Gideon M. Hirschfield • E. Jenny Heathcote
Editors

Autoimmune Hepatitis

A Guide for Practicing Clinicians

Humana Press

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Preface

Autoimmune hepatitis is a relatively rare chronic inflammatory disease of the liver, which is seen in adults and children, women and men, and across the world. It remains somewhat of a curiosity given the lack of specific diagnostic tests. Nevertheless, a pattern of clinical and laboratory presentation, coupled with an absence of alternative etiologies, remains a reliable way for clinicians to identify patients with this disease. Untreated mortality is high from severe disease, but fortunately immunosuppression with steroids and azathioprine in particular is very effective, and the outcomes for patients are now excellent, given appropriate diagnosis and prompt treatment. Nevertheless, with the recognized side effects of treatment it remains important for clinicians to be confident in their diagnosis, and to have clear strategies for how they manage their patients over the long term.

With this in mind we set out to write a clinically useful textbook on autoimmune hepatitis, which hopefully addresses the common concerns encountered in routine practice. We have collected expert opinion from North America and Europe, which jointly collates evidence and practice into one readily accessible volume. Dr. Vierling initiates with a stimulating discussion of the pathogenesis of disease, while Dr. Heathcote reviews patient presentation in the twenty-first century, something that continues to evolve. The use of serology in the diagnosis of disease is then reviewed by Dr. Bogdanos, whose chapter helps clinicians appreciate the benefits and limitations of autoimmune serology. Dr. Michael Manns and Dr. Arndt Vogel provide some biologic insights into mimics of disease, while very practical guidance from expert adult (Dr. Montano-Loza) and pediatric (Dr. Mieli-Vergani and Dr. Vergani) clinicians is presented. Given that there are nuances to care, further insights into strategies for treatment nonresponders is provided by Dr. Peters and Dr. Mileti. Dr. Neuberger gives the perspective of the Transplant Hepatologist, since patients, despite adequate treatment, may still need the life-saving benefits of transplantation, while Dr. Heneghan and Dr. Westbrook help clinicians with the issues raised for patients contemplating pregnancy. Finally, two important chapters conclude this book, the first by Dr. Levy, addressing side effects, and the second by Dr. Boberg, who tackles the often confusing but important area of overlap syndromes.

Readers will therefore see that in assembling this group of authors the editors have set out to provide the breadth of opinion and knowledge that exists on the present day management of autoimmune hepatitis. It remains our hope that this book therefore fills an important niche for those looking after patients with autoimmune hepatitis.

Birmingham, UK Gideon M. Hirschfield

Contents

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Chapter 1
Introduction

Gideon M. Hirschfield

Keywords Immune-mediated liver disease • Liver failure • Immunosuppressive therapy • Hyperglobulinemia

Gastroenterologists and hepatologists are usually charged with looking after autoimmune hepatitis (AIH), but because of its relative rarity few develop sufficient exposure to patients to become true experts. This textbook is an attempt to provide all clinicians with a ready source of information when faced with the challenge of diagnosing and managing patients with this immune-mediated liver disease. As a chronic and relapsing inflammatory disease of the liver, it may equally present as innocent liver biochemical changes, as it can fulminant liver failure. Disease is seen at all ages, in men and women, and across the world. When presenting classically, patients have markedly elevated transaminases, raised globulins, and circulating autoantibodies. The original patient descriptions remain apt even if increasingly patients present earlier in the course of their disease, and noted a predominance of young women, with an insidious, but prolonged and systemic disease, characterized by fever, arthralgias, and amenorrhea. After exclusion of viral, metabolic, and toxic injury, liver biopsy is usually required to confidently confirm the diagnosis, particularly before committing patients to immunosuppressive therapy and its attendant risks. No histologic features are in fact unique to AIH, but a plasma cell-rich interface hepatitis is often described.

Variations to the classic presentation are not that uncommon, and many things may mimic the disease, including notably drug injury and Wilson disease. Such classic descriptions additionally apply to severe disease, but a growing challenge

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is faced by clinicians seeing patients at earlier stages of disease, often without symptoms, or when patients with what appears to be something as common as fatty liver, have "overlapping" features serologically, or histologically that raise the specter of AIH. It is in these scenarios where the various proposed disease scoring systems can be of help.

Historically, hyperglobulinemia was recognized in patients with cirrhosis in the 1940s, and there were early descriptions of liver disease blamed on persistent infection that would fit for AIH with subacute hepatic necrosis. AIH was really "born" as a disease, initially with the given names "chronic active hepatitis" or "lupoid hepatitis," after the first clear descriptions some 60 years ago by Waldenstrom (1950) and Kunkel (1951). The disease became understood as an autoimmune one, albeit with potentially toxic or infectious triggers, and the label "lupoid hepatitis" arose because patients tested positive for LE (lupus erythematous) cells. AIH appears to nestle quite literally between the other two classic autoimmune liver diseases, primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC), a reflection most probably that interface hepatitis, one of the characteristic histologic features, is a common final pathway in liver injury. Yet it remains a somewhat perplexing disease, which lacks a specific diagnostic test, and requires astute and careful patient, laboratory, and histologic evaluation.

The steady improvement over time in understanding this disease (or more likely a group of diseases, that appear as one) mirrors the improved diagnostic tests now core to modern day hepatology – liver biopsy, serum autoantibodies, viral serology, and imaging. Treatment has revolutionized outcomes, but the early descriptions of patients should always remind clinicians of the untreated natural history of severe disease. In the controlled trial of steroids from the Royal Free, the placebo group suffered 15 deaths over 72 months, from a total of 27 patients. The response of patients to steroids therefore, and subsequently other immunosuppressants such as azathioprine, has changed the disease dramatically, and AIH is now one of the most treatment responsive diseases in hepatology. Treatment side effects are not to be forgotten, and therefore careful reflection from the clinician is required before either starting, or indeed stopping, treatment. The exquisite response to immunosuppression intriguingly contrasts with the immune mediated biliary diseases PBC and PSC.

AIH will always be a relatively challenging disease because of its rarity, its varied presentation, the absence of a single diagnostic test, the many potential disease mimics, and the presence of long-term treatment side effects. To the general physician broad guidance is important in helping them care for patients, although perhaps to the frustration of some, every expert has an individualized approach to patient care. Our goal in putting together this short book was to provide a general overview of the clinical aspects that challenge our management of AIH today. We have sought to provide chapters from recognized experts that either alone or in sequence, provide the general reader with an improved understanding of the disease. Duplication is inevitable but repetition does not hurt the practicing clinician, and individuals may wish to read chapters in isolation of others.

Our authors have tried to emphasize the important practical issues faced daily. Collectively, the editors and authors hope readers derive long-lasting value from this small contribution to the field.

Chapter 2
The Pathogenesis of Autoimmune Hepatitis

John M. Vierling

Keywords Innate immunity • Adaptive immunity • Antibodies • Autoantibodies • Endotoxin • Inflammasome • Cytokines • Chemokines • T cells • CD4 T cells • CD8 T cells • B cells • Macrophages • Kupffer cells • Stellate cell • Myofibroblast

Abbreviations

PAMPs	Pathogen-associated molecular patterns
PRRs	Pattern recognition receptors
TLRs	Toll-like receptors
NOD	Nucleotide-binding oligomerization domain receptors
DAMPs	Damage-associated molecular patterns
LPS	Lipopolysaccharide
NK	Natural killer cells
NKT	Natural killer T cells
DCs	Dendritic cells
mDCs	Myeloid dendritic cells
pDCs	Plasmacytoid dendritic cells
APCs	Antigen-presenting cells
Ig	Immunoglobulin
LSECs	Liver sinusoidal endothelial cells

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IL	Interleukin
TCRs	T cell receptors
CDR	Complementarity determining region
aa	Amino acid
CD	Cluster of differentiation
Th	T helper cell
Tr1	CD 4 T regulatory 1 cell
Th3	CD4 Th3 regulatory cell
CTLs	Cytotoxic T lymphocytes
Tregs	Regulatory T cells
IFN	Interferon
FcR	Fc receptor
FasL	Fas ligand
CTLA-4	Cytotoxic T lymphocyte antigen-4
AIRE1	Autoimmune regulator 1 gene
CYP	Cytochrome P450
UGTs	UDP-glucuonosyltransferases
HAV	Hepatitis A virus
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HEV	Hepatitis E virus
EBV	Epstein–Barr virus
HSV	Herpes simplex virus
CMV	Cytomegalovirus

Introduction

Autoimmune hepatitis (AIH) is a progressive necroinflammatory disease of the liver of unknown cause [1]. As indicated by its name, it is regarded as a putative autoimmune disease on the basis of shared features with classical autoimmune diseases and non-autoimmune-mediated inflammatory diseases (Table 2.1). AIH is characterized by a female predilection, genetic factors that influence susceptibility, resistance and disease progression, nonorgan and organ-specific autoantibodies, hypergammaglobulinemia (and/or isolated elevation of IgG), lymphoplasmacytic portal and periportal inflammation, and responsiveness to immunosuppressive therapy [1]. The histopathological hallmark of AIH is interface hepatitis, in which T cells, plasma cells, and macrophages within portal tract infiltrates invade the periportal parenchyma, destroy hepatocytes by causing apoptosis, and secrete cytokines that stimulate fibrogenesis [1, 2].

