients previously in remission who develop an acute transaminitis, checking 6-TGN metabolites can fulfill several objectives on top of assessing compliance. 6-TGN concentrations >220 pmol per 8×10^8 erythrocytes were shown to associated with remission in AIH patients, while a level >400 pmol correlates with bone marrow suppression [40]. A low 6-TGN level in the setting of adherence to therapy can trigger careful dose escalation of azathioprine up to 2 mg/kg/day prior to reaching for second-line agents. Lastly, high 6-MMP levels (>5000) seem to correlate with hepatoxicity from AZA.

Conclusion

First-line therapy for AIH is effective in most patients and requires careful monitoring for efficacy and safety. Predniso(lo)ne induction therapy with the addition of azathioprine is a predominantly effective strategy. Personalizing a treatment algorithm that balances the benefits of therapy with the risks of immunosuppression is desirable. Relapse with withdrawal of therapy is incredibly common and necessitates resumption of therapy. Overall, the success of first-line therapy makes treating AIH a satisfying experience for patients and providers.

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Chapter 6
Treating the Adult Patient: Alternative Drug Therapies

Paul A. Schmeltzer

Introduction

Conventional therapy for autoimmune hepatitis (azathioprine and prednisone) is continued until one of several endpoints is reached—remission, treatment failure, incomplete response, or drug toxicity. Remission is defined by the disappearance of symptoms, normalization of aminotransferases and γ globulin levels, and resolution of histologic abnormalities. Normalization of liver enzymes occurs in 66–91% of patients within 2 years [1]. Treatment failure is seen in at least 7% of patients and is manifested by clinical, laboratory, and histological worsening despite compliance with conventional therapy [2]. AASLD guidelines recommend high-dose prednisone monotherapy (60 mg daily) or combination therapy with prednisone 30 mg daily and azathioprine 150 mg daily for at least one month for treatment failures. EASL guidelines recommend increasing predniso(lo)ne to 60 mg daily and azathioprine to 2 mg/kg/day for at least one month for these patients if they do not exhibit liver failure. Failure to achieve remission after 36 months of treatment constitutes an incomplete response and occurs in 13% of patients. Both AASLD and EASL recommend long-term low-dose corticosteroids and azathioprine 2 mg/kg/day for the management of incomplete responders [3, 4].

Drug intolerance/toxicity to conventional therapy may also impact management decisions, including dose reduction of the offending drug or its discontinuation. Steroid side effects, most commonly Cushingoid features, can develop after receiving doses exceeding 7.5–10 mg daily for several months. Azathioprine can also be associated with a variety of side effects in 10–20% of patients, including bone marrow suppression, hepatotoxicity, pancreatitis, nausea and vomiting, rash, opportunistic infections, and malignancy [5]. Adherence to medical therapy is an issue for

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M. W. Russo (ed.), *Diagnosis and Management of Autoimmune Hepatitis*,
https://doi.org/10.1007/978-3-030-33628-8_6

many young patients with autoimmune hepatitis, and drug side effects can further hinder compliance and disease control.

A suboptimal response to conventional therapy and drug side effects has led to the use of a number of alternative agents to manage autoimmune hepatitis. These medications include budesonide, 6-mercaptopurine, mycophenolate mofetil, calcineurin inhibitors, m-TOR inhibitors, and biologics. There is a lack of high-quality data using these agents, which are considered empiric salvage therapies. Much of the experience with these medicines stems from their use in liver transplantation. This chapter will review the current literature supporting the use of second-line treatments.

Predictors of Poor Response

It is important to be cognizant of risk factors that may eventually lead to the use of second-line agents or liver transplantation. Notable pretreatment risk factors include younger age at onset, severe acute presentation, hyperbilirubinemia, and the presence of HLA DRB1∗03. A Model of End-Stage Liver Disease (MELD) score of ≥12 was >90% sensitive and 68% specific at identifying those likely to fail first-line therapy and more predictive than HLA status or other risk factors [2]. Regarding on-treatment response, failure to improve serum bilirubin or MELD-Na after 7 days of standard therapy has a high negative prognostic value [6].

Evaluating the Nonresponder

Prior to switching from conventional therapy to second-line agents for a suboptimal response, it is important to reevaluate the clinical situation. Failure to meet treatment goals could be due to the presence of coexisting liver diseases or noncompliance. Overlap with primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC) can be seen in 9% and 6% of AIH patients, respectively [7]. Variant syndromes should be considered if there is biochemical cholestasis, cholestatic changes on liver biopsy, or a history of inflammatory bowel disease. The AASLD recommends consideration of cholangiography to exclude PSC if there is no response to 3 months of steroid therapy [3]. More common concurrent liver diseases such NAFLD should be considered particularly if there has been significant weight gain on steroids. Medications, including supplements, should be reviewed to evaluate for drug-induced liver injury. In some cases, a repeat liver biopsy may be indicated to rule out competing diagnoses. The utility of monitoring azathioprine metabolites in patients with AIH is unclear, though inappropriately low 6-thioguanine (6-TGN) and 6-methylmercaptopurine (6-MMP) levels can be useful to confirm inadequate dosing or nonadherence (Table 6.1).

Table 6.1 Evaluating the nonresponder

Question	Action
1. Is the patient compliant with treatment?	Obtain a detailed history Verify pharmacy refills Check azathioprine metabolites
2. Has standard therapy been optimized?	Assess response to azathioprine 150 mg daily and prednisone 30 mg daily Check 6-thioguanine level
3. Is there another cause of liver disease?	MRCP to evaluate for PSC if there is biochemical cholestasis or a history of IBD Consider liver biopsy to rule out fatty liver disease or drug-induced liver injury if possible based on clinical presentation

Budesonide

Budesonide is a second-generation synthetic glucocorticoid with more than 90% hepatic first-pass clearance. Its pharmacokinetics thereby allows for antiinflammatory effects in the liver with fewer systemic side effects than predniso(lo)ne. The use of budesonide for AIH dates back to 1994, when a small sample of 13 patients were treated for 9 months with initial doses of 6–8 mg daily. Significant decreases of alanine aminotransferase (ALT) and immunoglobulin G (IgG) were seen after 6 weeks of treatment, and plasma cortisol levels remained in the normal range in noncirrhotic patients. Patients with biopsy-proven cirrhosis, however, had significantly reduced plasma cortisol [8]. The limitation of budesonide use in cirrhotics was further delineated in a 2003 study that examined serum levels of budesonide and its metabolites in a Child A cirrhotic who developed facial swelling, peripheral edema, and weight gain on budesonide 9 mg daily. Serum levels of budesonide were 13-fold higher in this patient compared to a matched control who did not have steroid side effects [9]. Budesonide is not recommended in patients with cirrhosis.

Budesonide has been shown to be effective as frontline therapy for AIH. A seminal article by Manns et al. from 2010 compared the effects of budesonide and azathioprine versus prednisone and azathioprine. This prospective multicenter randomized controlled trial included noncirrhotic AIH patients with aminotransferase levels ≥2 times higher than the upper limit of normal (ULN) and elevated levels of γ globulins or IgG. The treatment group received budesonide 3 mg three times a day or twice daily and azathioprine 1–2 mg/kg/d for 6 months. The control group was given prednisone 40 mg daily, which was tapered to 10 mg daily, and azathioprine 1–2 mg/kg/d for 6 months. The second phase of the study was an open-label phase where all patients received budesonide and azathioprine. The s primary endpoint of the study was complete biochemical remission (normal aminotransferase levels) without steroid-specific side effects. The primary endpoint was met in 47/100 (47%) of patients in the budesonide group and 19/103 (18.4%) patients in the prednisone group ($p < 0.001$). After 6 months of treatment, biochemical remission was achieved in 60% of the budesonide group and 38.8% of the prednisone group. Among 87 patients who initially received prednisone and switched to budesonide

during the open-label phase, steroid-specific side effects decreased from 44.8–26.4% ($p < 0.02$) at 12 months [10].

The Manns et al. study has been criticized for the lower than expected remission rate in the steroid group, the scheduled prednisone taper that was used, the short trial duration, and the lack of follow-up histology. Regardless, it is an often-cited study due to the lack of randomized controlled trials evaluating alternative AIH treatment. The AASLD AIH guidelines, which were also published in 2010, do not have specific recommendations regarding budesonide [3]. The more recent EASL guidelines from 2015 include budesonide 9 mg daily plus azathioprine for the initial treatment of noncirrhotic patients, especially if steroid side effects are anticipated. The guidelines do acknowledge that budesonide will not treat any extrahepatic immune-mediated diseases, and little is known about the preferred schedule to taper budesonide [4].

Since budesonide acts on the same corticosteroid receptor as predniso(lo)ne, one would expect that its role as second-line therapy would be limited. A recent single-center retrospective analysis evaluated 60 AIH patients who were switched from prednisolone to budesonide 9 mg daily due to the development of steroid side effects (30 patients) or prednisolone dependency (30 patients). Prednisolone dependency was defined by an inability to reduce the dose below 10 mg daily. The mean duration of prednisolone therapy before budesonide was 47 months, and the mean maintenance dose of prednisolone was 8.5 mg daily. Ten patients were given budesonide monotherapy at treatment initiation. Fifty patients were given budesonide with additional immunosuppression, including azathioprine (40 patients), mycophenolate mofetil (six patients), 6-MP (two patients), and prednisolone (two patients). Patients with overlap syndromes and cirrhosis were excluded. A response to treatment was defined as normal aminotransferases and IgG levels in accordance with AASLD and EASL guidelines. After 6 months of budesonide treatment, 50% of patients who were given budesonide because of prednisolone side effects achieved a response. A biochemical response of 60% was seen at 6 months in the 30 patients who were previously prednisolone dependent. Fifteen patients had baseline osteopenia and were followed with DEXA scans. Of those 15 patients, only one had a deterioration of the T-score on budesonide treatment. Seventeen patients (28%) were switched back from budesonide to prednisolone for a variety of reasons, including budesonide-associated side effects, AIH flares, extrahepatic autoimmune disease, or the development of cirrhosis [11]. While budesonide was shown to induce remission in some patients previously treated with prednisolone, its role in second-line therapy is limited, as evidenced by the number of patients who had to be switched back to prednisolone in this study. Consequently, AASLD and EASL guidelines do not include budesonide among second-line therapies.

Thiopurines

Side effects from azathioprine occur in 10–20% of patients. Azathioprine is nonenzymatically converted to 6-MP, the biologically active form of the drug, which then inhibits purine nucleotide synthesis. In 1996, Pratt et al. published a case series of

three patients with AIH who were successfully switched from azathioprine to 6-MP. One teenage patient did not achieve remission on azathioprine and prednisone but later achieved remission after starting 6-MP 75 mg daily. Two other patients developed intolerance to azathioprine (one had nausea, vomiting, and diarrhea, and the other had fever and arthralgia). Both patients' symptoms resolved on 6-MP 100 mg daily [12].

Data in patients with inflammatory bowel disease (IBD) have shown that 6-MP can be successfully used in patients with intolerance to azathioprine. A study by Lees et al. evaluated 61 patients with IBD who could not tolerate azathioprine due to side effects, including nausea and vomiting, flu-like illness, neutropenia, hepatotoxicity, and pancreatitis. Overall, 36 (59%) patients tolerated 6-MP at a median dose of 1 mg/kg. In terms of specific symptoms to azathioprine and those who tolerated 6-MP, 17/28 (61%) with nausea/vomiting, 11/18 (61%) with flu-like symptoms, and 3/3 (100%) with rash tolerated 6-MP [13]. Among 135 IBD patients with azathioprine intolerance, 70 (52%) tolerated a switch to 6-MP. Interestingly, 6-MP was particularly well tolerated in the setting of azathioprine hepatotoxicity with 12/17 (71%) patients tolerating 6-MP. The same held true for those who experienced arthralgia/myalgia with azathioprine with 68% tolerating 6-MP [14]. Of note, managing azathioprine hepatotoxicity is addressed by LiverTox, an online resource for information on drug-induced liver injury developed by the NIDDK. The site states that "some patients have tolerated switching therapy to mercaptopurine or thioguanine, but substitution with a structurally unrelated immunosuppressive agent is more appropriate" [15].

Patients treated with prednisolone and azathioprine can be successfully switched to 6-MP. Seventy-five percent of patients respond; of those, 53% have a complete response and 47% have a partial response [16].

The AASLD briefly mentions using 6-MP at a dose of 1.5 mg/kg/day in children with AIH without supporting evidence [3]. EASL guidelines comment on using 6-MP in the setting of azathioprine intolerance and reference the abovementioned Pratt case series [4]. This author feels that a trial of 6-MP is reasonable with gastrointestinal symptoms, flu-like symptoms, rash, or arthralgia attributed to azathioprine, but its use should be avoided after more serious side effects such as hepatotoxicity or pancreatitis.

6-thioguanine (6-TG) is approved for use in the treatment of acute and chronic myeloid leukemia and chronic lymphocytic leukemia. It has also been used in IBD patients with insufficient response or intolerance to azathioprine or 6-MP. It has been used in AIH patients with azathioprine intolerance or nonresponse, though the data are limited. In theory, its use could be beneficial since there is one-step metabolism from 6-TG to the pharmacologically active 6-TG nucleotides without the formation of potentially toxic 6-methyl MP metabolites. A single-center retrospective study examined 17 patients who were treated with 6-TG as a second-line agent for either azathioprine intolerance/failure (11 patients) or as a third-line agent after intolerance/failure with azathioprine and subsequently MMF (six patients). Sixteen patients normalized their aminotransferases within 3 months. Eleven (64%) patients maintained a biologic response (defined by normal aminotransferases after steroid withdrawal). Two patients discontinued treatment due to side effects. There was one

patient who had evidence of nodular regenerative hyperplasia on a liver biopsy after receiving 6-TG for 10 months [17]. A multicenter retrospective study from the Netherlands used 6-TG therapy with a median dose of 20 mg daily in 52 patients with AIH or AIH variants who did not respond to or were intolerant to conventional thiopurines. Thirty-eight patients were treated with 6-TG because of intolerance to azathioprine or 6-MP. Twenty-four of these patients achieved a complete biochemical remission, four had an incomplete response, and one had no response. Nine patients discontinued treatment. Seven of 11 patients with an incomplete response to azathioprine or 6-MP were responsive to 6-TG. Three had a complete response, and four had an incomplete biochemical remission. 6-TG was effective as first-line maintenance in three patients. Twenty-eight liver biopsies were performed on ten patients on 6-TG treatment. In this cohort, there were no reported cases of nodular regenerative hyperplasia [18]. 6-TG can be considered for individuals intolerant or unresponsive to azathioprine or 6-MP.

Low-dose allopurinol has been used as a strategy to shift thiopurine metabolism away from the production of 6-MMP metabolites and toward the metabolically active 6-TG nucleotides. A retrospective case series of eight adult AIH patients with intolerance to azathioprine/steroids (4/8) or 6-MP/steroids (4/8) reported complete biochemical remission in 3/3 intolerant patients and 4/5 unresponsive patients [19]. Close monitoring of blood cell counts is advised if using allopurinol in combination with thiopurines due to the possibility of severe myelosuppression (Fig. 6.1). Due to the risk of toxicities, allopurinol is rarely used.

Mycophenolate Mofetil

Mycophenolate mofetil (MMF) inhibits inosine monophosphate dehydrogenase, the rate-limiting enzyme in de novo purine synthesis. It has supplanted the use of azathioprine in organ transplant patients who require multidrug regimens. It is also used as a steroid-sparing agent in a variety of rheumatologic diseases. Many of the studies evaluating MMF as frontline and second-line therapy for AIH have been

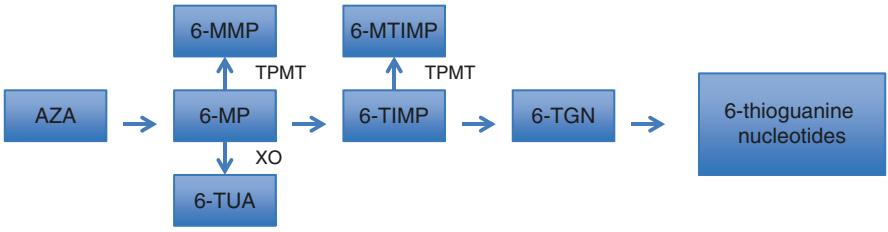

Fig. 6.1 Azathioprine metabolism. AZA = azathioprine, 6-MP = 6-mercaptopurine, 6-MMP = 6-methylmercaptopurine, 6-TUA = 6-thiouric acid, 6-TIMP = 6-thioinosine-monophosphate, 6-MTIMP = 6-methylthioinosine-monophosphate, 6-TGN = 6-thioguanine, TPMT = thiopurine methyltransferase, XO = xanthine oxidase

limited by study design (retrospective, single-center case series/reports), heterogeneous study populations (treatment naïve versus steroid refractory versus azathioprine intolerant), and variable definitions of response/remission. Despite the lack of randomized controlled trials, the AASLD guidelines regard MMF as "the most promising current agent" for those who fail standard treatment [3].

A prospective though uncontrolled study assessed the efficacy and safety of MMF in inducing and maintaining remission in 59 treatment-naïve AIH patients. Twenty-four percent of patients had cirrhosis at presentation. The MMF dose was titrated to 1.5–2 grams daily in divided doses. Prednisolone was also given at a dose of 0.5–1 mg/kg/d and then gradually tapered based on the clinical and biochemical response. Fifty-two out of 59 (88%) patients achieved biochemical remission (normal aminotransferases and γ-globulin levels); most of them (69%) did so within 3 months of treatment initiation. Prednisolone was withdrawn in 34/59 (58%) patients within 8 months, though only 37% were able to maintain remission off steroids. Serious adverse events were seen in two (3.4%) patients; both of them had septicemia and underlying cirrhosis. The authors concluded that MMF was effective and safe as frontline therapy for AIH but recommended randomized controlled trials to directly compare MMF and steroids to conventional therapy [20]. An ongoing clinical trial has been designed to compare the efficacy of MMF and azathioprine in treatment-naïve patients (ClinicalTrials.gov, NCT02900443).

As mentioned earlier, studies investigating the efficacy of MMF as second-line therapy have been flawed. Biochemical remission or improvement in aminotransferases in 31–73% of subjects has been reported [21, 22]. The rate of MMF discontinuation due to side effects has ranged from 13% to 34% [23, 24]. Common MMF side effects include nausea, vomiting, diarrhea, and bone marrow suppression. MMF is more effective for azathioprine intolerance than for nonresponse [23, 25, 26].

A retrospective study of 105 patients with AIH conducted at 17 liver clinics in Australia reported the experience with MMF in patients intolerant (60%) or who did not respond (40%) to azathioprine. Sixty-three patients (60%) achieved biochemical remission (normal aminotransferases and IgG levels) after a median of 12 weeks of treatment. The percentage of patients who achieved remission for nonresponse to conventional therapy (57%) was similar to the percentage (62%) that were intolerant. Notably, the patients with cirrhosis fared worse with a remission rate of 47% compared to 66% in those without cirrhosis. In addition, serious adverse events occurred in three patients, all of whom had cirrhosis. There was one death in a patient with Child-Pugh C cirrhosis who was taking MMF 1 g twice daily and prednisolone 12.5 mg/day. This patient developed severe sepsis from necrotizing fasciitis. Two other cirrhotic patients had infectious complications, including a dental abscess requiring drainage and methicillin-sensitive *Staphylococcus aureus* bacteremia. This is the largest study to date evaluating MMF as rescue therapy in adults and included a relatively high number of patients with cirrhosis (37%). Unlike some earlier trials, the response rate was similar regardless of the indication for MMF. Given the lower response rate and risk of infection, caution is advised in using MMF in patients with cirrhosis [27]. Other considerations regarding the choice of MMF as an alternative AIH treatment include its cost (six to seven times

more expensive than azathioprine) and its potential role as a teratogen during pregnancy [1]. MMF is classified as a category D drug in pregnancy by the United States Food and Drug Administration. Prior to using it in women of reproductive age, there should be a documented discussion about the risk of spontaneous abortion and birth defects such as severe cranial, facial, and cardiac abnormalities. At risk patients should be advised to use two forms of birth control and have periodic urine pregnancy tests.

Calcineurin Inhibitors

The calcineurin inhibitors (CNIs) are another family of immunosuppressive agents, which have been used to treat AIH. Familiarity with their use has been gained through solid organ transplant experience where they are the backbone of standard immunosuppressive regimens. Cyclosporine has been used as frontline and salvage treatment for AIH since 1985. It acts by binding to a family of cytoplasmic proteins called cyclophilins. That complex then competitively binds to calcineurin, which is a phosphate that activates nuclear factors. The complex inhibits interleukin (IL)-2 production and lymphocyte proliferation. Tacrolimus, which has been used to treat AIH since 1995, binds to the cytoplasmic FK-binding proteins, which then leads to the same downstream effect as cyclosporine. Much like the experience with MMF, data using CNIs is limited, and their use for AIH is empiric and off-label [28]. AASLD guidelines mention that "mycophenolate mofetil or cyclosporine have had the most empiric use as alternative medications," though CNI use is not strongly endorsed by the AASLD or EASL [3, 4].

Cyclosporine has been used at doses of 2–5 mg/kg/d with target trough levels between 100 and 300 ng/mL. Composite results of ten reports involving 133 patients over 26 years has shown a positive response of any degree in 93% of patients [29]. One representative open-label study by Malekzadeh et al. studied a population of nine treatment-naïve and ten steroid refractory subjects. They were treated with cyclosporine 2–5 mg/kg/day in divided doses for 26 weeks. The goal cyclosporine trough level was 100–300 ng/mL. There was a significant improvement in ALT levels (455–79 IU/L), and the mean histologic activity index (HAI) decreased from 15.2 to 7.1 ($p < 0.005$). Side effects were common, including four subjects with paresthesias and three with gingival hyperplasia. Renal insufficiency is a common side effect of CNIs, though there was only a nonsignificant increase in creatinine in this study [30].

Tacrolimus is a newer and more potent CNI than cyclosporine. In the transplant community, it has replaced cyclosporine as the preferred first-line immunosuppressant. In AIH studies, tacrolimus has been used at doses of 0.5 mg/day to 3 mg twice a day. Composite data evaluating tacrolimus for AIH treatment included three reports involving 41 patients over 16 years. The cumulative overall positive response was 98% [29]. A literature review of 162 adult patients with AIH treated with tacrolimus (dose 1–8 mg/day, trough level 0.5–10.7 ng/mL) demonstrated a biochemical

response (defined as normal aminotransferases) of 74.7%. Tacrolimus was discontinued in 17.3% of subjects for various reasons, though renal function was reported to have remained stable in most patients [31].

An international multicenter study retrospectively evaluated the efficacy and safety of MMF and tacrolimus in 201 patients who were nonresponsive or intolerant to conventional therapy. One hundred eight patients had a complete response to standard therapy but were switched to second-line therapy due to side effects of steroids or azathioprine. Ninety-three patients were switched to second-line therapy because they were nonresponders. One hundred twenty-one subjects received MMF (dose of 0.5–2.0 g/d) and 80 received tacrolimus (dose of 1–8 mg/d) for a median of 62 months. A complete biochemical response was defined as normalization of aminotransferases and IgG levels at any time within 6 months of treatment initiation. Overall, the complete response rate was similar between the MMF group (69.4%) and the tacrolimus group (72.5%) ($p = 0.639$). In the group that was intolerant to standard therapy, the biochemical remission rate was similar in the MMF treated patients (91.9%) and the tacrolimus treated ones (94.1%). However, in those who were intolerant to standard therapy, tacrolimus had a significantly higher remission rate than MMF (56.5% vs. 34%, respectively; $p = 0.029$). Ten patients who received MMF (8.3%) and ten patients who received tacrolimus (12.5%) discontinued therapy due to side effects. The rates of liver-related death or transplantation were similar between the MMF group (13.2%, $n = 15$) and the tacrolimus group (10.3%, $n = 9$) [32]. Tacrolimus has a similar side effect profile to cyclosporine, though it has fewer cosmetic side effects (e.g., hirsutism, gingival hyperplasia). Given that MMF is a teratogen and cyclosporine has cosmetic side effects, tacrolimus does have some added appeal as a second-line agent for AIH, particularly in adolescents and women of child-bearing age (Table 6.2).

Table 6.2 Most commonly used second-line medications

Drug	Mechanism of action	Dose	Potential side effects	Clinical experience
Mycophenolate mofetil	Inhibits purine synthesis	0.5–3 g/day in divided doses	Nausea, vomiting, diarrhea, leukopenia	Biochemical remission rate 31–73% May be more effective for intolerance rather than nonresponse category D for pregnancy
Cyclosporine	Calcineurin inhibitor	2–5 mg/kg/day in divided doses	Nephrotoxicity, neurotoxicity, hypertension, hirsutism, gingival hyperplasia	Positive response of any degree = 93%
Tacrolimus	Calcineurin inhibitor	0.5 mg/day to 3 mg bid	Nephrotoxicity, neurotoxicity, hypertension	Positive response of any degree = 98% Fewer cosmetic side effects than cyclosporine

mTOR Inhibitors

Sirolimus and everolimus are immunosuppressive medications that act by inhibiting the mammalian target of rapamycin (mTOR), a regulatory protein involved in the proliferation and survival of activated lymphocytes. mTOR inhibitors are used in organ transplant where they are often part of a renal-sparing regimen to limit CNI nephrotoxicity. Potential side effects include stomatitis, proteinuria, hyperlipidemia, impaired wound healing, bone marrow suppression, and pulmonary toxicity. Interstitial pneumonitis is a potentially severe side effect and necessitates immediate discontinuation of the mTOR inhibitor, though most cases will improve within 3 weeks of stopping the drug [33].

The first reported use of sirolimus for AIH was in pediatric patients with de novo or recurrent AIH post liver transplant. Kerkar et al. published their experience involving six patients, five of whom were nonresponsive to increased steroids, the addition of azathioprine or MMF, and a CNI. Sirolimus was used at a dose of 1–3 mg daily with a goal trough level of 5–8 μg/dL MMF, or azathioprine was discontinued a week after sirolimus was started. All patients responded to the addition of sirolimus with a significant reduction of ALT and IgG levels. Minimal side effects were noted in three patients [34].

The experience using sirolimus in posttransplant patients with AIH has led to its use in adults with difficult-to-treat AIH. A small series by Chatrath et al. studied five adults who were refractory to treatment with prednisone and azathioprine. Three of the patients also failed a trial of MMF. The starting sirolimus dose was 2 mg daily, and trough levels of 10–20 ng/dL were targeted. Four patients had at least a 50% reduction of their ALT, and 2/5 normalized their ALT. All patients had a significant reduction in steroid use. Side effects included high cholesterol and triglycerides [35].

The data on everolimus as rescue therapy are even more limited. One small study of seven adults used everolimus for intolerance or nonresponse to standard therapy, MMF, and CNI use. The starting dose of everolimus was 0.75–1.5 mg twice daily for goal trough levels of 3–6 ng/mL. After 3–5 months of treatment, three patients had normal ALT (10–45 IU) levels and four had ALT levels <55 IU. Only minor side effects were reported, which did not require drug discontinuation [36]. AASLD guidelines do not mention mTOR inhibitors as an option for rescue therapy [3]. While EASL guidelines reference two of the above sirolimus studies, the authors state that "no strong recommendations can be drawn from such small sample sizes" [4].

Rituximab

Rituximab is a chimeric murine-human antibody that binds to the CD-20 antigen on the surface of B-lymphocytes, promoting depletion and the suppression of pathogenic antibodies. It was approved in 1997 and is used for the treatment of non-Hodgkin's lymphoma, chronic lymphocytic leukemia, rheumatoid arthritis, and ANCA-associated vasculitis. There are case reports of AIH patients who received

rituximab for coexisting autoimmune or hematologic diseases, including mixed cryoglobulinemic glomerulonephritis in a patient with cirrhotic-stage AIH [37], marginal B-cell lymphoma [38], idiopathic thrombocytopenic purpura (ITP) [39], and Evans syndrome (hemolytic anemia and ITP) in a patient with PBC-AIH overlap [40]. These patients did experience significant improvement of their liver disease with rituximab.

There is one single-center open-label study in the literature that used rituximab in six patients with AIH. Three patients had intolerable side effects with prednisone and/or azathioprine, and the other three were refractory to standard therapy with aminotransferase elevations >2× the ULN after a minimum of 6 months. These patients were administered two doses of rituximab 1000 mg as an IV infusion 2 weeks apart. They remained on stable doses of azathioprine. Prednisone was continued at stable doses for at least 3 months following rituximab, at which point a taper was allowed if biochemical remission was achieved. All subjects had improved aminotransferases by week 12; the improvement in AST reached statistical significance by week 24. Rituximab was well tolerated, and no patients had infusion reactions or serious adverse events during 72 weeks of follow-up [41].

Due to the paucity of data, neither the AASLD nor the EASL endorses rituximab as a second-line agent for AIH [3, 4]. It is also important to mention that patients require screening of their HBsAg and anti-HBc prior to starting rituximab. The risk of HBV reactivation is >10% for both HBsAg-positive and anti-HBc-positive patients [42], which warrants antiviral prophylaxis. Because of limited date for its use in AIH and the potential for HBV reactivation, rituximab is an agent that should only be used in specialized centers.

Infliximab

Infliximab is a recombinant humanized chimeric antibody that is used for the treatment of autoimmune diseases, including ulcerative colitis, Crohn's disease, rheumatoid arthritis, psoriatic arthritis, plaque psoriasis, and ankylosing spondylitis. It acts by neutralizing soluble tumor necrosis factor-alpha and also has proapoptotic and antiproliferative effects on lymphocytes. Similar to rituximab, the data examining the use of infliximab for AIH consists of a few case reports and one small case series.

One case report described a 17-year-old female with biopsy-proven AIH who was subsequently diagnosed with adult Still's disease. Her symptoms and liver dysfunction initially improved with prednisolone and methotrexate but flared after prednisolone was tapered. Infliximab 3 mg/kg was added to this regimen, and her clinical course (symptoms, ALT, ferritin) improved [43]. A second case report involved a 36-year-old woman who presented with acute liver injury due to autoimmune hepatitis and did not respond to high-dose steroids. Infliximab 5 mg/kg was used to successfully induce remission, which was subsequently maintained with azathioprine and prednisone [44].