The pathogenesis of AIH involves dynamic interplay of genetics, environmental exposures, the immune repertoire and dysfunction of immunoregulation (Fig. 2.1) [3–5]. In the absence of a defined etiology, pathogenetic mechanism(s)

Table 2.1 Comparison of autoimmune hepatitis, classical autoimmune diseases, and immune-mediated inflammatory diseases

Feature	Autoimmune hepatitis	Autoimmune diseases	Immune-mediated inflammatory diseases
Disease-specific autoantigenic epitopes	Based on autoantibodies: possible type 1, definite type 2 T cell autoantigenic reactivities poorly defined	Yes	No
Autoantibodies	Yes, type 1 and 2	Definite	Yes
Autoantigen immunization generates auto reactive T cells and/or autoanti bodies and disease in animal models	Yes for type 2 Unclear for type 1	Yes	No
Female predilection	Yes	Yes	No
Afflicts children and adults	Yes	Yes	Yes
Strong HLA associations	Yes	Yes	Yes
Non-HLA genetic associations	Yes	Yes	Yes
Environmental factors	Yes	Yes	Yes
Organ-specific disease	Yes	Yes	Yes
Associated immunological diseases	Yes	Yes	Yes, more limited
Immunopathology	Autoreactive T cells, Ig	Autoantibodies, autoreactive T cells	T cells, activated macrophages
Responsive to immunosuppression	Yes	Yes	Yes
Examples	Not applicable	SLE, MS, myasthenia gravis, Grave's thyroiditis, Type 1 DM	Rheumatoid arthritis, psoriasis, ulcerative colitis, Crohn's disease

Abbreviations: *Ig* immunoglobulin, *SLE* systemic lupus erythematosus, *MS* multiple sclerosis, *DM* diabetes mellitus

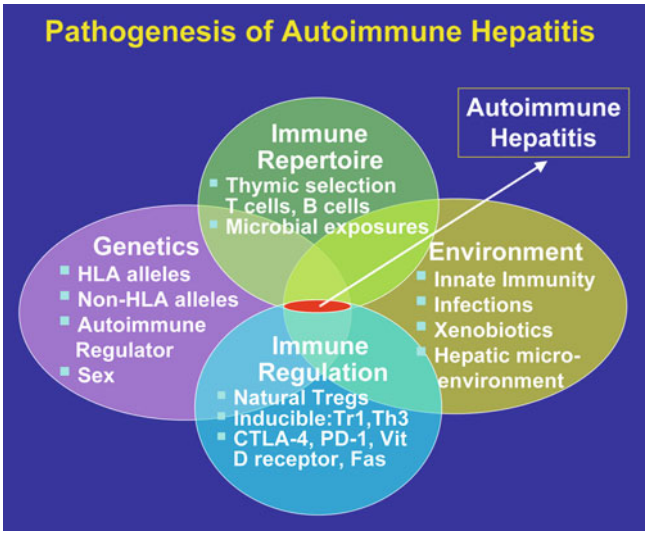

Fig. 2.1 Interactive factors of genetics, immune repertoire, immune regulation, and environment involved in the pathogenesis of autoimmune hepatitis

or a disease-specific diagnostic laboratory test, AIH has been classified into two types on the basis of its autoantibody profiles [1]. Type 1 is associated with ANA and/or SMA, while type 2 is characterized by anti-liver-kidney-microsomal type 1 (anti-LKM1). An autoantibody specific for AIH, anti-SLA, is highly specific for AIH and is observed in a minority of patients with either type 1 and 2 AIH [1, 3].

The Liver as an Organ of Immunity

The liver is now recognized as a primary site of innate immunity and regulation of systemic adaptive immunity [6–9]. Moreover, the innate immune system plays a key role in hepatic inflammation and fibrosis [10–12]. The liver contains large numbers of activated Kupffer cells and immature myeloid DCs (mDCs) and plasmacytoid DCs (pDCs) [13] in addition to a complex repertoire of intrahepatic lymphocytes that vary in quantity, function, and phenotype from counterparts in other organs (Fig. 2.2) [7, 8, 14]. NK, NKT, and γδ (gamma delta) T cells congregate in the liver in proportions far greater than found in blood. In addition, most complement proteins, all acute phase reactant proteins, the majority of circulating growth factors and cytokines are produced in the liver.

The normal liver must achieve a balance between a hyporeactivity to food antigens, intestinal microbial products, and xenobiotics, while remaining capable of robust responses to pathogens and tumors. Intestinal PAMPs in portal venous blood

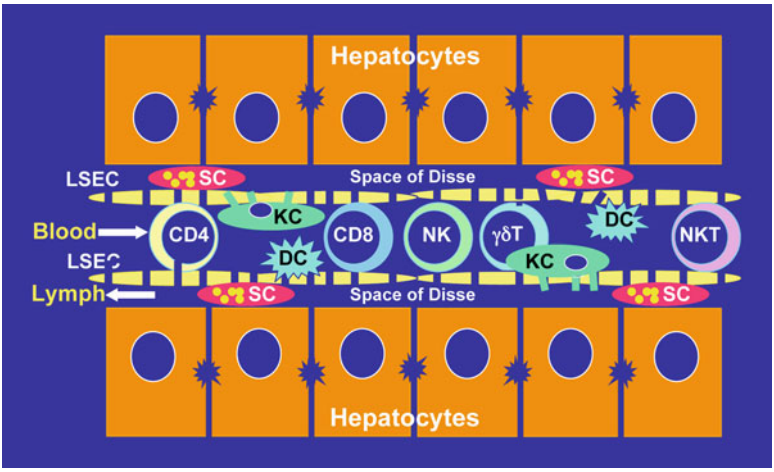

Fig. 2.2 *The Liver as a Lymphoid Organ*. Abbreviations: *LSEC* liver sinusoidal endothelial cell, *SC* stellate cell, *KC* Kupffer cell, *DC* dendritic cell, *NK* natural killer cell, *NKT* natural killer T cell

constitutively activate TLRs on Kupffer cells, Liver Sinusoidal Endothelial Cells (LSECs), hepatocytes, DCs, and stellate cells, while bacterial peptidoglycans activate intracellular NOD proteins [7, 8, 10]. The net effect of these stimuli in the normal liver is NFκB-mediated production of the immunosuppressant cytokine IL-10. While Kupffer cells are hyporesponsive to physiological levels of LPS in portal blood, increased quantities of PAMPs and/or DAMPs result in TLR-mediated Kupffer cell secretion of proinflammatory IL-1β (beta), IL-6, IL-12, IL-18, and TNFα (alpha), along with physiological concentrations of the immunosuppressant IL-10. In contrast, LSECs are invariantly hyporesponsive to normal or pathophysiological concentrations of LPS. Thus, Kupffer cell responses to injurious stimuli change the dynamic balance between immunosuppressive and immunostimulatory cytokines. Hepatic stellate cells (HSCs) located in the space of Disse are the primary source of normal matrix proteins and, when activated, secrete collagen resulting in hepatic fibrosis [12]. TLR-mediated signaling in nonimmune cells, such as hepatocytes and cholangiocytes, also results in production of proinflammatory cytokines and chemokines that may contribute to the pathogenesis of inflammatory liver disease [10]. Finally, Kupffer cells, LSECs, hepatocytes, and HSCs can also function as antigen-presenting cells (APCs) for intrahepatic activation of T cells [7]. Their APC functions coordinate the interplay of innate and adaptive immunity, and the cytokine milieu influences the type and magnitude of the T cell responses, while the migration of antigen-activated hepatic DCs to lymph nodes serve to activate nonhepatic T cells in lymph nodes. These activated T cells subsequently circulate in the blood and enter the portal tracts by transendothelial migration through the portal veins [15].