Table 6.3 AASLD vs. EASL guidelines for AIH treatment

Drug	AASLD	EASL
AZA/prednisone dose for treatment failure	Prednisone 60 mg daily or prednisone 30 mg/d + AZA 150 mg/d	Predniso(lo)ne 60 mg/d + AZA 2 mg/kg/d
Budesonide	No recommendations	Induction treatment option for noncirrhotics
6-MP	1.5 mg/kg/d an induction option for children	"alternative option" for AZA intolerance
MMF	2 g/d "most promising current agent"	"second line drug of choice"
Cyclosporine	"most empiric use" along with MMF	No recommendations
Tacrolimus	No recommendations	No recommendations
mTOR inhibitors	No recommendations	"no strong recommendations"
Rituximab	No recommendations	No recommendations
Infliximab	No recommendations	No recommendations

A retrospective case series from Germany evaluated infliximab in 11 adults with difficult-to-treat AIH. Six patients had intolerance to azathioprine, and the other five did not achieve remission with standard therapy. Seven patients had cirrhosis at the time of infliximab initiation. The infliximab dose used was derived from the inflammatory bowel disease literature—5 mg/kg at time zero, two weeks, six weeks and then every 4–8 weeks for a minimum of 6 months. Normal aminotransferases were achieved in eight patients, and six patients had normal IgG levels. Mean AST pretreatment was 475 U/L ± 466 and decreased to a mean of 43 U/L ± 32 during treatment. Seven patients had infectious complications, and three patients discontinued treatment [45]. In addition to concerns about paucity of data, infliximab has been reported to cause drug-induced AIH [46]. For these reasons, its use has not been endorsed in liver society guidelines. Table 6.3 lists the differences between AASLD and EASL guidelines regarding AIH treatment. Figure 6.2 is a proposed treatment algorithm.

Investigational Agents

There are several therapeutic agents under investigation that target key components of the immune system. JKB-122 (TaiwanJ Pharmaceuticals Co., Ltd) is a toll-like receptor 4 (TLR4) antagonist that is currently being studied in a phase 2 pilot study in AIH patients with intolerance or resistance to standard therapy (ClinicalTrials.gov, NCT02556372). It is thought that intestinal dysbiosis is heightened with AIH. Increased bacterial translocation can produce higher levels of plasma lipopolysaccharide (LPS). LPS can then activate the TLR4 of Kupffer cells and hepatic

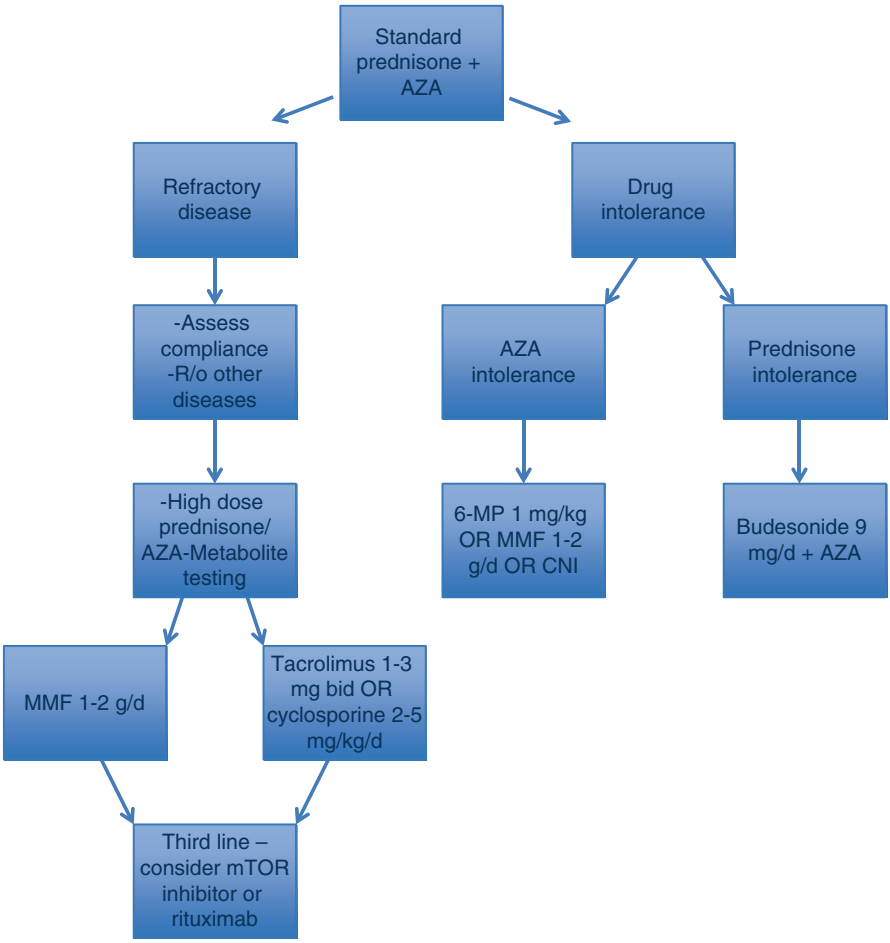

Fig. 6.2 Proposed treatment algorithm

stellate cells, leading to the production of proinflammatory cytokines and hepatic fibrosis.

Another agent, VAY736 (Novartis Pharmaceuticals), acts by targeting the B-cell activating factor (BAFF) receptor, a member of the TNF superfamily. This then leads to B-cell depletion. Higher BAFF levels have been reported in AIH patients compared to patients with other chronic liver diseases and healthy controls. There is an ongoing multicenter, randomized, double-blind placebo-controlled study using VAY736 in AIH patients with an incomplete response or intolerance to first-line therapy (ClinicalTrials.gov, NCT03217422).

Another area of focus is regulatory T cells (Treg), which have been shown to be deficient in number and function in AIH patients. Low-dose IL-2 is being studied as a Treg inducer in various autoimmune diseases, including AIH (ClinicalTrials.gov,

NC01988506). Additionally, Tregs are being modified ex vivo and then infused back to the patient in a process called adoptive cell transfer (ACT). An ongoing phase 1 trial is assessing the safety and efficacy of ACT in children and adults with AIH (ClinicalTrials.gov, NCT02704338) [47].

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Chapter 7
The Approach to the Pediatric Patient

Vani V. Gopalareddy

Autoimmune Hepatitis

AIH is an inflammatory condition affecting the liver that can present as an acute hepatitis, chronic hepatitis, fulminant liver failure, or end-stage liver disease. In children, similar to adults, the most common features of autoimmune hepatitis are elevated immunoglobulin/IgG level, positive autoantibodies, and interface hepatitis on histology [1].

The prevalence of juvenile autoimmune hepatitis is unknown. Incidence seems to be increasing likely due to the increased awareness of this condition/possible true increase in incidence. The clinical presentation of autoimmune hepatitis in children can be different from that in adults. It is more aggressive in children compared to adults. It is not uncommon to see advanced fibrosis at presentation in pediatric autoimmune hepatitis. If untreated, AIH evolves to end-stage liver disease rapidly.

Diagnostic criteria for autoimmune hepatitis are based on a combination of clinical, laboratory, and histologic features in the setting of no identifiable causes for the liver disease. Viral hepatitis (A, B, C, E, EBV), Wilson's disease, nonalcoholic steatohepatitis, and drug-induced liver disease should be excluded. Liver biopsy is needed to confirm the diagnosis and to stage fibrosis [5]. The autoimmune hepatitis scoring system is not suitable for the juvenile form of the disease, especially with fulminant hepatic failure presentation [6, 7].

Juvenile autoimmune hepatitis (AIH) is divided into two types according to the autoantibody profile: AIH type 1 is characterized by positive antinuclear (ANA) and/or antismooth muscle (SMA) antibody. AIH type 2 is positive for anti-liver kidney microsomal antibody (anti-LKM) or antisoluble liver cytosol (anti-SLC)

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M. W. Russo (ed.), *Diagnosis and Management of Autoimmune Hepatitis*,
https://doi.org/10.1007/978-3-030-33628-8_7

autoantibodies rarely described in adults. Scoring systems for autoimmune hepatitis diagnosis in adults are not applicable to pediatric patients. A scoring system for the diagnosis of autoimmune liver disease in children is proposed for testing and validation. Autoantibodies can be present in much lower titers, and the IgG level can remain normal in pediatric autoimmune hepatitis. About 15% of children with AIH type 1 and 25% of children with AIH type 2 have normal IgG levels [2].

In both types, there is a female preponderance, but type 2 AIH can present at a younger age and also during infancy. Type 2 AIH can be part of the autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) syndrome, in which 20% of the patients may have liver disease [3]. IgA deficiency is common in AIH type 2 [2].

Pathologic Features

The typical histological feature of autoimmune hepatitis is interface hepatitis, which is characterized by a dense inflammatory infiltrate composed of lymphocytes and plasma cells, which crosses the limiting plate and invades the surrounding parenchyma. The presence of a less dense inflammatory infiltrate does not exclude the diagnosis of AIH. In the pediatric cohort, histology of patients with autoimmune liver disease was compared with that of non-autoimmune liver disease, and typical histology comprising interface hepatitis, portal lymphoplasmacytic infiltrate, rosette formation, and emperipolesis was observed in 56% of patients with autoimmune liver disease [9]. In pediatric patients with AIH, the findings 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 immunohistochemical analysis, correlating with a >2-fold increase in serum IgG level [10]. Histology also evaluates the extent of fibrosis and identifies overlap syndromes and possible presence of concomitant diseases such as nonalcoholic fatty liver disease [11].

Classic histological features of autoimmune hepatitis may not be evident in acute liver failure from AIH. Hence, absence of histology should not preclude prompting initiation of immunosuppressive treatment in this setting. However, liver biopsy should be performed as soon as coagulation indices improve. Changes may be noted predominantly in the centrilobular area [11] often with massive necrosis and multilobular collapse.

Treatment

Remission is defined as complete clinical recovery with normal transaminase levels. Now the push is to achieve normalization of IgG levels, negative autoantibodies, and histological resolution of inflammation [14].The histological remission lags

behind the biochemical remission. Children may achieve histological improvement after a mean duration of 4 years of effective treatment [17].

Relapse is defined as an increase in transaminase levels after remission was achieved. Relapse during treatment is commonly occurring in half of the patients, requiring a temporary increase in steroid dose or a change of immunosuppression. Small daily doses of steroids are effective in maintaining disease control and in minimizing the need for high-dose steroid pulses during relapses and do not affect final height [18].

When to Treat

AIH in childhood is more aggressive than in adults. Cirrhosis is present in 44–80% of children at the time of initial diagnosis [13, 16, 17]. It is very responsive to immunosuppression, and treatment should be initiated promptly to avoid the progression of the disease. The goal of treatment is to reduce liver inflammation, induce remission, resolve symptoms, and prolong life expectancy with native liver [14, 19, 20]. Most children remain clinically stable and well on long-term treatment.

How to Treat (Fig. 7.1)

AIH responds to standard treatment with a reported remission rate of up to 90%, with the exception of fulminant presentation with encephalopathy, cirrhosis with decompensation.

Standard Treatment

Conventional treatment of AIH consists of *prednisolone or prednisone* 2 mg/kg/day (max of 60 mg/day) and gradually weans over 1–2 months to a minimum maintenance dose of 2.5–5 mg a day (Table 7.1). The majority of patients improve with almost normalizing transaminases within 2 months of treatment, but in some cases it may take several months. Transaminases should be monitored closely in the first few weeks of treatment so steroids can be weaned accordingly.

The addition of maintenance treatment with a steroid-sparing agent is the standard of care for long-term sustained remission. Over 85% of pediatric patients require the addition of azathioprine; it is added either in the beginning, along with steroids, or 2–3 weeks later once the bilirubin and transaminases improve on steroid treatment. Azathioprine is typically added at a dose of 0.5–2 mg/kg/day. Azathioprine is rarely used alone for both induction and maintenance [4].

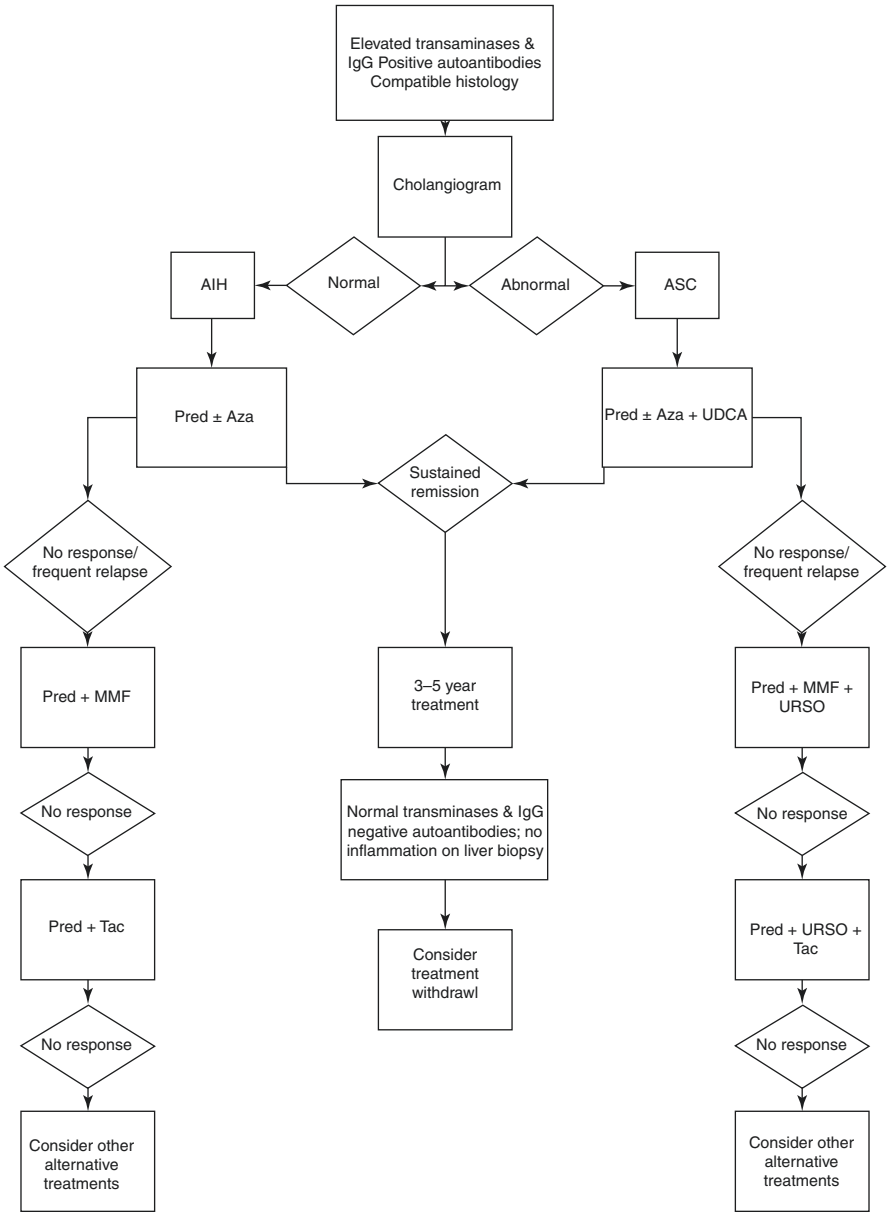

Fig. 7.1 Proposed algorithm for managing pediatric patients with autoimmune hepatitis

Measurement of thiopurine methyltransferase (TPMT) activity level before initiating azathioprine therapy is helpful to minimize toxicity; however, advanced fibrosis but not TPMT genotype activity was noted to be associated with azathioprine toxicity in AIH [21]. Ideal therapeutic levels for AIH have not been deter-

Table 7.1 Immunosuppression for autoimmune liver disease in childhood

	Induction	Maintenance	
AIH	Prednisone 2 mg/kg/day, decreased gradually to a minimum maintenance dose of 2.5–5 mg/day	Prednisolone 0.1–0.2 mg/kg/day	AZA monotherapy (AIH-1) 1.2–1.6 mg/kg/day
	Add azathioprine 1–2 mg/kg after 2 weeks of steroids	with	
		AZA 1–2 mg/kg/day	
ASC	Prednisone/AZA as above with UDCA 15 mg/kg/day	Prednisone/AZA as above with UDCA 15 mg/kg/day	

AIH autoimmune hepatitis, *ASC* autoimmune sclerosing cholangitis, *AZA* azathioprine, *UDCA* ursodeoxycholic acid

mined; however, a low-range 6-TGN level from 50 to 250 pmol on a dose of 1.2–1.6 mg/kg/day was reported to maintain sustained biochemical remission [22]. Remission can also be maintained on monotherapy with low-dose azathioprine in AIH [22].