Pathogenesis of Autoimmune Hepatitis: Interplay of Susceptibility, Immune Responses, and Immunoregulation

The pathogenesis of AIH remains incompletely understood and the subject of investigation in both human beings and animal models. Figure 2.1 illustrates key features of autoimmunity pertinent to the pathogenesis of AIH. It is now clear that the pathogenesis involves the interplay between the innate and adaptive immune responses in the liver (compared in Table 2.2), genetic susceptibility, environmental triggering events, robust T helper cell, T cytotoxic cell and B cell responses to hepatic autoantigens or molecular mimics that are unrestrained by appropriate T regulatory cell control, and an inflamed hepatic microenvironment conducive to progressive fibrosis. Greater detail about the immunology involved in these processes can be found in the Appendix.

Genetic Factors in Pathogenesis

Multiple genes have been implicated in AIH, including those conferring susceptibility or resistance and others related to progression [3, 4]. The involvement of multiple genes indicates that AIH is a complex genetic disorder in which the actions of multiple genes interact to produce and regulate immune responses to environmental agents, such as viruses or drug metabolites or possibly xenobiotics. While the complex interplay of genetics has been associated with susceptibility, resistance, and severity, no single allele appears to be obligatory or sufficient for AIH to develop. Further exploration using genome-wide association scans in patients with AIH will undoubtedly refine and expand our knowledge in the near future.

HLA Class I, II, and III Molecules

Class I HLA

Neither susceptibility nor resistance to AIH is conferred by HLA class I molecules that present antigens to the TCR of CD8 CTLs [3, 4]. Thus, the participation CTLs as effector cells in AIH does not appear to be determined by a genetically restricted TCR repertoire with a propensity for autoreactivity. In contrast, susceptibility to AIH is conferred by class II HLA-DR3 (see details below), which exhibits strong linkage disequilibrium with class I HLA-A, HLA-Cw, and HLA-B molecules. The extended haplotype of this linkage is referred to as A1-B8-DR3-DQ2 and the linked alleles are HLA *A*0101-Cw*0701-B*0801-DRB1*0301-DQA1*0501-DQB1*0201* [3, 4]. Because of this linkage, HLA-DR3 patients preferentially have common class I HLA alleles, which could influence CTL effector function. Class I MICA or MICB genes also encode highly polymorphic ligands expressed by cells damaged

Table 2.2 Comparison of innate and adaptive immunity and role of the liver as an immunological organ

	Innate immunity	Adaptive immunity
Distinctive features		
Onset	Rapid due to preformed receptors for pathogens, endogenous molecules	Delayed due to requirement for antigen activation, clonal proliferation, and maturation of effector cell functions
Specificity	PAMPs, DAMPs, reactive oxygen species, activated complement proteins, apoptotic bodies	Epitopes of peptide antigens recognized by T cell receptors or B cell immunoglobulins
Genetics	Restricted, germline-encoded	Complex with T cell receptors and antigen-binding domains of immunoglobulins produced by somatic recombination of gene segments
Diversity	Limited, evolutionarily conserved	Virtually infinite
Memory	None	Memory T and B cell responses capable of amnestic reactivation
Self-tolerance	Discrimination of pathogens and endogenous DAMPs, rather than autoantigens	Positive and negative selection of T cell receptor and B cell immunoglobulin to have restricted capacity to react with autoantigens
Components		
Physical barriers	Skin, mucosal epithelia, antimicrobial proteins	Intraepithelial lymphocytes of intestine
Cells	Dendritic cells, monocytes, macrophages (Kupffer cells), neutrophils, NK cells, NKT cells	Professional antigen-presenting cells, α/βT cells, γ/δT cells, natural and inducible Treg cells, B cells
Proteins	C' proteins, IFNα,β,γ, cytokines, chemokines	IgM, IgG, IgA, IgE antibodies
Role of Liver as an Immunological Organ	Yes. Dendritic cells, Kupffer cells, neutrophils, NK cells, NKT cells IFNα,β,γ, balance between proinflammatory and immunosuppressive chemokines	Yes. Antigen presentation by hepatocytes, stellate cells, Kupffer cells, LSEC. PD-L1/2 inhibition of activated CD8 T cells, site of CD T cell elimination

Abbreviations: *PAMPs* pathogen-associated molecular patterns, *DAMPs* damage-associated molecular patterns, *IFN* interferon, *NK* natural killer, *LSEC* liver sinusoidal endothelial cell, *PD-L1/2* programmed death ligands 1 and 2

by stress, infection, or neoplasia [16, 17]. When killer receptor NKG2D on NK cells, NKT cells, macrophages, γ/δ T cells, and CD8 T cells bind to MICA and MICB they induce apoptosis of the target cell. MICA and MICB map between the class I HLA B and class III TNF loci. While not associated with classic AIH, MICA alleles are associated with primary sclerosing cholangitis, suggesting a potential role for MICA in the pathogenesis of the PSC-AIH overlap syndrome [18].

Class II HLA

In contrast, both susceptibility and resistance alleles for AIH have been detected among the HLA *DRB1* alleles that encode the β (beta)-chains of the class II molecules that present peptide antigens to the TCR repertoire of CD4 T cells [3, 4]. Allelic differences result in amino acid substitutions in key areas of the floor and wall of the class II HLA antigen-binding groove and so dictate which specific antigens can appropriately bind and align in the groove (Fig. 2.3). Since immunogenetic variations in DRB1 genes dictate the antigenic peptides that can be presented to T cells, their associations with susceptibility and resistance strongly suggest that class II molecules encoded by *DRB1* genes preferentially bind the antigens that incite a CD4 T cell response in AIH (Fig. 2.3, Table 2.3). In addition, *DRB1* alleles may also influence disease severity and the probability of a concurrent extrahepatic autoimmune disease.

Professional APCs simultaneously express class I and II HLA molecules and costimulatory molecules CD80/86 (B7.1/B7.2) and CD40 required for the functional activation of T cells [19, 20]. Positive and negative costimulation is provided by professional APCs to induce and, subsequently, quench adaptive immune responses (Fig. 2.4). Activation of naïve CD4 T helper (Th0) cells is a critical step in cellular immunity because it generates four distinct Ag-specific cells defined by their secretion of mutually exclusive combinations of cytokines (Fig. 2.5) [21].

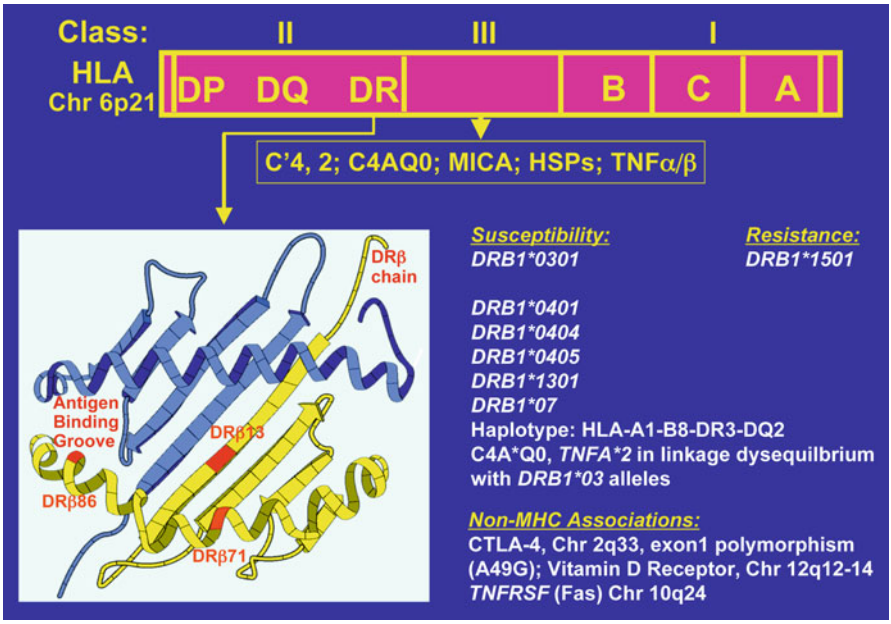

Fig. 2.3 Immunogenetics of autoimmune hepatitis: HLA class I, II, and III regions, locations of amino acid sequence variations in the DRβ-chain associated with susceptibility and resistance alleles, and non-MHC gene associations

Table 2.3 HLA DRB1 alleles associated with autoimmune hepatitis susceptibility and resistance

	aa DRβ13	aa DRβ86	aa DRβ71	Geographic distribution
Susceptibility alleles				
*DRB1*0301*	S	V	K	N. America–Europe
*DRB1*0401*	H	G	K	N. America–Europe
*DRB1*0404*	H	G	R	Japan
*DRB1*0405*	H	G	R	Japan
*DRB1*1301*	S	V	E	S. America
Resistance allele				
*DRB1*1501*	R Japan	V S. America	A N. America Europe	
*DRB1*1302*	–	G S. America	–	

Abbreviations: *aa* amino acid, *N* North, *S* South, *S* serine, *V* valine, *K* lysine, *H* histidine, *G* glycine, *R* arginine, *E* glutamic acid

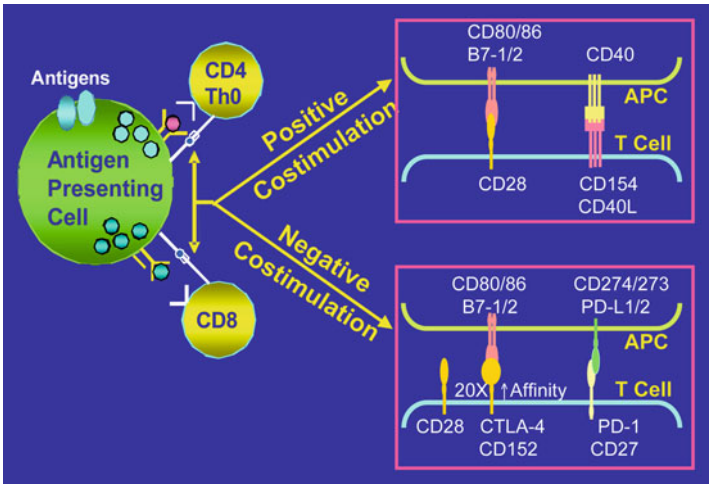

Fig. 2.4 *Positive and Negative Costimulation of T Cell Activation*. Abbreviations: *CD4 Th0* naïve CD4 T cell, *APC* antigen presenting cell

Type I Autoimmune Hepatitis

In North American and European Caucasians, susceptibility to type 1 AIH is associated primarily with HLA-DR3 (encoded by *DRBI*0301*) and DR4 (encoded by *DRB1*0401*) [22, 23]. Both of these β chains share a hexameric LLEQKR (single letter aa code) sequence at positions DRβ(beta)67–72 and have a lysine (K in the single letter aa code) at position 71 (Table 2.3). In this population, the presence of K at position DRβ(beta)71 is required for the binding and presentation of the

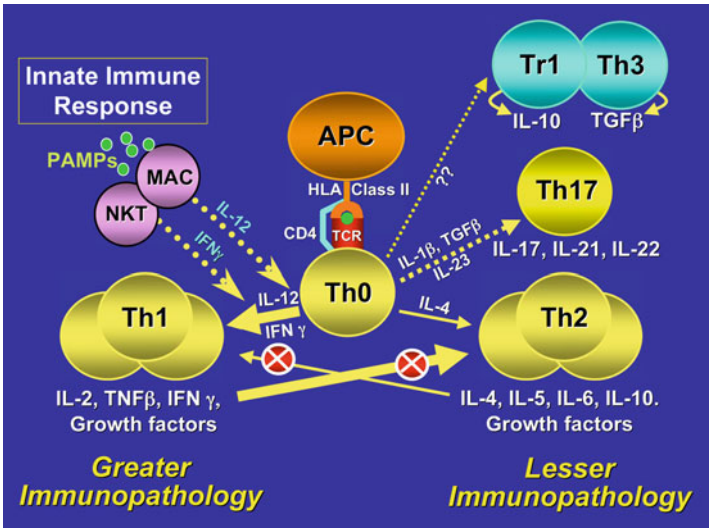

Fig. 2.5 *Activation of naïve CD4 T cells resulting in differentiation into distinct subsets*. The immunopathology of AIH indicates a predominance of CD4 Th1 effects, shown by the width of the arrows. Abbreviations: *PAMPs* pathogen-associated molecular patterns, *MAC* activated macrophage, *NKT* natural killer T cell, *APC* antigen presenting cell, *TCR* T cell receptor for antigen, *Th0* naïve CD4 T helper cell, *Th1* CD4 T helper 1 cell, *Th2* CD4 T helper 2 cell, *Th17* CD4 T helper 17 cell, *Tr1* CD4 T regulatory 1 cell, *Th3* CD4 T helper 3 cell, *IL* interleukin, *IFN* interferon; *TNF* tumor necrosis factor, *TGFβ* transforming growth factor β(beta)

peptide antigen activating CD4 T cells and initiating AIH. *DRBI*0301* is in strong linkage disequilibrium with *DRB3*0101*, which also encodes K at position DRβ(beta)71. Thus, patients with *DRBI*0301* most often have two DRB alleles per haplotype with DRβ(beta)71, which has been hypothesized to explain the greater disease severity observed in these patients based on a greater activation of their immune responses [24]. In contrast, *DRB1*0401* is in strong linkage disequilibrium with *DRB4*0103*, which encodes arginine (R) at DRβ71. In comparison with patients with *DRBI*0301+DRB3*0101*, the single DRβ(beta)71 allele in *DRB1*0401* patients has been cited as an explanation for a lesser disease severity. In addition, the expression of both K and R at position DRβ(beta)71 in patients with *DRB1*0401* and *DRB4*0103* might increase the diversity of peptide antigens presented. This possibility may explain the higher frequency of concurrent extrahepatic autoimmune diseases in this haplotype.

In Japan, Mexico, and China, the DRB1 alleles *DRB1*0405* and *DRB1*0404* are linked to susceptibility (reviewed in [4]). Both alleles share the LLEQ-R motif with *DRB1*0301* and *DRB1*0401* but have arginine (R), rather than lysine (K) at position DRβ(beta)71. Since both K and R are positively charged, the immunogenic peptide antigens binding and aligning at position DRβ(beta)71 have been inferred to be negatively charged amino acids, such as aspartic acid (D) and conversely, class II molecules without a positive charge at position DRβ(beta)71 should be unable to

present the antigens capable of inducing AIH. The fact that *DRB1*1501* confers resistance to developing type 1 AIH in white North Americans and Europeans supports this hypothesis [22, 23]. This allele encodes alanine (A) instead of lysine (K) at DRβ(beta)71, which has a neutral, rather than positive charge. In addition, DRB1*1501 also alters the extended hexameric motif by encoding isoleucine (I) for leucine (L) at DRβ(beta)67. A recent Japanese genome-wide scan identified 9 markers of susceptibility and 17 markers of resistance to AIH, in patients with and without HLA DR4 [25]. None were associated with disease severity.

The association of *DRB1*1301* with AIH in Argentine children and Brazilians argues against the unifying hypothesis that positively charged arginine (R) or lysine (K) at DRβ(beta)71 is required for class II molecules to bind and present the peptide autoantigens involved in AIH [26, 27]. Specifically, *DRB1*1301* encodes a ILEDER motif at positions DRβ67-72 with the negatively charged glutamic acid (E), aspartic acid (D), and glutamic acid (E) at positions DRβ(beta)69, 70, and 71. Thus, peptide antigens presented by *DRB1*1301* class II molecules must be distinctly different than those presented by class II HLA encoded by *DRBI*03*01, *DRBI*040*1, or *DRBI*1301*, and appears to be associated with protracted hepatitis A virus (HAV) infections [28]. A meta-analysis of studies in Latin America reported that HLA *DQ2* and *DR52* were also susceptibility loci, while HLA DR5 and DQ3 were protective loci for AIH [29]. Specifically, the susceptibility alleles were DQB1*02, DQB1*0603, DRB1*0405, and DRB1*1301, while the protective alleles were DQB1*0301 and DRB1*1302. Table 2.3 shows that susceptibility and protection are conferred by valine versus glycine in the DRβ(beta)-86 position, respectively.