Alternative Treatments: For Induction of Remission

Cyclosporine monotherapy dosed at 4 mg/kg/day in three divided doses to achieve a blood concentration of 250 ± 50 ng/mL for 3 months to induce remission, followed by the introduction of prednisone and azathioprine, has been successful [23].

Tacrolimus has anecdotally been used to induce remission in adults and children who failed conventional therapy. Target tacrolimus trough levels were 2.5–5 ng/ml [24].

Budesonide, a drug with hepatic bypass clearance of over 90% of the oral dose and fewer side effects than prednisolone, has not been found to be beneficial in juvenile AIH [12].

Whether induction with alternative medications as opposed to the standard treatment with prednisolone-azathioprine has any advantage needs to be studied in controlled trials.

Alternative Treatments: For Refractory Cases

Mycophenolate mofetil (MMF), the pro rug of mycophenolate acid, has been used as an alternative treatment to induce and/r maintain remission in refractory cases or in those who do not maintain sustained remission on azathioprine. It has also been effective in those intolerant to azathioprine when used at a dose of 20 mg/kg twice a day, together with prednisolone [25].

Sirolimus, a drug that selectively expands regulatory T cells, has been used in a few patients with refractory AIH with short-term beneficial effect [26].

Rituximab, an anti-B lymphocyte monoclonal antibody, has been successfully used in children with refractory AIH [27]; however, it can cause prolonged lymphopenia and IgG deficiency.

Infliximab has been reportedly used as a rescue treatment in the pediatric case [28]; again, it has potential infectious side effects and hepatotoxicity. It has also been documented to induce autoimmune hepatitis in adults and children with inflammatory bowel disease; hence, we need to understand the role of TNF alpha in AIH pathogenesis before recommending its use.

Autoimmune Sclerosing Cholangitis

An overlap syndrome between autoimmune hepatitis (AIH) and sclerosing cholangitis (ASC) is more common in children than in adults. Fifty percent of the patients with the ASC are male, and over 75% of the patients have positive pANCA. ASC is frequently diagnosed and treated as AIH type 1, and the presence of sclerosing cholangitis may be discovered during follow-up after the appearance of a cholestatic biochemical profile. The parenchymal liver damage in ASC responds well in terms of normalization of chemistries to immunosuppressive treatment used for AIH; however, the bile duct disease may progress in about 50% of the patients despite treatment, particularly in those with associated difficult-to-control IBD. Ursodeoxycholic acid (UDCA) added to immunosuppression treatment in the dose of 12–15 mg/kg/day has been reported to be beneficial [8].

IBD associated with ASC may represent a distinct entity different from classic ulcerative colitis and Crohn's disease being characterized by right-sided colitis with frequent rectal sparing, small bowel mucosal breaks on capsule enteroscopy [29].

Liver Transplantation for Pediatric Autoimmune Liver Disease

Orthotopic liver transplantation (OLT) is a treatment option for AIH and ASC patients with end-stage liver disease, liver malignancy, intractable pruritus, or acute liver failure unresponsive to steroid treatment. AIH accounts for 2–5% of pediatric liver transplants performed in Europe and in the United States [5, 30]. Sclerosing cholangitis accounts for 2–3% of liver transplant performed in pediatric patients [30].

Despite good outcomes after liver transplantation for AIH, the disease can recur in the allograft despite immunosuppression. The reported incidence is variable, ranging from 38% to 83% with a five-year interval from OLT [15, 31, 34]. The diagnosis of recurrent AIH is based on the elevation of transaminases and IgG levels, the presence of autoantibodies, and histology suggestive of interface hepa-

titis, along with response to prednisone and azathioprine [5, 35]. Most patients with recurrent AIH posttransplant respond to reintroduction of steroids and azathioprine.

Treatment failures may respond to addition of MMF in place of azathioprine or replacement of tacrolimus with sirolimus.

Recurrence of sclerosing cholangitis after OLT has been reported in 10–50% of the recipients. Risk increases with time post Orthotopic Liver transplantation (OLT). The diagnosis of recurrent sclerosing cholangitis is suggested by histological and cholangiography findings of bile duct disease. No established treatment for recurrent sclerosing cholangitis is available. If dominant strictures are noted, they should be dilated whenever possible [36]. Ursodeoxycholic acid has been used, but the impact on outcome is unknown.

De novo autoimmune hepatitis after OLT in pediatrics ranges from 2% to 6% [32, 33, 37, 38]. It is important to exclude other causes for graft dysfunction, including rejection, hepatic artery thrombosis, and infection. These patients develop a form of graft dysfunction with features identical to those of classical autoimmune hepatitis. Treatment with prednisolone or combining it with azathioprine or MMF is successful. The importance of maintenance therapy with steroids in de novo autoimmune hepatitis was shown in a study, where in all steroid untreated patients developed cirrhosis and either died or required retransplantation, and none of the steroid treated patients had progressive disease [39].

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Chapter 8
Autoimmune Hepatitis and Pregnancy

Claire Meyer

Introduction

Women comprise 70–75% of those affected by autoimmune hepatitis [1], and for many of those diagnosed before or during the childbearing years, management of their liver disease in the context of pregnancy is an essential part of their longitudinal care. Physicians need to consider both the effect of the disease (and medications used to treat it) on the pregnancy, and the effect of the pregnancy on the disease. This chapter focuses on aspects of management specific to autoimmune hepatitis; for those patients whose disease has progressed to cirrhosis, there are additional considerations related to advanced liver disease and portal hypertension in pregnancy, which are beyond the scope of this review.

Pre-conception Counseling

Historically, autoimmune hepatitis has been associated with reduced fertility [2]. However, a recent population-based study in Denmark showed no difference in the age at first birth between women with autoimmune hepatitis and controls, suggesting that fertility in women with autoimmune hepatitis is not significantly impaired [3]. For patients planning a pregnancy, optimal timing may be when the disease has been well controlled for at least 1 year, given the improved fetal outcomes observed compared to those not in remission [4]. Due to its teratogenicity,

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M. W. Russo (ed.), *Diagnosis and Management of Autoimmune Hepatitis*,
https://doi.org/10.1007/978-3-030-33628-8_8

mycophenolate mofetil should be avoided in women of childbearing potential or discontinued at least 6 weeks prior to conception [5]. To stratify risk, checking anti-Ro (SSA) and anti-SLA/LP antibodies can be considered, as one study showed an increased risk of adverse pregnancy outcomes in women who tested positive for these antibodies [6].

Management During Pregnancy

Disease Course During Pregnancy

The inflammation seen in AIH is reflective of cell-mediated immunity [7], and pregnancy induces a state of relative immune tolerance, likely as a result of increased estrogen levels, with "a cytokine shift from a profile that favours the differentiation and proliferation of liver-infiltrating cytotoxic T lymphocytes to a profile that has anti-inflammatory effects" [8]. Nonetheless, the course of AIH during pregnancy is variable. Disease activity has been seen to improve beginning in the second trimester in some patients despite a decrease in immunosuppressive medications [9]. On the other hand, up to 6% of cases of autoimmune hepatitis present during pregnancy [10], and flares occur during pregnancy in 7–21% [4]. The risk of disease flare is similar regardless of whether it is a woman's first pregnancy or a subsequent one [7].

Immunosuppressive Medications During Pregnancy

The AASLD (American Association for the Study of Liver Diseases) guidelines suggest that in preconception counseling, "termination of immunosuppressive therapy should be attempted where possible. … Intuitively, little or no treatment during pregnancy is a desirable protective measure for the mother and fetus" [11]. In contrast, EASL (European Association for the Study of the Liver) guidelines advocate that "maintenance treatment of azathioprine plus/minus predniso(lo)ne should be continued" in pregnancy, though "the final decision to modify immunosuppression either preconception or during pregnancy should be based on the perceived risk to the patient and the pregnancy" [12]. For those who do require immunosuppression during pregnancy, prednisone (immediate release) is classified as pregnancy category C [13], and prednisone monotherapy is appropriate for those who are pregnant or planning a pregnancy [11]. First trimester use, however, is associated with a slight increased risk for orofacial clefts [14].

The role of azathioprine in the treatment of pregnant patients with autoimmune hepatitis is less certain. In the US, azathioprine is pregnancy category D (positive evidence of human fetal risk), but in patients treated with azathioprine during pregnancy

(such as those with inflammatory bowel disease and organ transplant recipients [2]), "increased birth defects have not been reported" [11]. Though in some studies increased risk of adverse birth outcomes including congenital malformations, low birthweight, and pre-term delivery has been seen in women taking azathioprine for autoimmune diseases (or to prevent transplant rejection), the effect of the medication may have been confounded by the activity of the underlying disease, and other studies have not shown increased risk [15]. Since in autoimmune hepatitis "azathioprine is not an essential treatment, disease activity commonly subsides during pregnancy, and adjustments in the dose of prednisone are typically sufficient to suppress disease activity" [8], azathioprine should be discontinued when possible [11]. The steroid dose may need to be increased in conjunction with discontinuation of azathioprine.

Among the alternative agents used in autoimmune hepatitis, mycophenolate mofetil should not be used in pregnancy, as noted above. Tacrolimus can cause fetal harm, though good outcomes have also been reported in post-transplant patients treated with tacrolimus during pregnancy [16]. Budesonide also carries the possibility of fetal harm, though the very little published clinical experience so far has shown good pregnancy outcomes [17].

Pregnancy Outcomes

While maternal outcomes of pregnancy in those affected by autoimmune hepatitis are good the majority of the time, maternal adverse events have been found to occur in 7.8–11% of pregnancies [4]. Reported maternal complications – some fatal – include disease flare, decompensation of cirrhosis, variceal bleeding, need for liver transplantation, porto-pulmonary hypertension, and post-partum renal failure, vaginal bleeding, and infection [4, 6, 13]. An increased risk of gestational diabetes has also been seen in women with autoimmune hepatitis, though it is theorized that this may be related to the association between autoimmune hepatitis and other autoimmune diseases [18]. Risk factors for adverse maternal outcomes include cirrhosis, poor disease control in the year before conception, increased age, and no therapy [4].

The majority of pregnancies in women with autoimmune hepatitis result in good fetal outcomes. The risk of miscarriage is equivalent to that of the general population [3], as is the risk of congenital malformations [3, 18]. However, a nationwide study in Sweden showed autoimmune hepatitis to be associated with an increased risk of preterm birth and low birth weight [18]. A more recent nationwide study in Denmark confirmed the increased risk of preterm birth, and showed an increased risk for small for gestational age infants, which was not seen in the Swedish study [3]. The majority of the preterm births were only moderately preterm, rather than very preterm (<32 weeks) [19]. Risk factors for admission to the special care baby unit include maternal cirrhosis and flare during pregnancy [4].

Post-partum Management

Estimates of the risk of flare in the post-partum period vary widely, from 12.5% (of 32 pregnancies), to 21.7% (of 46 pregnancies), to 52% (of 42 pregnancies) to 86% (of 14 pregnancies) [2, 6, 9, 20]. The series showing the highest rate of post-partum flares was one in which immunosuppression was reduced during pregnancy. The median time of relapse or flare of autoimmune hepatitis after delivery is 75–78 days [13, 20]. In light of the risk of flare, the AASLD recommends "resuming standard therapy 2 weeks prior to anticipated delivery and … closely monitoring serum AST or ALT levels at 3-week intervals for at least 3 months after delivery [11]."

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Chapter 9
Liver Transplantation for Autoimmune Hepatitis

Steven Zacks

Introduction

Autoimmune Hepatitis (AIH) is a chronic, progressive, inflammatory, liver disease that responds to immunosuppressive therapy. Liver transplantation (LT) may be needed for AIH patients who present with acute liver failure without a prior diagnosis of AIH or in those who develop decompensated liver disease despite medical therapy. The proportion of all AIH that present as acute hepatitis that may progress to liver failure and need transplantation ranges from 20% to 75% [1–3]. Approximately 2–3% of all pediatric and 4–6% of adult liver transplants in the US are for AIH [4, 5]. AIH can recur in the allograft with an incidence of recurrence between 8–12% at 1 year and 36–68% at 5 years [6, 7]. Recurrent AIH is characterized by non-organ specific autoantibodies, increased aminotransferases and immunoglobulin G (IgG), and typical histologic features of an interface hepatitis composed of peri-portal plasma cell infiltrates. *De novo* AIH is the development of features of classical AIH in the allograft of patients who have not been transplanted for AIH. Other names for this condition include 'graft dysfunction mimicking AIH' and 'plasma cell hepatitis'. There is a need for uniform diagnostic criteria. Careful attention to excluding other causes of hepatitis is important in diagnosing *de novo* AIH. The cause of recurrent or *de novo* AIH is unknown. Several mechanisms have been implicated in this loss of self-tolerance including impaired thymic regulation, impaired activity of T regulatory cells, molecular mimicry, calcineurin inhibitors, glutathione-s transferase and genetic polymorphisms. While the phenotype of *de novo* AIH in pediatrics has been uniform, it has been more variable in adults, highlighting the need for uniform diagnostic criteria or scoring system post LT. Better understanding of the development of autoimmunity and its difference from classical rejection after LT will improve therapeutic

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M. W. Russo (ed.), *Diagnosis and Management of Autoimmune Hepatitis*,
https://doi.org/10.1007/978-3-030-33628-8_9

strategies and outcomes. Experience in pediatric liver recipients suggests that immunosuppressive therapy used in AIH can successfully treat *de novo* AIH.

Presentations of Autoimmune Hepatitis Leading to Transplant

Acute Severe Autoimmune Hepatitis

Although AASLD guidelines define acute liver failure, there is no standard definition for acute severe autoimmune hepatitis (AS-AIH). A standard definition would be beneficial for research purposes and for deciding who is likely to require liver transplantation. One proposed definition of AS-AIH requires a presentation ≤26 weeks with an INR ≥ 1.5, without cirrhosis on biopsy [8, 9]. Others have suggested further defining the acute presentation of AIH as nonsevere, severe, and fulminant based on the presence or absence of jaundice, coagulopathy, and encephalopathy. The acute, but nonsevere, AIH could be seen in patients presenting with jaundice who neither have encephalopathy nor coagulopathy. AS-AIH could be defined as a presentation with jaundice and coagulopathy (INR ≥ 1.5) but no encephalopathy. AS-AIH with acute liver failure (ALF) could be defined as presenting with jaundice, coagulopathy, and encephalopathy (Fig. 9.1) [10].

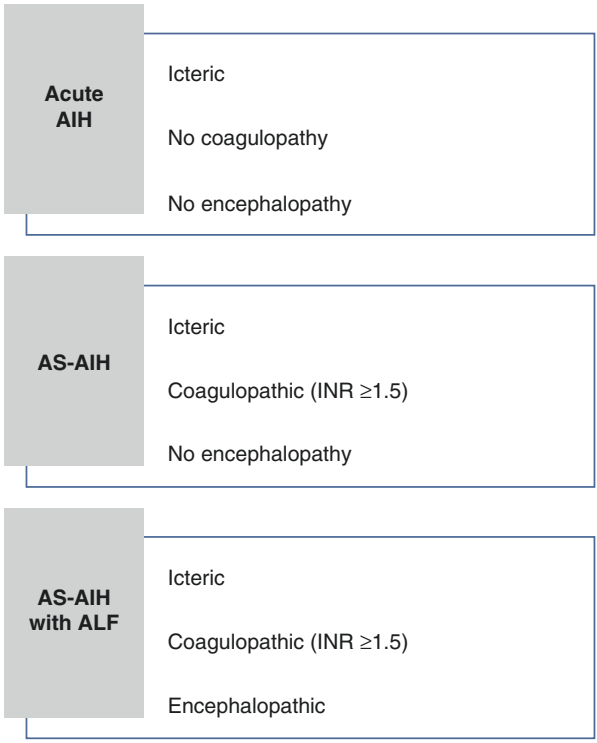

Fig. 9.1 Proposed definitions of acute presentations of AIH. (AIH autoimmune hepatitis, AS-AIH acute severe autoimmune hepatitis, ALF acute liver failure). (Adapted from Rahim et al. [10])

Although AIH is a chronic disease, 20–75% of patients may present with new onset, acute AIH [1–3]. It is important to exclude decompensation from superimposed infection or a drug reaction in a patient with preexistent AIH. In one series, 69% of patients who presented with AS-AIH developed acute liver failure [7]. Conversely, anywhere from 8% to 30% of ALF patients had AIH as the cause [11–13].

Blacks are more likely to present with ALF from AIH at initial presentation than non-Blacks [14] Furthermore, blacks were more likely to referred for liver transplantation, less likely to respond to immunosuppression, and have a higher mortality rate. Black males had the poorest outcomes among AIH patients [12]. In a comparison of black and white patients with AIH in Europe, black patients present at a younger age, have higher IgG levels, and a greater proportion have systemic lupus erythematosus (SLE). In that study, black patients had a greater risk of liver transplantation and liver-related mortality, indicating more aggressive disease [15]. There is some evidence to suggest that type 2 AIH, which may have positive anti-liver kidney microsome (LKM) and anti-liver cytosol antigen type 1 antibodies, may be more aggressive than type 1 AIH, which may have positive antinuclear antibody (ANA) and antismooth muscle antibody (ASMA) [16].

Whereas there are three diagnostic criteria or scoring systems for the diagnosis of AIH, the original, revised, and simplified criteria [17–19], these criteria have not been validated in acute severe AIH. This may lead to a delay or even prevent the diagnosis of AIH such that steroids may be started too late or not at all.

Laboratory and Imaging Features of Acute Severe Autoimmune Hepatitis

In AS-AIH, increased IgG and positive antibodies may not be seen. It may be due to the sensitivity of the assays or delay in the development of autoantibodies. ANA may be undetected or only weakly positive in 29–39% of acute severe or fulminant AIH [20, 21]. In another series, 88% of AS- AIH tested positive for ANA, ASMA, and/or LKM antibodies. Using the International Autoimmune Hepatitis Group (IAIHG) scoring system titer strengths, only 66% of patients had positive autoantibodies [7]. IgG levels may be normal in 25–39% of cases [18, 19].

Imaging is of limited in value in the diagnosis of acute severe AIH. Noncontrast CT can show heterogeneous, hypoattenuated areas in the liver in 65% of patients with ALF from AIH [22].

Histology of Acute Severe Autoimmune Hepatitis

While liver biopsy is an important part of the diagnosis of AIH and in excluding other liver diseases, the findings are nonspecific and may also be seen in viral hepatitis and drug induced liver injury. There are two problems with sampling with liver

biopsy. First, the biopsy is only a fraction of the entire liver. Second, the biopsy is only a snapshot in time in a dynamic disease process.

As opposed to typical, chronic AIH, histological features of AS-AIH predominantly occur in the centrilobular zone. The severity ranges from diffuse, lobular hepatitis to confluent centrilobular, bridging, or multiacinar necrosis to submassive loss of hepatocytes [10]. Because studies have described centrilobular necrosis with portal inflammation in chronic AIH, centrilobular necrosis without portal inflammation may be a feature of new onset, acute AIH [23]. With confluent necrosis, typical features of AIH, e.g. rosetting, may not be seen. Thus, it may be difficult to apply the IAIHG histologic criteria.

Steroid Use and Response in Acute Severe Autoimmune Hepatitis

There is considerable disagreement about the initial dose of steroids and their effectiveness in inducing remission. While some use methylprednisolone 1 g IV on the first day of treatment with a rapid taper, others use 20–40 mg of prednisolone per day. In one study of 72 treatment naïve, AS-AIH who did not have hepatic encephalopathy, subjects received prednisone or prednisolone 40–60 mg/day. Treatment failure, defined as the need for another therapy, progression to ALF, or death, occurred in 18% of subjects. The severity of coagulopathy correlated with treatment failure [24].

In a study comparing treatment to no treatment, 23 patients received prednisone or prednisolone at a median dose of 40 mg/day or intravenous hydrocortisone at a median dose of 300 mg/day. Another 9 patients did not receive steroid due to severity of their disease as measured by MELD and the presence of encephalopathy. No difference in mortality was observed between the treated and untreated groups. There was a nonsignificant increase in the incidence of sepsis in the treated group. All the untreated patients required transplantation, while over half of the treated group did not require transplant. MELD score did not predict the response to treatment [7]. In a single center in France, 16 patients were admitted to a specialized liver unit with a median INR of 5.4 and 10 of the 16 subjects had encephalopathy at presentation. Twelve of the sixteen subjects received steroids. Of these 12 subjects, 1 died, 1 improved, and 10 were transplanted. Of the 4 subjects not given steroids, 1 died and 3 were transplanted. Severe infections including gram negative bacteremia occurred in 3 treated patients [25]. In a multicenter study of 121 patients with AS-AIH, 61% responded to steroids. The most significant predictor of treatment failure was hepatic encephalopathy with 1 of 8 with encephalopathy responding. Improvement in bilirubin by day 7 of steroids also predicted steroid response [26]. These and another study [27] suggest the steroids have little benefit once patients are encephalopathic or if there is no improvement in bilirubin after 7 days and steroids could increase the risk of infection.

Other Immunosuppressants and Response

There is no evidence to support the use of other immunosuppressants in AS-AIH. Azathioprine is not recommended because of poor metabolism and risk of cholestasis. There are no studies on the use of mycophenolate mofetil nor calcineurin inhibitors in AS-AIH. The closest experience may be a series of treatment naïve, icteric AIH patients who failed to improve with steroids. Of 11 patients who failed to respond to steroids and received other immunosuppressants, 9 responded. Of the 9, 7 received tacrolimus, 1 mycophenolate mofetil, and 1 plasmapheresis [22].

Appropriate Timing of Liver Transplantation for Acute Severe Autoimmune Hepatitis

Because of the logistics of evaluating and listing a patient for transplant, it is important to identify AS-AIH patients who will not respond to medical therapy and need consideration for transplant. Based available data, patients presenting with high-grade hepatic encephalopathy should be considered for liver transplant without delay.

In patients without high grade hepatic encephalopathy, it is also important to identify those who will eventually need transplantation. Predictors of response to steroids, thus avoiding transplant, include a MELD < 28 on admission, the absence of high grade encephalopathy, the absence of massive hepatic necrosis on biopsy, and improvement in bilirubin and INR within 4 days of starting treatment [11, 28, 29]. Another series suggested that if the MELD-Na does not improve within 7 days, there is a high risk of acute liver failure, steroids could be stopped, and transplant should be considered. A decline in MELD-Na of less than 2 points in 7 days had a sensitivity of 77% and specificity of 78% in predicting treatment failure [22].

Liver Transplant for Chronic Autoimmune Hepatitis

A lack of response to immunosuppression over time is predictive of the need for liver transplantation in chronic AIH. Between 10% and 15% of chronic AIH patients progress to decompensated liver disease despite immunosuppression [30]. Less than 50% improvement in aminotransferases within 6 months in response to treatment with prednisone and/or azathioprine predicts a need for transplant. The odds ratio for a patient who fails to improve their aminotransferases proceeding to transplant compared to a patient who does improve their aminotransferases is 16.8 (95% CI 7.5–37.7) [31].

Presentations of Autoimmune Hepatitis After Liver Transplant

Recurrent Autoimmune Hepatitis After Liver Transplant

Recurrence of AIH after liver transplantation can occur in 17–42%. The diagnosis is based on several features (Table 9.1). Rates vary depending on the number of patients studied, length of follow up, diagnostic criteria, use of protocol biopsies, and type and depth of immunosuppression. Histologic recurrence is not well defined. It can be difficult to distinguish between recurrence and acute cellular rejection. Both rejection and recurrent AIH can coexist in the graft. The recurrence rate increases with time from 0–12% at 1 year to 11–36% at 10 years [32]. Because histologic recurrence can be seen before clinical recurrence, protocol biopsies might be considered as part of the protocol for management of post transplant AIH. Perhaps a more appropriate term would be alloimmune hepatitis because the immune response is against the donor liver.

There is a higher risk of acute cellular rejection in AIH patients compared to others transplanted for other indications. Increased IgG and transaminases along with moderate to severe inflammation in the explant can predict a higher incidence of recurrent AIH after transplant [33, 34, 35]. Higher risk of recurrence is observed when steroids are aggressively tapered [36]. In children, 60% of those with recurrent AIH developed cirrhosis [37]. In adults, graft failure has been reported in 13–50% with recurrent AIH [37, 38].

Certain HLA types, particularly DRB1∗03, are associated with increased severity of AIH prior to liver transplant and recurrent AIH. It is tempting to hypothesize that HLA matching between donor and recipient may enable the autoimmune response to the graft by presenting shared antigens [39]. However, HLA DRB1∗03 mismatching did not make a difference to the recurrence of AIH in one case series of 47 patients transplanted for AIH in which 13 developed recurrence [40].

Table 9.1 Diagnosis of recurrent AIH

Liver transplant for autoimmune hepatitis
Elevation of transaminases
Interface hepatitis
Elevation of immunoglobulin G
Presence of autoantibodies (ANA, SMA, and/or anti–LKM-1)
Corticosteroid dependency
Exclusion of other causes of graft dysfunction

Adapted from Liberal et al. [71]

Prevention and Treatment of Recurrent Autoimmune Hepatitis

The benefit of long-term steroids is not clear. Successful steroid withdrawal has been reported in 0–68% of patients transplanted for AIH [32, 41, 42]. In a randomized, controlled trial of 30 patients who were more than 12 months out from transplant, 14 were randomized to receive prednisone (10–20 mg/day) and tacrolimus (dosed to achieve a level of 5–8 ng/mL) while 16 were randomized to receive mycophenolate mofetil (1 g BID) and tacrolimus (dosed to achieve a level of 5–8 ng/mL). The incidence and severity of acute rejection were similar in both groups. Recipients on long term steroids had poorer diabetes control. Cholesterol levels and bone densities were better in the mycophenolate mofetil group. Three of the 16 on mycophenolate mofetil required dose reduction for GI side effects or cytopenias [43]. There is no observed difference in recurrence of AIH comparing cyclosporine to tacrolimus based regimens [4, 31, 44, 45].

The post-transplant literature focuses on preventing recurrent AIH. There is little data on the treatment of established recurrent AIH in the graft. It is reasonable to suggest that minor changes on liver biopsy and minor aminotransferase elevations can be addressed through modest adjustments in immunosuppressants. More significant recurrence may require significant increase in or reintroduction of steroids or the addition of azathioprine or mycophenolate mofetil. Because noncompliance with the immunosuppression regimen is an important cause of graft dysfunction, a careful of evaluation of compliance is always needed prior to making any medication changes.

Autoantibodies After Liver Transplantation

Autoantibodies can be detected in liver transplant recipients who are transplanted for non-autoimmune liver disease. ANA is most frequently observed followed by ASMA, and an atypical form of LKM antibody [46]. The prevalence of new autoantibodies at 6, 12, and 36 months after transplant were 23%, 42%, and 66% in one series [47]. Autoantibodies have been associated with graft dysfunction, chronic rejection, graft loss, and death [46, 48, 49, 50] Autoantibody seropositivity associated with high levels of IgG and transaminases should prompt liver biopsy for histologic assessment.

De Novo *Autoimmune Hepatitis After Liver Transplantation*

De novo AIH is defined as a hepatitis that resembles AIH that occurs in liver transplant recipients who are transplanted for other liver diseases. It was first described in children transplanted primarily for biliary atresia [51]. It has also been seen in children transplanted for Alagille syndrome, primary familial intrahepatic cholestasis,

primary sclerosing cholangitis, and Budd-Chiari [46, 51, 52, 53]. *De novo* AIH is seen in adults with lower frequency. It has been described in adults transplanted for primary biliary cholangitis, primary sclerosing cholangitis, alcoholic cirrhosis, hepatitis C, and Wilson disease [54, 55, 56, 57].

Although the term *de novo* AIH is often used, the Banff working group on liver allograft pathology prefers the term 'plasma-cell rich rejection' [58]. Plasma-cell rich rejection is preferred because this condition has histologic findings that are not typical of AIH, including lymphocytic cholangitis, central perivenulitis, and other features of T-cell mediated rejection. Further study is needed to distinguish between autoimmune and alloimmune phenomena as a cause because this condition responds to immunosuppression, some of the histologic features in the allograft can also be seen in atypical AIH independent of transplant, and the majority of de novo AIH patients will have autoantibodies and increased IgG [45, 59, 60, 61].