The binding of peptide antigens to the antigen-binding grooves of class II HLA molecules involves attachment of several regions of the antigen, designated P1, P4, P6, P7, and P9 [30]. The crystalline structure of a *DRB1*0401* molecule containing a bound peptide antigen indicates the peptide antigen should contain a negatively charged aspartic acid (D) or glutamic acid (Q) at position P4 to optimize the interaction with the positively charged aa at DRB71 [31]. Since the hypervariable region 3 (HVR3) in the class II HLA molecule determines whether it can accommodate a negatively charged antigenic aa at position P4, the HSVR3 may play an important role in the induction of AIH. It is equally important to recognize that class II HLA molecules encoded by alleles other than those with a statistically significant association with susceptibility might also express similarly charged amino acids at P4 and DRB71, resulting in the capacity to present autoantigen [24, 32]. This could explain the occurrence of AIH in patients lacking known HLA *DRB1* alleles for susceptibility.

Type 2 Autoimmune Hepatitis

Susceptibility to type 2 AIH is associated with HLA-DR7 (*DRB1*07*01) in Germany, Britain, and Brazil, while in Spain it is associated with DR3 (*DRB1*0301*) (reviewed in [4]). This paradox may be explained by the fact that both *DRB1**0701 and

*DRB1*0301* share strong linkage disequilibrium with *DQB1*0201*, which may be the unifying immunogenetic determinant of susceptibility, while the contribution of DR7 may be related more to disease severity and progression [33, 34].

Class III HLA

The class III region of the HLA region encodes C′2, C′4, TNFα(alpha)/β(beta), and heat shock proteins. Homozygosity for the C′4 null allele (C4AQ0) is associated with several autoimmune diseases, but its contribution to AIH is minor [35]. TNFα(alpha) is one of the principal proinflammatory cytokines and its genetic polymorphisms have been associated with AIH [36, 37]. TNFα(alpha) production after stimulation of peripheral blood leukocytes with endotoxin varies up to tenfold among normal people. The *TNFA*2* allele commonly found in white northern Europeans is in linkage dysequilibrium with *DRB1*03* alleles. Some studies indicate an association between overproduction of TNFα(alpha) and HLA-DR3, while others do not. However, combined expression of proinflammatory type cytokines (IL-2, IFNγ(gamma) and TNFα(alpha) and type 2 cytokines (IL-4, -5, -6, -8, -10, and -13) is more commonly associated with the extended A1-B8-DR3-DQ2 haplotype and HLA-DR3 than is a pure type 2 cytokine profile. Studies of TNFα(alpha) microsatellite, single nucleotide polymorphisms (SNPs) at position −308 have shown that substitution of adenine for guanine results in constitutive and inducible expression of proinflammatory TNFα(alpha). In North American and European patients with type 1 AIH, this SNP correlated significantly with younger age, *DRB1*0301,* and inferior response to steroid immunosuppression [37]; however, no correlation was observed in Japan [25].

Non-HLA Genes

SNPs in non-HLA genes have also been implicated in the pathogenesis and/or progression of AIH. These genes encode CTLA-4, Fas, vitamin D receptor, and the autoimmune regulator 1 transcription factor.

CTLA-4

CTLA-4 (CD152) is an inhibitory costimulatory molecule induced by activation of T cells to downregulate the activated T cell response by competitively binding to CD80/86 (B7.1/B7.2) on the APC surface with a 20-fold greater affinity than the binding of the activating costimulatory molecule CD28 [38, 39]. Substitution of adenine for guanine at position 49 is strongly associated with autoimmunity, most

likely reflecting inferior ability to downregulate T cell responses to autoantigens and their mimics [39]. The frequencies of the CTLA-4 genotypes vary significantly between North American patients with type 1 AIH and healthy persons [40], but these difference are absent in Brazilians [41].

Cytokines

The functional roles of SNPs in proinflammatory cytokines (e.g., IL-1β(beta), IL-6, IFNγ(gamma)), immunosuppressive cytokines (e.g., IL-4, IL-10, TGFβ(beta)), chemokines, and pattern recognition receptors are under investigation in a variety of diseases. Although they have not been systematically studied, polymorphisms have been identified in AIH for the promoter genes of IL-1, IL-6, and IL-10 [42].

Fas

As noted earlier, binding of FasL (CD178) on CD8 CTLs to target cells expressing Fas (CD95) initiates target cell apoptosis. The Fas gene exhibits multiple SNPs, four of which have been associated with susceptibility for AIH in Japanese [43]. In white North American and European patients, substitution of adenine for guanine at position −670 in the *TNFRSF6* has been associated with earlier onset of cirrhosis, and the adenosine–adenosine or adenosine–guanine genotypes more commonly have cirrhosis than the guanine–guanine genotype [44].

Vitamin D Receptor

A SNP in the vitamin D receptor gene has been reported to be significantly more common in patients with AIH than normal controls [45, 46]. It is now clear that vitamin D and its receptor play important roles in regulation of the immune responses, and that vitamin D deficiency is associated with autoimmunity [47, 48]. Further studies are required to define its potential contribution to the pathogenesis of AIH.

Autoimmune Regulator 1

Approximately 20% of pediatric patients with Autoimmune Polyendocrinopathy-Candidiasis-Ectodermal Dystrophy (APECED) syndrome develop a hepatitis resembling type 2 AIH [49–51]. The syndrome is an autosomal recessive disorder resulting from homozygous mutations in the Autoimmune Regulator 1 (*AIRE1*) gene. The AIRE1 gene is expressed in thymic medullary cells and encodes a transcription factor. Studies of AIRE1$^{-/-}$ knockout mice indicate that the AIRE1

gene prevents organ-specific autoimmunity by promoting expression of peripheral autoantigens in the thymus needed to delete autoreactive T cells. As a result, patients with APECED syndrome have a variety of autoimmune disorders, most commonly hypoparathyroidism and adrenal failure. However, spontaneous mutations in AIRE1 have been reported in three children with severe type 2 AIH and extrahepatic autoimmune disease and four children with type 1 AIH and positive family histories for autoimmunity [51]. The potential contributions of AIRE1 mutations or polymorphisms to the pathogenesis of classical AIH require further study.

Gender

AIH predominates in females with a female to male ratio of 4:1 [1]. Thus, female sex may facilitate, but not fully explain the pathogenesis of AIH. X-linked genetic abnormalities of immune function are generally devastating syndromes, unrelated to autoimmunity [5, 52]. The fact that the female to male ratio is the same for pediatric and adult patients with AIH and that the disease may manifest after menopause argues against estrogen being the primary risk factor [53]. Other hormones, including prolactin, growth hormone, progesterone, and testosterone may, along with estrogen, play roles in the greater immunological reactivity observed in women compared to men. The unique female experience of pregnancy may also facilitate induction or exacerbation of autoimmunity [54, 55]. Studies of fetal microchimerism indicate that it may jeopardize maintenance of self-tolerance; however, there is no evidence for its involvement in the pathogenesis of AIH. Overall, the heightened cellular and humoral responses characteristic of women suggest that female sex results in a heightened initiation response and a reduced immunoregulatory response to autoantigens in AIH.

Environmental Factors in Pathogenesis

Throughout life, exposures to pathogens, drugs, and xenobiotics are involved in the generation of individually unique immune repertoires, including natural and inducible Tregs [56]. The strong associations between the HLA-DR-DQ alleles responsible for the binding and alignment of antigens presented to CD4 T cells indicate that AIH is induced by the presentation of a restricted number of antigens by class II HLA molecules [3, 4]. Indeed, analysis of the TCRs expressed by intrahepatic T cells in AIH shows oligoclonality, which indicates T cell activation by only a small number of antigens [57]. Viral infections and drugs or xenobiotic exposures are primary candidates for the triggering events in AIH, either through molecular mimicry or by presentation of hepatic autoantigens concentrated in apoptotic bodies [58].