Although autoantibodies are seen in de novo AIH, they are not sufficient to diagnose de novo AIH because they have been detected in pediatric liver transplant recipients with stable graft function [62] and in adults with chronic rejection where autoantibodies were associated with the need for retransplantation [48].

It is possible that allograft factors may trigger *de novo* AIH because it is seen more often in grafts from female and older donors [45]. Retrospective data suggests that the use of tacrolimus and mycophenolate mofetil may have a greater association with *de novo* AIH compared with the use of cyclosporine [45], though this may be due to the use of steroids with cyclosporine or perhaps other unknown factors associated with cyclosporine.

Several mechanisms can lead to autoimmunity and *de novo* AIH can arise in liver transplant recipients who were transplanted for nonautoimmune conditions. The release of autoantigens from damaged tissue at the time of surgery or molecular mimicry may play a role [63, 64]. All patient in a series of *de novo* AIH were infected with cytomegalovirus, Epstein-Barr virus, or parvovirus [56] suggesting that viral infections can lead to upregulation of the immune system which leads to the hepatitis. There is some evidence that calcineurin inhibitors interfere with the maturation of T cells and the function of regulatory T cells leading to emergence and activation of autoaggressive T cells [65, 66]. Cyclosporine blocks activation-induced death of T cells and interferes with the development of tolerance [67]. Calcineurin inhibitors reduce interleukin-2 production [68]. A reduction in interleukin-2 diminishes proliferation and survival of regulatory T cells, thus impairing the immune suppression function of these regulatory T cells. It is hypothesized that although calcineurin inhibitors suppress rejection of the graft, in genetically predisposed organ recipients, they might induce or promote autoreactivity. Children may be particularly at risk of *de novo* AIH because of their immature immune systems are susceptible to calcineurin inhibitor induced autoimmunity [69]. Acute cellular rejection could reduce the number of regulatory T cells [70], so that the combination of calcineurin induced reduction in the function of these T cells combined with a reduced number of T cells, could trigger *de novo* AIH.

Treatment of *de novo* AIH usually requires prednisone often with azathioprine or mycophenolate mofetil in protocols that are similar to treating standard AIH. Patients may require long term steroids to keep the condition controlled [56].

Chapter Summary

1. Timely recognition of acute severe or chronic AIH patients who need liver transplantation is critical. The presence of encephalopathy should trigger an evaluation for transplant.
2. Although liver transplantation treats decompensated liver disease from AIH, AIH recurs in 30% of recipients.
3. Recurrent or *de novo* AIH after liver transplantation should be treated as one would treat AIH, with a combination of azathioprine or mycophenolate mofetil and steroids, in addition to a calcineurin inhibitor.

Useful Tips for Practitioners

1. The severity of acute severe AIH can be assessed by the presence of jaundice, encephalopathy, and coagulopathy (INR $\geq$ 1.5).
2. AIH patients who present in the acute severe phase may not always meet the typical International AIH Group diagnostic criteria, so a strong clinical suspicion for AIH is needed to assist in making the diagnosis.
3. AIH transplant recipients should be considered for long term steroids as part of their immunosuppression.

Common Pitfalls in Practice

1. It may be difficult to apply the International AIH Group histologic diagnostic criteria in acute severe AIH because of the centrilobular necrosis.
2. Negative antibody serologies, as defined by the International AIH Group criteria, can be seen in 34% of acute severe AIH.
3. Acute severe AIH patients who develop encephalopathy are unlikely to respond to steroids. Steroids may increase the risk of infection thus precluding transplant.

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Chapter 10
Autoimmune Overlap Syndromes

Philippe J. Zamor

Introduction

Autoimmune hepatitis, primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC) are immune-mediated diseases of the liver. It is important to note that PBC has been renamed primary biliary cholangitis because many of these patients either do not have or do not progress to cirrhosis [1]. While most patients present with a single distinct entity, a small percentage of AIH patients present with coexisting cholestatic liver disease. The term overlap syndrome describes patients that present with features of AIH and PBC: AIH-PBC overlap and AIH and PSC: AIH-PSC overlap. The term 'Variant Syndrome' has also been introduced to describe these various entities. There has been increasing attention to this uncommon clinical subgroup of patients. Overall overlap syndromes are uncommon, and the true prevalence of these overlap syndromes reported in the literature varies partly due to the inappropriate use of the diagnostic scoring tools that have been validated only for AIH. Patients with a suspected variant syndrome should undergo a thorough work-up to include liver histology, serology and if not conclusive, imaging of the bile duct system (Table 10.1). It is important to note that clinicians should not over diagnose these variant syndromes so patients are not unnecessarily exposed to corticosteroids and the associated toxicities. Patients with PBC are at higher risk for osteoporosis, so the benefit to treating AIH-PBC overlap must be carefully weighed against the risk of toxicity.

Similar mechanisms of injury are described for AIH, PBC and PSC, whereby the manifestation of liver disease likely represent the end result of a cell [3–6] and antibody-mediated immunological [7–10] attack against liver-specific targets [11, 12]. The exact mechanism or triggers that lead to the cascade of events perpetuate overlap syndromes are not clearly understood, but likely are influenced by genetic

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M. W. Russo (ed.), *Diagnosis and Management of Autoimmune Hepatitis*,
https://doi.org/10.1007/978-3-030-33628-8_10

Table 10.1 Serologies of hepatic autoimmune disorders

	AIH	PBC	PSC
Immunoglobulins	Increased IgG	Increased IgM	Increased IgG and 45% IgM elevated
Specific autoantigen(s)	Smooth muscle Ab (F-actin)	AMA (PDC-E2 subunit)	None identified
ANA	70–80%	20–50% (anti-GP210 and anti-SP100 are highly specific)	8–70%
Anti-Smooth Muscle Ab	70–80%	0–10%	0–83%
Anti-LKM1	3–5%	–	–
Anti SLA/LP	10–30%	Few	Few
P-ANCA	60–90% (often atypical)	0–10%	26–94%
AMA	AMA in low titer occasionally seen	+90–95% (anti-PDC-E2 pattern is highly specific)	Few

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predisposition [13–16]. Dysregulation of immune system as perhaps triggered by environmental factors are speculated to cause a breakdown in self-tolerance [17–23].

Autoimmune Hepatitis and Primary Biliary Cholangitis Overlap

PBC-AIH overlap is the most commonly described overlap syndrome in patients with AIH [24, 25]. PBC is one of the most common intrinsic biliary disorder of the liver. Patients with PBC are most often middle-aged women, and the female:male ratio is 9:1 [24]. This disease rarely affects children. Multiple risk loci have been identified by genomic-wide association studies [26], and it has been recognized that relatives of patients with PBC have an increased risk of developing the disease [27]. These patients also have been termed 'hepatitic form of PBC' or 'PBC with secondary AIH'. Because of the lack of consensus in diagnostic criteria, there is a wide range in prevalence of the AIH-PBC overlap. It is generally accepted that the AIH-PBC variant occurs in 8–10% of adult patients with either PBC or AIH [28, 29]. The prevalence of PBC and AIH ranged from 2.1% to 19% in one study using the Paris criteria and International Autoimmune Hepatitis Group (IAIHG) revised criteria [28]. It is not clear whether overlap syndrome is a variant of AIH, PBC or its own entity, since PBC and AIH share histologic findings such as ductal injury, which has been described in AIH, and interface hepatitis, which can be seen in PBC [30, 31].

Of note, the incidence and presentation of PBC overlap with AIH is different among Hispanic and nonHispanic populations [24, 32]. Levy et al. reported that Hispanic patients with PBC had a significantly higher prevalence of overlap

syndrome based on the Paris or simplified IAIHG criteria as compared to non-Hispanic patients (31% vs 13%; $P = 0.002$) [33]. After a median follow-up of 3.65 years a greater percentage of Hispanics had ascites (24% vs 12%; $P = 0.03$) as well as variceal bleeding (20% vs 7%; $P = 0.01$). Of note, there were no differences in the number of deaths or liver transplants. Among 204 total patients, 180 received ursodeoxycholic acid (UDCA) for at least 1 year. A lower proportion of Hispanic patients had a biochemical response to UDCA therapy (60% vs 88%; $P < 0.0001$). Higher rates of PBC-AIH overlap in Hispanic patients have been reported as high as 15% based on the Paris criteria [34].

Diagnosis

Criteria for diagnosing AIH-PBC overlap syndrome have not been independently validated [35] which is likely due to the relative paucity of these patients. There are formal defining criteria for PBC-AIH overlap syndrome, but typically the term is used to describe patients with clinical features of both antimitochondrial antibodies (AMA)-positive PBC and AIH (Table 10.2) [36]. The 'Paris criteria' as reported by Chazouilleres et al. is one widely accepted definition for diagnosing this variant with a requirement of at least two of the three key criteria of each disease: for PBC (1) alkaline phosphatase (ALP) ≥ 2× upper limit of normal (ULN) or gamma-glutamyltransferase (GGT) ≥ 5× ULN (2) presence of antimitochondrial antibodies (AMA); (3) liver biopsy demonstrating florid bile duct lesions. Criteria for AIH include: (1) alanine aminotransferase (ALT) ≥ 5× ULN; (2) serum immunoglobulin

Table 10.2 Summary of clinical features of AIH, PBC, PSC

	AIH	PBC	PSC
Age of onset	All ages (bimodal peaks: 10–20 years and 40–50 years)	>40 years	All ages, most under age 40
Gender	Female:Male 4:1	Female:Male 9:1	Male:Female 2:1
Clinical presentation	Acute and chronic hepatitis	Pruritis, fatigue, elevated ALP	Cholestatic liver test elevation, pruritis
Concurrent autoimmune disorders	17–40%; thyroiditis, rheumatoid arth, IBC	~20% thyroiditis, CREST syndrome, sicca symptoms	~80% with IBD
Cross sectional imaging	Often times normal, unless cirrhosis	Large central nodularity pattern	Structuring +/− biliary ductal dilatation
Hepatopathology	Interface hepatitis, lymphoplasmacytic infiltrate in the portal area, rosette formation	Classic florid duct lesion, lymphocytic infiltrate in the portal area	periductal fibrosis (variable detection), lymphocytic infiltrate in the portal area
Treatment Response	Very responsive to corticosteroids ± AZA	Good response to UDCA	No medical treatment available

Table 10.3 Diagnosis of AIH and PBC

PBC 2 out of 3 required for diagnosis	AIH diagnosis
Alkaline phosphatase ≥ 2× ULN Or GGT ≥ 5× ULN	IgG ≥ 2× ULN Or Positive smooth muscle antibody
AMA serum positivity	ALT 5× ULN
Liver biopsy with florid bile duct lesion	Liver biopsy with moderate or severe periportal or periseptal lymphocytic piecemeal necrosis

G (IgG) ≥ 2× ULN or presence of anti-smooth muscle antibody (ASMA); 3) liver biopsy demonstrating moderate or severe periportal or periseptal lymphocytic piecemeal necrosis [28] (see Table 10.3). AMA positivity is observed in more than 90% of patients with PBC [37]. This antibody is directed against acetyltransferases of the inner mitochondrial membrane with the vast majority of sera (90%) have specificity for the E2 subunit of the pyruvate dehydrogenase complex (PDC-E2). This pattern is highly specific for PBC. Positive antinuclear antibodies (ANA) are found in at least one third of PBC cases [38, 39]. The are ANA subtypes that are specific to PBC as measured by immunofluorescent staining are anti-sp100 and anti-gp210, in those who are AMA negative [37, 40–42].

Other biomarkers in patients with AIH-PBC overlap include anti-double stranded DNA (anti-dsDNA) and Anti-p53. Banked serum from 197 subjects with PBC criteria was analyzed for anti-dsDNA by the *Crithidia luciliae* immunoflourescence (CLIFT) assay (1:20 dilution) as well as chemiluminescence (CIA:QUANTA Flash®, Inova Diagnostics, San Diego); 16 of the 197 subjects (8.1%) met criteria for PBC-AIH OS [43]. Anti-dsDNA by CLIFT was noted to be higher in subjects with PBC-AIH OS when compared PBC alone patients (37.5% vs. 9.9% respectively, $P < 0.01$). Other markers were analyzed by immunoassay but did not show any differences in the two groups: Anti-p53, anti-Ro52/TRIM21, anti-YB 1, anti-GW182, anti-Ge-1, and anti-Ago 2.

Clinical Presentation

Patients with PBC-AIH overlap typically present with features of both conditions, [35, 44] but patients may present with features of only one of these conditions, such as elevated alkaline phosphatase and subsequently develop elevations aminotransferases years after the primary diagnosis [45–47]. In patients with a confirmed diagnosis of PBC, suspicion should be raised for and AIH-PBC overlap syndrome if patients do not appropriately respond by 6–12 months of treatment with UDCA and aminotransferases are more than five times upper limit of normal.

The prognosis of patients with AIH-PBC overlap is typically worse than for PBC alone and patients tend to present with more advanced fibrosis [46, 48, 49]. Mayo

clinic reported their experience with a cohort of patients followed for 5.75 years and found that more patients with AIH-PBC overlap developed portal hypertension (54% vs 28%; $P < 0.01$), features of decompensated liver disease and progressed to liver transplantation or death (38% vs 19%; $P < 0.05$) when matched to subjects with AIH alone [50]. Another group from Shanghai demonstrated that 5-year adverse outcome free survival was 58% in the AIH-PBC overlap group compared to 81% in the PBC group [49]. Multivariate analysis in the overlap patients demonstrated that total bilirubin ≥2.7 ULN predicted a poor prognosis ($P = 0.008$, relative risk 8.39, 95% confidence interval (CI) 1.73, 40.73). Of note, cirrhosis was more prevalent in the PBC group vs. the AIH-PBC overlap group (16.7% vs. 7.9% respectively), but did not predict worse outcomes. Conversely, the Toronto group published their data examining 16 subjects meeting criteria for AIH-PBC OS out of 331 PBC alone and found that response to those randomized to receive 2 years of UDCA was similar whether or not AIH features were present [51].

Management

Treatment of overlap syndromes should be individualized and the approach should not be static, but rather responsive to clinical changes. The PBC component should be treated with UDCA at the standard dose of 13–15 mg/kg/day. Immunosuppression with corticosteroids and azathioprine is indicated to induce and maintain clinical remission in AIH. ALT serves as a marker of disease activity and treatment response in AIH but may not in patients with overlap. Reduction in alkaline phosphatase is the desired response to therapy in patients with PBC. It is generally accepted that the cornerstone of therapy for patients with PBC-AIH overlap is UDCA and corticosteroids but it is important to highlight that larger, long-term studies on the prognosis of patients treated with this regimen are lacking. UDCA leads to slowed progression of fibrosis and liver failure, most notably in patients who demonstrate an acceptable biochemical response to treatment [51, 52].

Attention should be directed to the management of the comorbidities related to PBC as these can have a major impact on morbidity and the quality of life for many patients. Patients with AIH-PBC OS are subject to these same symptoms as PBC patients alone. The symptoms of PBC do not necessarily correlate with severity of liver disease. PBC therapy does not always relieve associated symptoms.