Viral Infections

Multiple lines of evidence suggest that hepatic viral infections may trigger autoimmune reactions, including AIH in immunologically susceptible hosts. For example, sensitive techniques have identified ANA and SMA in up to 50% of patients infected with HBV or HCV [59, 60]. Molecular mimicry between the proteins of both HBV and HCV and human nuclear and smooth muscle autoantigens has been identified and may explain production of autoantibodies in these viral infections [59, 60]. In Europe, approximately 10% of pediatric HCV infections are associated with anti-LKM1 autoantibodies [61]. These findings, however, do not imply that the pathogenetic mechanisms of host immune-mediated destruction of hepatocytes infected with HBV or HCV are directed against the autoantigens associated with AIH. It appears more likely that these autoantibodies are epiphenomena dependent on the balance between innate immune reactions and adaptive immune reactions at the time of viral infection. Since viral hepatitis caused by HAV, HBV, HCV, or HEV results in hepatocyte apoptosis, APC uptake of apoptotic blebs containing concentrated autoantigens of organelle membranes may explain the subsequent presentation of multiple hepatocyte autoantigens in class II HLA molecules [58]. In patients with the appropriate HLA DR or DQ alleles for presentation of autoantigens, TCR repertoire capable of concurrent recognition and ineffective immunoregulation, viral hepatitis could trigger AIH. In addition, other nonhepatotrophic viruses, such as mumps, rubella, EBV, CMV, and HSV, cause a transient hepatitis that could also trigger AIH through the same apoptotic mechanism [62]. In support of this hypothesis, viral infections with HAV have been reported to trigger type 1 AIH [63].

HAV infection may play a disproportionate role in the triggering of AIH in Argentine children [28] and Brazilians. As noted earlier, susceptibility for AIH in these groups is associated with the HLA class II allele *DRB1*1301* that has been associated with protracted HAV infection and development of AIH. Thus, it is possible that the *DRB1*1301* allele may confer susceptibility for AIH by encoding class II molecules that are also able to present hepatic autoantigens from hepatocytes infected with HAV.

In the case of type 2 AIH, molecular mimicry between viral antigens and antigenic epitopes of CYP2D6 appears to be very likely. The immunodominant B cell epitope recognized by 93% of patients with type 2 AIH ($CYP2D6_{193–212}$) overlaps with the epitopes recognized by 50% of HCV infected patients that have anti-LKM1 autoantibodies [64, 65]. These antibodies cross-react with homologous regions of both HCV peptides (NS5B $HCV_{2985–2990}$) and CYP2D6 ($CYP2D6_{204–209}$). Similarly, CMV (exon $CMV_{130–135}$) contains epitopes cross-reactive with the immunodominant CYP2D6 epitope [65]. Other antigenic epitopes of CYP2D6 (aa 254–271) also share homologies with HCV (E1 aa 310–324) and HSV1 (IE175 aa 156–175). Cross-reactive epitopes among CYP2D6, HCV, CMV, and HSV suggest that infections with HSV1, CMV, or self-limited HCV might generate cross-reactive antibodies capable of binding to CYP2D6. In the setting of transient liver injury in an

immunogenetically susceptible host, antibody-CYP2D6 complexes could be taken up by APCs, and in a milieu of favorable innate immune reactions, lead to a break in tolerance to both B cell and T cell epitopes of CYP2D6.

Drugs and Xenobiotics as Triggers

Drugs or xenobiotics may also serve as triggers for AIH [66]. In type 2 AIH, hepatocyte metabolism of drugs progresses through formation of metabolites conjugated to CYP2D6. When immunogenetically susceptible individuals experience a conducive hepatic cytokine environment, these metabolites may be immunologically recognized as haptens bound to carrier self-proteins such as CYP isoforms or UDP-glucuonosyltransferases (UGTs). Haptens alone cannot elicit an immune response, but when coupled to a protein carrier can trigger a response that includes cellular and/or humoral responses to the carrier protein portion of the complex. The duration of drug exposure and the number of epitopes recognized by a permissive TCR repertoire could then determine the magnitude of the T cell and B cell responses. Multiple medications, especially minocycline and nitrofurantoin, have been associated with type 1 AIH [67]. However, the role of xenobiotics in triggering type 1 AIH must remain speculative until autoantigenic T and B cell epitopes specific for type 1 AIH are identified.

Two alternative hypotheses of pathogenesis have been proposed: the danger hypothesis and the p-i-concept [68]. The danger hypothesis would require that the driving force in DILI resulting in AIH requires both drug metabolite modification of self-proteins and generation of hepatocyte injury or stress, which constitutes the danger signal. The p-i-concept, which is defined as the "direct pharmacological interaction of drugs with immune receptors," involves direct binding of a drug to the variable regions of TCRs and MHC molecules that trigger TCR signaling and costimulation of the HLA molecules without APCs.

Immune Reactions and Regulation in Pathogenesis

Histopathology

The histopathology of AIH strongly indicates that the principal effector mechanisms of injury are cell mediated (Fig. 2.7). Primary evidence for this comes from immunopathological studies of liver biopsies [2, 69, 70]. These show that AIH is characterized by dense inflammatory infiltrates of the portal tracts, composed of lymphocytes, variable numbers of plasma cells, activated macrophages and rare eosinophils, and an interface hepatitis caused by invasion of periportal hepatocytes with lymphocytes, macrophages, and small numbers of plasma cells [71] (Fig. 2.6). Immunohistochemical analyses have shown that the T cells have α(alpha)/β(beta) TCRs and that CD4 T

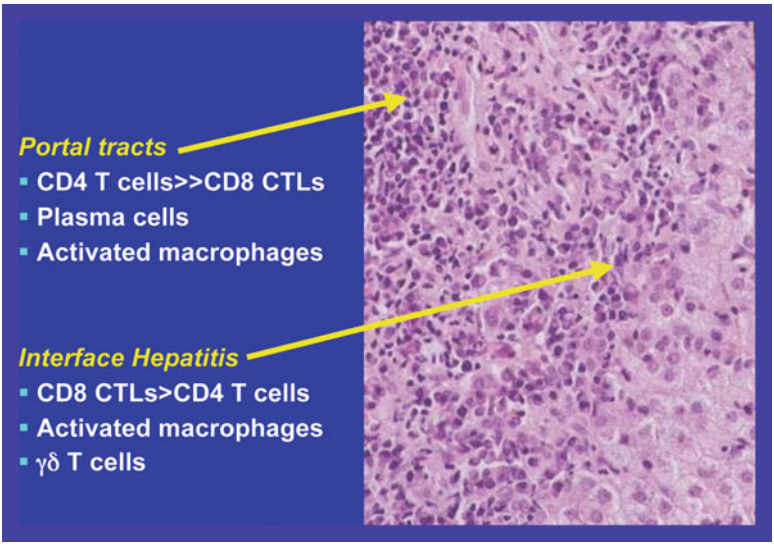

Fig. 2.6 *Immunopathology of portal inflammation and interface hepatitis in autoimmune hepatitis*. Portal inflammatory infiltrates differ in composition from the inflammatory infiltrates involved in periportal interface hepatitis

cells predominate in portal tract inflammatory infiltrates, while CD8 CTLs are the major cell type infiltrating periportal hepatocytes in areas of interface hepatitis [72]. As discussed earlier, the capacity of the portal tracts to serve as a lymphoid organ appears to facilitate localization of inflammatory infiltrates in the portal tracts in AIH, as well as other autoimmune liver diseases and chronic infections with HBV and HCV [73].

Cellular Immunity

Oligoclonal T Cells and Liver-Specific Autoantigens

In AIH, CD4 and CD8 α(apha)/β(beta) T cells are hypothesized to have TCRs that react with a limited number of hepatic autoantigenic peptides that are capable of binding in a genetically restricted number of HLA class II and I molecules [3, 4]. A corollary of this hypothesis is that only a restricted number of TCRs would be able to recognize these autoantigens and that subsequent expansion of a restricted number of clones from these activated CD4 and CD8 T cells would be oligoclonal TCRs. Analyses of the variable (V) regions of TCRs in T cells infiltrating the livers of patients with type 1 AIH support the hypothesis by proving that hepatic inflammatory infiltrates in AIH contain T cell clones with a restricted number of TCRs (reviewed in [57]). In addition, common aa sequences in the complementarity-determining

region 3 (CDR3) of the TCRs were identified in some, but not all patients. This directly supports the notion that type 1 AIH is mediated by a reaction against a restricted number of autoantigens. Since autoimmunity is most often initiated by the loss of tolerance to a single antigen that later expands to include additional autoantigens (a phenomenon called epitope determinant spreading), it is particularly important to assess TCR Vβ(beta) regions in patients with recent onset of type 1 AIH. Studies of such patients have confirmed the expectation that hepatic TCR Vβ(beta) diversity is highly restricted compared to peripheral blood T cells [74]. In addition, TCR CDRs were identical for each patient, but differed among patients. Studies using PCR techniques have also confirmed that oligoclonal T cells accumulate in the livers of patients with type 1 AIH [75]. Of note, nearly all T cells from single clones were CD8 CTLs, while both CD4 and CD8 T cells were typically detected in patients infected with HCV. Another factor in the oligoclonal expansion of liver-infiltrating T cells in AIH is their prolonged survival due to defective apoptosis [76, 77]. Protection from apoptosis appeared to be due to overexpression of antiapoptotic bcl-2, which was observed in CD4 T cells in both the blood and in portal inflammatory infiltrates [77]. Liver-infiltrating CD8 T cells did not overexpress bcl-2 [78].