Pruritis is a hallmark symptom of PBC and can markedly impair health-related quality of life. This can more profound with ductopenic variant of PBC and debilitating in patients with AIH-PBC overlap. Biliary obstruction from gallstones or malignancy should be excluded [53].

Bile acid-binding resins, such as cholestyramine, are first line therapy for pruritis [54]. Commons side effects include bloating and constipation [55]. Pruritus usually improves within 4–11 days. Caution must be used with these agents because they

may reduce absorption of other medications, and should be separated by 2–4 h from other medications.

Rifampin is used a second line agent used to treat pruritus [56]. Randomized placebo controlled trials have demonstrated rifampin is effective in treating cholestatic pruritis [57, 58]. Concerns with adverse events include hepatotoxicity and hemolysis so patients should be monitored with periodically with blood work [59]. Competition for hepatic bile acid uptake may be the mechanism for the increase in serum bilirubin levels and rarely severe hepatotoxicity. The recommended starting dose is 150 mg orally daily or twice daily. The dose can be increased to 600 mg/day in divided doses based on clinical response and liver tests.

Third line therapy are the oral opiate antagonists naltrexone and nalmefene. It is recommended to start naltrexone at low doses (12.5–25 mg orally daily) to avoid opiate withdrawal-like reactions [60]. Progressive dose escalation to 50 mg daily may be needed depending on clinical response.

Sertraline, a selective serotonin reuptake inhibitor, is used empirically in the management of pruritus unresponsive to other agents. Although not FDA approved for this indication, sertraline has been demonstrated in small studies to improve pruritus [61, 62]. The recommended dose of sertraline for pruritis is 50–100 mg daily. Dose reduction is recommended for those with hepatic dysfunction.

Although frequently prescribed or recommended, hydroxyzine and diphenhydramine are not recommended in the AASLD guidelines to treat pruritus because there are no randomized controlled trials demonstrating their efficacy or safety. One approach is to prescribe hydroxyzine 25 mg at bedtime to alleviate pruritus which seems to be especially severe at night.

Fatigue is a hallmark symptom of PBC and is present in over half of patients, and severe fatigue is reported in 20% of patients [63–65]. This can be quite difficult for patients and clinicians to manage, and no FDA approved treatments are available. Regular exercise may improve fatigue.

Sicca complex is common in PBC patients and occurs in AIH-PBC overlap syndrome [66, 67]. Dry eyes and/or dry mouth are the typical manifestations of this. Sjögren's syndrome has also been described in PBC patients, but most patients have sicca syndrome rather than Sjögren's. It is important to inquire about symptoms, since there are treatments specifically directed to relieve symptoms. Raynaud's phenomenon occurs in up to 25% of PBC patients [66]. This usually manifests as arterial spasms in the upper and lower distal extremities, but sometimes the ears and nose. Clinicians can recommend avoiding cold environments and wearing gloves and using hand and foot warmers, since these are triggers by colder temperatures. Calcium channel blockers, which vasodilate blood vessels are used for patients with more severe symptoms.

Guidelines are available that provide advice on treating these particular syndromes [68, 69].

Autoimmune Hepatitis and Primary Sclerosing Cholangitis (AIH-PSC Overlap)

PSC is found most frequently in male patients in the 4th decade of life often coexisting with inflammatory bowel disease (IBD). Patients showing features of AIH-PSC OS are usually younger than patients with PSC alone. AIH-PSC overlap is a relatively uncommon syndrome that has been mostly described in children, adolescents and younger adults and less common than AIH-PBC OS [70–72] The natural history of this syndrome often occurs in a sequential fashion, whereby patients typically first present with AIH and PSC is diagnosed later [73–75]. The diagnosis of PSC is established by characteristic cholangiographic findings [76]. Ulcerative colitis is present in 80% of patients with PSC and many of these patients are of northern European descent. The association of IBD is not exclusive to PSC and has been described in patients with AIH and PBC, although the association is not as common [77–79].

Fifty percent of children with AIH have endoscopic changes of sclerosing cholangitis. Autoimmune sclerosing cholangitis is the term given to this condition in childhood [70]. It remains unclear if this syndrome represents the individual occurrences of both diseases, either sequentially or simultaneously or various stages in the evolution of a single disease [80]. Overall AIH-PSC patients tend to be younger. Comparison of 7 of 41 PSC patients diagnosed with AIH-PSC OS vs PSC alone showed the AIH-PSC group was significantly younger (mean age, 21.4 ± 5.0 vs 32.3 ± 10 years; $P < 0.01$) and had higher ALT values (357 ± 26.5 vs 83.7 ± 60.7 U/L, $P < 0.005$) and higher serums levels of IgG (25.6 ± 4.7 vs 12.9 ± 6.0 mg/dL, $P < 0.0001$) [73].

Among patients with AIH, 6–20% develop features of PSC, including cholestasis and abnormal cholangiograms. [70, 78, 81, 82] Among patients with PSC, 8–17% develop features of AIH defined by the scoring system of the IAIHG [72, 73, 83]. Small duct-PSC may occur in 7% of patients with AIH [84, 85]. AIH is uncommonly diagnosed in patients with an established diagnosis of PSC [35]. When applying the AIHG Revised Diagnostic Criteria (RDC), only 1.4% had scores of definite AIH, and 6% had probable out of in a cohort of 211 subjects [86]. Patients with AIH-PSC OS had higher serum levels of IgG ($P = 0.001$), autoantibody titers ($P < 0.001$) and histologic scores ($P < 0.001$) as compared to PSC alone matched cohort.

Clinical Features

Approximately 50% of patients presenting with AIH-PSC OS are asymptomatic at presentation [87]. The overlap of AIH and PSC should be considered if the following clinical criteria occur: (1) poor response to corticosteroid treatment; (2) coexisting

inflammatory bowel disease (IBD)-ulcerative colitis (UC) in particular and rarely Crohn's disease; (3)liver test elevation in a cholestatic pattern (alkaline phosphatase[ALP] and/or gamma-glutamyl transferase [GGT]) and hepatitis (4) serologies positive for antinuclear antibodies (ANA) and/or ASMA plus hypergammaglobulinemia associated with anti-neutrophil cytoplasmic antibodies (p-ANCA); (5) typical cholangiogram features of PSC (multifocal, short, annular strictures with intervening segments of normal or dilated ducts) detected by magnetic resonance imaging (MRI)/magnetic resonance cholangiopancreatography (MRCP) or endoscopic retrograde cholangiography; (6) histologic features of AIH with variable ductopenia plus interface hepatitis [88]. When present, the most common symptoms are fatigue, pruritis, right upper quadrant abdominal pain and jaundice. In contrast to AIH-PBC OS, two-thirds of AIH-PSC OS are male.

Magnetic resonance cholangiography (MRC) is the primary diagnostic modality for PSC [89]. ERCP can be considered if MRC plus liver biopsy is equivocal or there a therapeutic indications for ERCP. Endoscopic treatment or sampling of bile duct strictures (brush cytology, endobiliary biopsies) that are identified on MRC in PSC patients is indicated to evaluate for cholangiocarcinoma. Cholangiocarcinoma should be suspected in any patient with worsening cholestasis, weight loss, elevated serum CA 19-9 tumor marker and/or new dominant stricture.

Treatment

Treatment for AIH when it occurs as AIH-PSC overlap is approached in a similar fashion to when it occurs alone with corticosteroids +/− azathioprine. The response to therapy appears to be better in children with AIH-PSC. Treatment of PSC is mostly supportive because there is no medical therapy that has been proven to halt the progression of disease. Although liver tests may improve with ursodeoxycholic acid (UDCA) data have not demonstrated improvement in clinical outcomes. In fact, high dose UDCA (30 mg/kg/day) is associated with a twofold risk of death or liver transplantation [90]. Thus this higher dose is not recommended.

ERCP can be utilized to dilate dominant strictures. Repeated balloon dilatation of dominant stricture could potentially slow down the development of end stage liver disease. Surveillance for cholangiocarcinoma, gallbladder cancer is controversial but MRI and MRCP can be considered. Surveillance for colorectal carcinoma (as appropriate in IBD patients) is recommended for AIH-PSC OS patients [91].

Liver Transplantation for Overlap Syndromes

Current estimates indicate that autoimmune liver diseases account for roughly 10–25% of the liver transplants in the United States and Europe: approximately 12% for PBC, 8% for PSC and 4% for AIH [92, 93]. Overall excellent patient and

graft survival have been reported, but autoimmune diseases can recur in the graft and impact survival [92]. 5-year and 10-year survivals for adults with AIH are greater than 80% and 70%, respectively [92–94]. Since overlap syndromes are uncommon, there is a paucity of studies with large number of patients describing the outcomes in such patients. Bhanji et al. evaluated 231 adult liver transplant patients for AIH related end stage liver disease; this included 12 patients with overlap syndromes (7 AIH-PBC and 5 AIH-PSC) [95]. Patients with overlap syndrome had a higher rate of recurrence: at 5 years, 53% vs 17%; at 10 years, 69% vs 29%; (P = 0.001). It was also noted that the median time to recurrence in overlap syndrome patients was shorter as compared to AIH patients alone. Japanese researchers reported excellent survival rates in a small study (12 AIH patients with 4 AIH-PBC OS) of patients that had undergone liver-donor liver transplantation [96].

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Chapter 11
Drug-Induced Liver Injury with Autoimmune Features

Paul A. Schmeltzer

Introduction

Autoimmune hepatitis (AIH) is an inflammatory liver disease characterized by hepatocellular liver injury, positive autoimmune antibodies, hypergammaglobulinemia, and lymphoplasmocytic inflammation with interface hepatitis on liver biopsy. Establishing the diagnosis of AIH also involves excluding alternative causes of liver disease. Based on Northern European epidemiological data, the incidence of AIH is 1–2 per 100,000 persons per year [1].

Drug-induced liver injury (DILI) is another rare cause of liver disease with an estimated incidence of one per 10,000 to one per 100,000 of treated patients [2]. It can result in severe liver injury, and in fact, 11.1% of acute liver failure (ALF) subjects were adjudicated to have DILI in a multicenter, prospective study by the Acute Liver Failure Study Group [3]. While some drugs cause hepatotoxicity in a dose-dependent fashion (e.g., acetaminophen), the majority of DILI cases are unpredictable and therefore termed idiosyncratic. Over 1000 different drugs and herbal remedies have been known to cause DILI [4].

There are different subtypes of DILI, and the applied terminology can be misleading. Drug-induced liver injury with immunoallergic features (IA-DILI) is characterized by an acute liver injury with a concomitant systemic immunoallergic response. These symptoms can include fever, rash, arthralgias, edema, and lymphadenopathy. Laboratory findings such as eosinophilia, lymphocytosis, and elevated inflammatory markers are associated with a heightened allergic response. Drugs associated with IA-DILI include macrolides, penicillin, phenytoin, and sulfonamides [5].

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M. W. Russo (ed.), *Diagnosis and Management of Autoimmune Hepatitis*,
https://doi.org/10.1007/978-3-030-33628-8_11

Table 11.1 DILI terminology

DILI Subtype	Characteristics
Immunoallergic (IA-DILI)	Fever, rash, eosinophilia, increased CRP, ESR
AIH with DILI	Patients with known AIH who develop superimposed DILI, may have advanced fibrosis
Drug-induced AIH	Self-perpetuating AIH triggered by medication, relapse after withdrawal of immunosuppression
Immune-mediated DILI	Similar features to iAIH but usually no advanced fibrosis and remission maintained after withdrawal of immunosuppression

IA-DILI has a different clinical presentation than drug-induced autoimmune liver disease (DIAILD), the subject of this chapter. In 2011, Weiler-Normann and Schramm further subclassified various forms of DIAILD. First, "AIH with DILI" was used to describe DILI that arises in a patient with preexisting AIH. This could be a chance association, and advanced fibrosis is likely to be present on histology. Second, "drug induced AIH" (DI-AIH) refers to self-perpetuating liver disease that is triggered by a drug. This is associated with a good initial response to immunosuppression, but relapse is common after withdrawal. Third, "immune-mediated DILI" (IM-DILI) can be indistinguishable from iAIH but usually resolves with drug withdrawal [4]. The broader term DIAILD will be used to describe these three types for the remainder of this chapter (Table 11.1).

DIAILD has been postulated to arise via neoantigens created by the hepatic metabolism of some drugs [6]. Among patients diagnosed with DILI, DIAILD has been reported in 2–8% of cases [7]. Much like idiopathic autoimmune hepatitis (iAIH), DIAILD is characterized by hepatocellular injury, autoantibodies, histologic findings, and a positive response to immunosuppression. Distinguishing between iAIH and DIAILD can be quite challenging. This chapter will cover the clinical and histologic features of DIAILD, treatment recommendations, and a discussion of the drugs most often associated with DIAILD.

Diagnosis of Drug-Induced Autoimmune Liver Disease

While there is no consensus definition of DIAILD, the International Autoimmune Hepatitis Group (IAIHG) original and simplified diagnostic criteria used for iAIH can be applied to assess the likelihood of DIAILD, although they have not been validated for this entity. The challenging aspect of identifying DIAILD is assigning causality to the culprit medication or supplement. Agarwal et al. provided a list of the minimal elements needed for the reporting of drug-induced liver injury. This list includes patient demographics, the drug and its dose, the indication for the drug, pertinent past medical history, date of drug initiation and discontinuation, symptoms, physical findings, medication history, laboratory tests, imaging studies, histology results, and whether a drug rechallenge was performed (Table 11.2). In

Table 11.2 Minimal elements for reporting DILI

Demographics: gender, age
Medication and dose
Indication for treatment
Concomitant diseases
Pertinent past medical history
Alcohol use history
Dates medication was started and stopped
Symptoms: date of onset, list of pertinent symptoms
Pertinent physical exam findings
Medication history: those given 3 months before onset of liver injury
Laboratory tests: basic labs, serologies to exclude other etiologies
Imaging studies
Liver histology
Rechallenge performed?

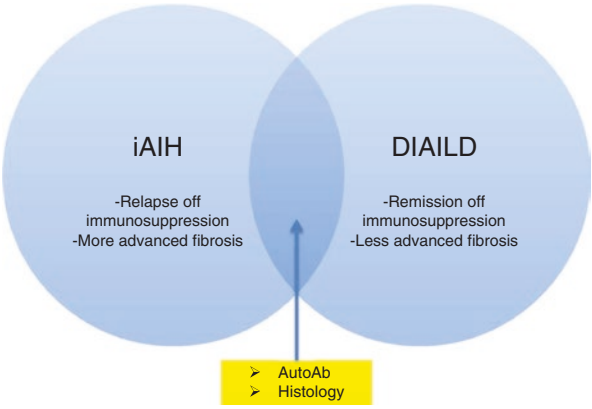

Fig. 11.1 Differentiating between iAIH and DIAILD

clinical practice, however, many of these elements are underreported, making it difficult to establish causality [8]. Moreover, while causality scoring systems exist (e.g., Roussel Uclaf Causality Assessment Method, Clinical Diagnostic Scale), their complexity and disagreement with expert opinion have limited their use. Frequently, the diagnosis of DIAILD cannot be established until after the culprit drug and corticosteroids (if given) are discontinued (Fig. 11.1). Most cases of DIAILD resolve 1–3 months after the drug is stopped, whereas iAIH typically relapses after corticosteroid withdrawal [9].