In type 2 AIH, the immunodominant B cell epitope is $CYP2D6_{193–212}$, and additional minor epitopes have also been defined [66]. It is likely that epitope determinant spreading leads to sequential recognition of the minor autoantigens over time [79]. The CD4 T cell epitope, $CYP2D6_{266–285}$, overlaps with one of the B cell epitopes, $CYP2D6_{257–269}$ [80]. This suggests that the B cell capture and presentation of antigenic peptides to CD4 T cells may augment autoreactivity by expanding the number of autoreactive T cell clones [81]. Autoantigenic epitopes $CYP2D6_{193–212}$, $CYP2D6_{217–260}$, and $CYP2D6_{305–348}$ encompass those recognized by B cells and both CD4 and CD8 T cells [33, 65, 82].

The restricted number of immunodominant hepatic autoantigenic epitopes recognized in the earliest phase of AIH would be likely be presented by an immunogenetically restricted number of HLA class I and II molecules on mDCs. Normally, mDCs take up antigen and migrate to lymph nodes where they present antigens to CD4 Th0 cells and cross-present antigens to naïve CD8 T cells. In the unique immunological environment of the liver, antigen presentation to CD4 Th0 and naïve CD8 T cells can also occur in the sinusoids with Kupffer cells, stellate cells, LSECs, or hepatocytes as APCs [7]. During the chronic phases of hepatic inflammation it is likely that nonautoreactive T cells would also be recruited into the inflammatory infiltrates [83, 84]. This is a plausible explanation for a failure to detect shared CDR3 motifs during later phases of AIH [74]. Moreover, it indicates the need for caution in interpreting the presence of CD8 T cells as evidence of antigen-specific CTLs.

The presence of intense immunopathology in AIH and plasma cells within the portal and periportal inflammatory infiltrates provides circumstantial evidence for a dynamic balance of CD4 T cell mass composed of CD4 Th1 > Th2. While little is known about the role of Th17 T cells in AIH, tantalizing observations suggest that they could play important roles in pathogenesis [85, 86]. As noted earlier, CD4

Th17 cells are proinflammatory cells involved in several types of organ-specific autoimmunity. In human livers, Th17 cells have been observed in chronic graft-versus-host disease [87] and the peribiliary infiltrates of primary biliary cirrhosis [86]. In contrast, Th17 cells selected against self-antigens in the thymus of mice spontaneously migrated to the liver and suppressed chemical-induced hepatitis by secreting IL-22 [88]. The role of Th17 cells in the pathogenesis of AIH is unknown but should be studied.

Role of γ(Gamma)/δ (Delta)T Cells

Although the majority of liver infiltrating T cells express the α(alpha)/β(beta) TCR, the livers of patients with AIH contain larger proportions and absolute numbers of T cells with a γ(gamma)/δ(delta) TCR than observed in other autoimmune or non-autoimmune liver diseases [57, 89, 90]. γ(gamma)/δ(delta) T cell clones established from the liver biopsies of children with AIH exhibited increased non-HLA restricted cytotoxicity against a human hepatoma line, indicating a possible role in non-HLA restricted, non-antigen-specific hepatocytolysis [90]. Involvement of γδ(gamma delta)T cells in the pathogenesis of the autoimmune disease, multiple sclerosis [91] suggests that they may also play a role in AIH.

Role of Macrophages

Activated macrophages are present in portal infiltrates and at sites of interface hepatitis [2, 69–71]. In addition, Kupffer cells in the sinusoids are perpetually activated tissue macrophages [7]. Peripheral blood monocytes from children with AIH have an activated phenotype characterized by overproduction of TNFα (alpha) and IL-10 and overexpression of TLR4, the receptor for LPS [92]. The activation of macrophages in the liver is most likely driven by the proinflammatory cytokine milieu and exposure to bacterial cell wall products, such as LPS, in portal venous blood. Activated macrophages can mediate cytotoxic effects by cell contact or through TNFα-mediated apoptosis of target cells [93]. In addition, activated macrophages may also recruit infiltrating Th17 cells [94].

Pivotal Role of Immunoregulation

The activation of CD4 and CD8 T cells with appropriate positive co-stimulatory signals and antigen recognition by B cells results in a dynamic response of clonal proliferation, maturation, and development of effector functions (Fig. 2.7). While normal immune responses are limited by negative costimulatory signals and natural Tregs, autoimmune diseases are characterized by failure of immunoregulation and perpetuation of immunopathology mediated by CD4 Th1 and Th2 cells, CD8 CTLs, activated macrophages, and autoantibodies.

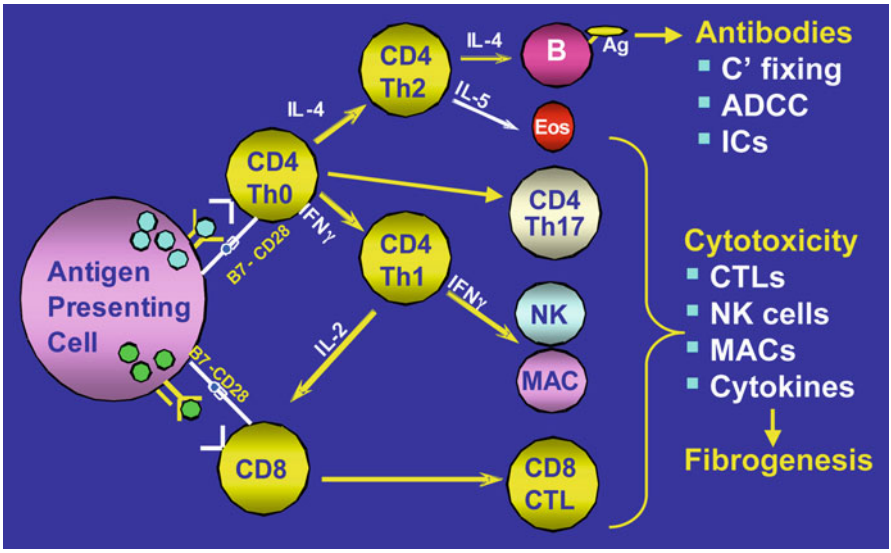

Fig. 2.7 *Generation of functional effector cells and antibodies by an immune response*. The immunopathology of AIH indicates a predominance of CD4 Th1 effects and CD8 CTL activity along with activation of macrophages and plasma cells secreting IgG. Abbreviations: *Th0* naïve CD4 T helper cell, *Th1* CD4 T helper 1 cell, *Th2* CD4 T helper 2 cell, *Th17* CD4 T helper 17 cell, *IL* interleukin, *CTL* CD8 cytotoxic T lymphocyte, *NK* natural killer cell, *MAC* activated macrophage, *B* activated B cell secreting immunoglobulin, *C'* complement, *ADCC* antibody directed cellular cytotoxicity, *ICs* immune complexes

Treg Deficiencies

The hallmarks of autoimmunity are genetic susceptibility for reactions against autoantigens and failure of Tregs to maintain tolerance to autoantigens [5, 95]. Multiple observations indicate that the pathogenesis of AIH involves significant impairment of the numbers of Tregs and their functions (Table 2.4). At the time AIH is first diagnosed, both the quantity and function of natural Treg (CD4+CD25+FoxP3+) cells are deficient [96, 97]. Successful treatment with corticosteroids and/or azathioprine partially restored the numerical and functional deficiencies of natural Tregs [96, 97]. This observation strongly indicates that functional Treg deficiency is produced, in part, by inflammatory disease activity and the magnitude of deleterious effector cell functions. The mechanism(s) of restoration of Treg numbers and functions during immunosuppressive therapy are undefined. In children with either type 1 or type 2 AIH, the quantities of natural Tregs were significantly inversely correlated with disease severity as well as with titers of anti-SLA and anti-LKM1 autoantibodies [97]. The inverse correlation with autoantibody titers has been interpreted as evidence of a pathogenetic role for autoantibodies (see below). Tregs isolated from children with AIH were also profoundly dysfunctional and unable to inhibit secretion of IFNγ by CD4 or CD8 T cells [96, 97]. Thus, deficiencies of natural Tregs facilitate

Table 2.4 Impaired immunoregulation in autoimmune hepatitis

Type of Impairment	Type 1 AIH	Type 2 AIH
Suppressor cell dysfunction [156]	+	?
Defective antigen-specific T cell suppression [157]	+	?
Decrease quantities and functions of CD4/CD25 Tregs [96, 97]	+	+
Defective Treg control of CD4 and CD8 T cells [96, 97]	+	+
Defective Treg promotion of regulatory cytokines [158]	+	?
Defective Treg control of monocytes [92]	+	?

pathological cellular and humoral immune responses in AIH. Even though treatment with immunosuppressive medications partially restored Treg functions, they never reached normal levels, suggesting that an underlying Treg deficiency plays a permissive role in initiation of AIH. The numbers and functions of inducible Tregs have not been reported in AIH.