Although drug rechallenge is a component of the abovementioned causality scoring systems, it is not a recommended method to diagnose DILI because the outcome can be fatal. Furthermore, there can be long-term hepatic memory for hypersensitivity, as demonstrated by a case of recurrent DILI from nitrofurantoin 17 years after it initially caused acute hepatitis [10]. Even different drugs can cause recurrent DILI if their metabolites share enough similarity to cause immunological cross-

sensitization. A study using data collected from the Spanish DILI Registry reported nine patients who had two DILI episodes caused by different drugs. Four cases were associated with drugs with similar molecular structures, and in another two cases, the drugs had a common target. Four cases displayed autoimmune features [11].

Risk Factors for DIAILD

The major risk factors for idiosyncratic DILI include older age, female gender, medication dose, drug interactions, hepatic metabolism, and genetic factors [12]. DIAILD, in particular, has been associated with a broad age spectrum and a propensity to affect women. In a review of minocycline-induced autoimmune syndromes (which included serum sickness, drug-induced lupus, AIH, and vasculitis), the average age was 19.7 years [13]. A case series of 27 patients with highly probable or probable DILI from nitrofurans, on the other hand, reported a mean age of 64 years [14]. This variability in age is reflective of the treatment indication as minocycline was mainly prescribed for acne and nitrofurans for urinary tract infections.

Clinical and Laboratory Features of DIAILD Versus iAIH

Much of the literature on DIAILD is limited to case reports and small case series. A retrospective review from the Mayo Clinic is frequently referenced as it helped describe the clinical characteristics and prognosis pertaining to DIAILD. The study examined 261 patients with a diagnosis of AIH between 1997 and 2007. Patient with liver failure, those who required liver transplantation, and those with overlap syndromes were excluded. Among 261 patients, 24 (9.2%) were determined to have DIAILD. Nitrofurantoin (n = 11) and minocycline (n = 11) were the main culprits. Liver tests at presentation were higher and jaundice was more common with DIAILD, but the differences were not statistically significant. A similar number of DIAILD patients had positive antinuclear antibodies (83% versus 70%) and smooth muscle antibodies (50% versus 45%) compared to patients with iAIH. Imaging abnormalities were noted in eight of 11 (73%) of the nitrofurantoin cases, and this mainly consisted of atrophy attributed to postnecrotic scarring. On histology from liver biopsy, the grade and stage were similar between the two groups but none of the DIAILD patients had cirrhosis at baseline, whereas 20% of iAIH patients were cirrhotic [15].

In the United States, the Drug Induced Liver Injury Network (DILIN) was established in 2003 to prospectively collect data on idiosyncratic DILI cases. A study published by the DILIN evaluated 88 cases of DILI attributed to nitrofurantoin, minocycline, methyldopa, and hydralazine collected from 2004 through 2014. At DILI onset, 72% had positive ANA levels, 60% had positive smooth muscle antibodies (SMA), and 39% had elevated immunoglobulin G (IgG) levels. The sum of the ANA, SMA, soluble liver antigen (SLA), and IgG was used to calculate an autoimmune score (range 0–8); cases with a score of ≥ 2 were deemed to have an

autoimmune phenotype. An autoimmune phenotype was observed in 82% of the nitrofurantoin cases and 73% of the minocycline cases. Lower percentages were seen with methyldopa (55%) and hydralazine (43%). With resolution of DILI, the number of samples positive for ANA and SMA decreased, as did the autoimmune scores ($p < 0.01$). In addition, the typical HLA alleles associated with iAIH (HLA DR3, DR4) were not present in the DILI patients [16].

Hisamochi et al. enrolled 62 patients with DILI diagnosed using RUCAM who underwent liver biopsy. The patients with histology showing AIH features ($n = 23$) were then compared to those without those histologic findings. They found a mean latency period of 143 days for DIAILD patients compared to 32 days for those without AIH features. The DIAILD patients were also distinguished by higher IgG levels ($p < 0.0001$) and positive ANA titers ($p = 0.003$). Interestingly, after drug discontinuation, the ANA became negative in five out of eight cases when rechecked, and the serum IgG or gamma globulin decreased in all 20 cases on follow-up blood work [17].

Histology

Distinguishing iAIH from DIAILD based on histology can be difficult. As mentioned above, cirrhosis at presentation would favor a diagnosis of iAIH. According to the IAIHG revised original scoring system, the presence of interface hepatitis, plasma cell inflammation, and rosettes are awarded points toward establishing a diagnosis of iAIH (AASLD guidelines). Suzuki et al. performed a histologic evaluation of iAIH and DIAILD cases in a blinded manner by four experienced hepatopathologists. They found that interface hepatitis, focal necrosis, and portal inflammation were present in all cases but were more severe with iAIH ($p < 0.05$). Features that favored iAIH included portal and intra-acinar plasma cells, rosettes, and emperipolesis (an intact lymphocyte within the cytoplasm of a hepatocyte) ($p < 0.02$). Meanwhile, features favoring DIAILD included portal neutrophils and hepatocellular cholestasis ($p < 0.02$). A model combining portal inflammation, fibrosis, portal neutrophils, and hepatocellular cholestasis had an area under the receiver operating characteristic curve (AUROC) of 0.91 in predicting DIAILD versus iAIH (Table 11.3) [18].

Centrilobular zone 3 necrosis may occur in both iAIH and DIAILD. With iAIH, this finding is more often seen in the setting of acute liver failure as the initial presentation [19]. Bjornsson et al. reported that 15 of 23 (65%) patients had zone 3 necrosis on biopsy [15]. This finding would be uncommon in late-onset immune-mediated drug-induced injury [9].

Table 11.3 Histological features of iAIH and DIALD

iAIH	DIAILD	Both
Portal and intra-acinar plasma cells	Portal neutrophils	Portal inflammation
Rosettes	Hepatocellular cholestasis	Interface hepatitis
Emperipolesis		Focal necrosis
Advanced fibrosis more likely	No/minimal fibrosis more likely	

Treatment and Prognosis

The most important step in treating DIAILD is stopping the causative agent. This action alone may lead to resolution of liver injury. Guidelines do not provide detailed recommendations for administering steroids for DIAILD. The EASL autoimmune hepatitis practice guidelines state: "severe AIH-DILI usually responds to high dose steroids in the same way as severe AIH" [20]. A trial of predniso(lo)ne 0.5–1 mg/kg is recommended by EASL for severe disease or if the distinction between DIAILD and iAIH is unclear. If there is a response to steroids, then a proposed algorithm advises a steroid taper, though details regarding the taper are not provided (EASL guidelines). A suggested regimen for steroids is for asymptomatic, mild cases at the outset or if liver tests remain abnormal 3 months after the drug is stopped. Steroids are also recommended at the outset for symptomatic patients with moderate activity. Treatment is continued until symptoms, laboratory tests, and histological findings normalize [9]. In clinical practice, repeat liver biopsies are not often performed for suspected DIAILD cases if the aminotransferases normalize on treatment. While there is little data on its use in DIAILD in the literature, combination therapy with azathioprine is a reasonable option to limit steroid exposure in patients who may be at higher risk of steroid-related side effects (Fig. 11.2).

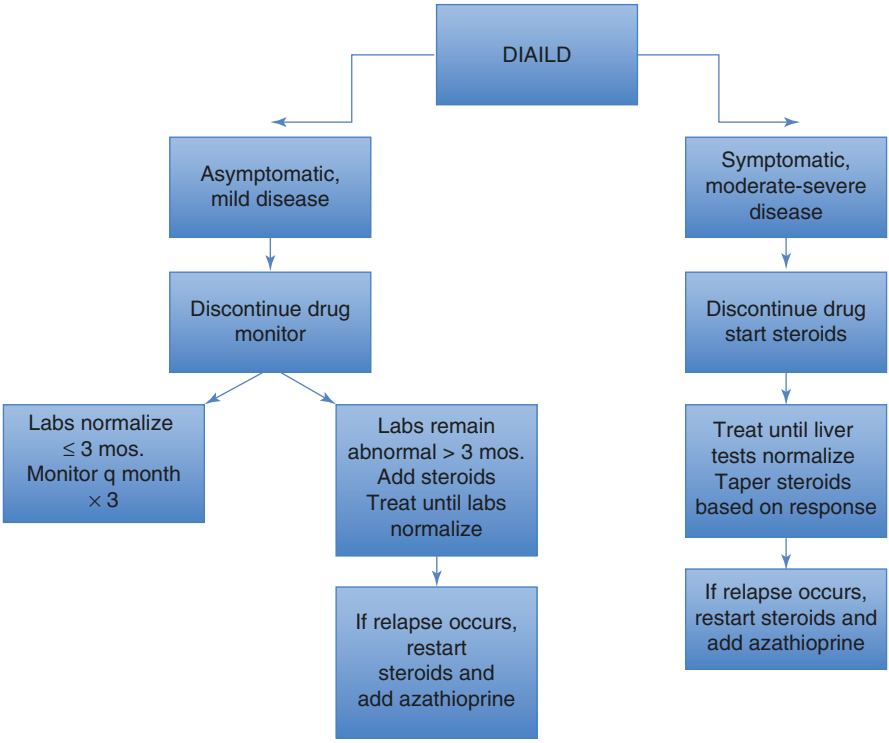

Fig. 11.2 Proposed treatment algorithm for DIAILD

A population-based study evaluated the response to steroids in patients who had DILI with autoimmune features. All patients with nitrofurantoin-induced DILI and nine out of 11 patients with minocycline-induced DILI received steroids. While corticosteroids were efficacious in both the DIAILD and iAIH groups, discontinuation was attempted in 14 DIAILD cases without any relapses, whereas 65% of the iAIH patients relapsed after corticosteroid withdrawal. None of the DIAILD patients progressed to cirrhosis or died from liver disease [15]. This study demonstrated that iAIH and DIAILD can present with similar features but that a trial off immunosuppression is warranted for suspected DIAILD cases.

Nitrofurantoin

Nitrofurantoin is an antibiotic that was introduced in the 1950s to treat acute and chronic lower urinary tract infections. Case reports from as early as the 1960s described acute hepatitis caused by nitrofurantoin [21]. It is one of the more common medications associated with DIAILD. A 2008 study from the DILIN examined 300 patients with idiosyncratic DILI. The most commonly implicated medication was amoxicillin/clavulanate ($n = 23$), followed by nitrofurantoin ($n = 13$), isoniazid ($n = 13$), and trimethoprim-sulfamethoxazole ($n = 13$) [22].

One of the largest case series of hepatic injury from nitrofurantoin and nifurtoinol included 52 cases reported to the Netherlands Centre for Monitoring of Adverse Reactions to Drugs. All patients were treated for urinary tract infections with a daily dose ranging between 100 and 400 mg. In 38 cases, the causal relationship was deemed likely. Most patients were female with a mean age of 64 years. Acute hepatitis was reported in 25 cases with a latency period of less than 6 weeks in 80%. A chronic reaction with positive autoimmune markers (ANA 82%, ASMA 73%) was evident in the other 13 cases. The latency period in this group was >6 months in 85%. Histology was notable for variable amounts of necrosis in the acute hepatitis cases and chronic active hepatitis in the chronic cases. The histologic findings in the acute cases correlates with the imaging findings of confluent fibrosis reported by others [15]. Three of the chronic cases had early cirrhosis. The patients with acute hepatitis generally recovered within 1–3 months after drug discontinuation, while the chronic cases had a longer recovery [14]. The outcomes from nitrofurantoin DIAILD are variable, and there have been fatal outcomes if nitrofurantoin is continued after liver dysfunction develops or if patients are rechallenged [23].

Minocycline

Minocycline is a tetracycline derivative that has been available since 1972 to treat a variety of conditions. One of the main treatment indications is acne vulgaris, which may require prolonged periods of use. Several different minocycline-induced syn-

dromes have been described, including serum sickness, drug-induced lupus, vasculitis, and autoimmune hepatitis [13]. Three patterns of hepatotoxicity have been described with minocycline. The first is hepatic steatosis, which arises following high-dose intravenous administration. This is thought to be a dose-related, direct hepatotoxic effect, which has been described with tetracycline as well [24]. A second reaction is characterized by immunoallergic features (i.e., rash, eosinophilia), which develop within 35 days of starting minocycline. The third presentation, which mimics AIH, occurs with a longer latency (average of 1 year) [25]. A systematic literature review reported on 65 cases of liver disease associated with minocycline; 29 patients had DIAILD. Adverse reactions occurred more frequently in women (58%) and younger patients (94% < 40 years of age). A positive ANA titer was seen in 26 (90%) patients. Recovery with drug discontinuation occurred in all patients in a mean of 14 days (range 4–38 days). Recommendations from LiverTox note that corticosteroids are often given, but their efficacy has not been proven. Rapid tapering of corticosteroids is advised with discontinuation within 3–6 months of initiation [26].

HMG-CoA Reductase Inhibitors (Statins)

Statins are widely used to treat dyslipidemia, thereby lowering cardiovascular morbidity and mortality. They have been associated with mild elevations of aminotransferases in 1–3% of patients, which are often asymptomatic, do not alter hepatic function, and may resolve even with continued use [27]. The literature on clinically significant DILI from statins is mainly comprised of case reports and small case series. A prospective study from the DILIN reported on 22 patients with statin-induced liver injury. Most statins were implicated, and there was not a single distinct phenotype identified. The median age was 60 years, and 68% were female. The latency to onset was variable (ranges 34 days to 10 years, median = 155 days). Nine patients had cholestatic hepatitis and 12 had hepatocellular injury. Of the patients with hepatocellular injury, six (50%) exhibited an autoimmune phenotype with a positive ANA or ASMA >1:80, a liver biopsy suggesting autoimmune hepatitis, or both. Two patients with an autoimmune phenotype developed chronic hepatitis requiring immunosuppressive therapy >6 months after initial onset. This is in contrast to the DIAILD described with the antibiotics mentioned earlier, which typically resolves [28].

Tumor Necrosis Factor (TNF)-α Antagonists

TNF-α antagonists are prescribed to treat inflammatory conditions, including inflammatory bowel disease, rheumatoid arthritis, and psoriasis/psoriatic arthritis. A 2013 study evaluated six patients from the DILIN database with TNF-α hepatotoxicity and an additional 28 previously reported cases. DILI was most common with

infliximab ($n = 26$) but was also reported with etanercept ($n = 4$) and adalimumab ($n = 4$). Median latency was 13 weeks, though 20% had latency periods longer than 24 weeks. Twenty-two of 33 subjects (67%) who had serologic testing were positive for ANA and/or ASMA. Seventeen patients with positive autoantibodies had a liver biopsy, and features consistent with AIH were seen in 15 patients. The autoimmune phenotype was characterized by a longer latency period and more marked hepatocellular injury. Twelve patients received corticosteroid therapy. Treatment with an alternative TNF-α antagonist after DILI resolution can be attempted and seems to be well tolerated [29].

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M. W. Russo (ed.), *Diagnosis and Management of Autoimmune Hepatitis*,
https://doi.org/10.1007/978-3-030-33628-8