Restoration of Treg Function

While successful immunosuppressive therapy can increase the quantities and functions of natural Tregs in AIH, their restoration remains incomplete [96, 97]. Natural and/or inducible Tregs are attractive therapeutic candidates for control of AIH because they could control disease without a need for systemic immunosuppression. However, the proliferative capacity of natural Tregs is poor, and apoptosis limits the duration of their function. A recent study, however, proved that natural Tregs can be expanded ex vivo in healthy persons and patients with AIH [98]. Their proliferation was accompanied by increased FoxP3 expression and suppressor functions. In AIH, natural Tregs could also be generated de novo from a subset within a population of CD4+CD25− T cells [98]. The autoantigen specificities of these Tregs remain unknown but could be tested in type 2 AIH, in which the autoantigenic epitopes for CD4, CD8, and B cells are defined.

Humoral Immunity

Role of B Cells and Antibodies

The dual functions of B cells are to produce antigen-specific antibodies and to serve as professional APCs capable of providing the costimulatory signals required for full T cell activation and receiving Th cytokines required for secretion of antibodies. In AIH, B cells receiving appropriate CD4 Th 1 and 2 cytokine stimulation, secrete increased amounts of IgG, resulting in an increased concentration of the IgG isotype and/or hypergammaglobulinemia, which are among the diagnostic criteria for AIH [1].

The spectrum of autoantibodies has defined two types of AIH, and molecular studies with specific autoantigen epitopes [1] (see chapter by Bogdanos). Yet, the role of autoantibodies in the pathogenesis of hepatic injury and inflammation remains unsettled [99]. One viewpoint is that autoantibodies are not involved in hepatocyte cytolysis [4] but may facilitate cellular immune reactions by forming immune complexes that can be phagocytosed, processed, and presented by APCs. In primary biliary cirrhosis, complexes of mitochondrial antigen and autoantibodies enhanced the capture of antigen and augmented cross-presentation by DCs to autoreactive CD8 CTLs [81]. The contrary viewpoint invokes evidence that intracellular antigens have been detected on the surface membranes of hepatocytes that could be targets for autoantibody binding and hepatocyte cytolysis caused by ADCC and/or C′-dependent mechanisms [3].

Regardless of a pathogenetic role for autoantibodies, classical AIH is associated with plasma cells in the portal and interface infiltrates and increased secretion of IgG [1]. Persistence of plasma cells in the portal tracts predicts relapse after withdrawal of immunosuppression [100]. Since plasma cells are terminally differentiated B cells that secrete copious amounts of antibodies for only a few days [101], their presence in portal and interface inflammatory infiltrates indicates that chemokines likely recruit and induce terminal differentiation of B cells [102]. However, portal inflammatory infiltrates containing plasma cells are found not only in AIH but also PBC [103]. However, plasma cells in the livers of patients with AIH predominantly contain IgG, while those from PBC livers contain IgM [103]. Neither the mechanisms responsible for hepatic plasma cell accumulation nor the antigen specificities of their antibodies have been defined.

Autoantibodies and Type I AIH

Both ANA and SMA (with or without f-actin specificity) are non-organ, non-species, and non-disease-specific autoantibodies in type 1 AIH [99]. They serve as indicators of a genetic predisposition to autoimmunity but likely have no pathogenetic role. Autoantibodies reacting with ASGPR, a molecule expressed on the surface of hepatocytes, occur in 50–76% of patients with type 1 AIH (as well as in patients with PBC, PSC, or viral hepatitis) and in 85–88% of patients with active AIH disease [104]. Since the titer correlated inversely with disease activity, it can be used to monitor therapeutic responses [105]. From 65 to 92% of patients with type 1 AIH also have perinuclear antineutrophil cytoplasmic antibodies (pANCA); however, the neutrophil autoantigen(s) actually appear to be nuclear rather than cytoplasmic [106]. Approximately, 10–30% of type 1 patients also have anti-SLA-LP autoantibodies targeting the UGA suppressor tRNA-associated antigenic protein ($tRNA^{Ser/Sec}$) that was recently renamed SepSecS [107]. Anti-SLA autoantibodies are specific for the disease AIH and are found in patients with either type 1 or 2 AIH [99]. In the past, antibodies reactive with so-called Liver Specific Protein (LSP) were implicated in antibody-mediated pathogenesis [3]. However, rather than being a specific protein, LSP contains an array of both liver specific (e.g., ASGPR and alcohol dehydrogenase [ADH]) and non-organ-specific antigens.

Indirect evidence has suggested that autoantibodies against autoantigens on surface of hepatocytes might cause hepatocytolysis, either through ADCC or C′-mediated mechanisms [108]. Evidence that hepatocytes isolated from AIH biopsies were coated with Ig provided circumstantial support [109]. The correlation between the titers of antibodies specific for the ASGPR and ADH and the biochemical and histological severity of type 1 AIH also suggested a potential pathogenetic role [108]. The more severe course of disease in patients with either type 1 or 2 AIH with anti-SLA autoantibodies has also been attributed to a role in pathogenesis [110]. However, it is also plausible that the correlation between severity and autoantibody titers reflects dual consequences of pathogenetic inflammation and cytokines, rather than their cause. To explore the role of autoantibodies in type 1 AIH requires identification of type 1-specific B cell autoantigenic epitopes and studies of animals immunized with the autoantigen(s). Seven human hepatocyte-specific candidate antigens were recently identified that reacted with antibodies in the sera of patients with type 1 AIH [111]. Further characterization of these atypical "autoantibodies" should be performed.

Autoantibodies in Type 2 AIH

In type 2 AIH, primary and secondary B cell epitopes have been identified on the full-length CYP2D6 molecule (see above), and a potential for molecular mimicry with viral B cell epitopes has been defined [3, 4]. Molecular modeling has also shown that the B cell epitopes are exposed on the surface of the intact, conformational CYP2D6 molecule [112]. In contrast to evidence of expression of CYP2D6 on the surface membranes of isolated rat hepatocytes [113], studies using immunoelectron microscopy and flow cytometry failed to detect CYP2D6 on the surface of human hepatocytes [114, 115]. Thus, no direct evidence exists for a pathogenetic role of anti-LKM1 antibodies in human hepatocyte cytolysis. As noted earlier, autoantibodies against "atypical autoantigens" in the plasma membranes of hepatocytes in the sera of type 1 patients might also be present in type 2 disease.

Role of Cytokines in Hepatic Fibrosis and Progression to Cirrhosis

Activation of stellate cells in the periportal, pericellular space of Disse leads to progressive periportal fibrosis in sites of interface hepatitis containing apoptotic hepatocytes, inflammatory effector cells, proinflammatory cytokines, LPS, and reactive oxygen species [12, 116]. Specifically, stellate cells are activated to become myofibroblasts by binding of their innate immune PRRs with apoptotic bodies, DAMPs, LPS, reactive oxygen species and the cytokines IL-10 and TGFβ(beta) secreted by Kupffer cells, LSECs, and infiltrating T cells. Myofibroblasts produce collagen, matrix metalloproteinases that alter and degrade matrix proteins and migrate in response to chemokines. Myofibroblasts also express a high density of

CD1d, indicating that they can activate NKT cell secretion of IFNγ, IL-2, IL-4, TNFα(alpha), G-M-CSF, and chemokines. Proliferation of myofibroblasts is driven by platelet-derived growth factor. Thus, continued periportal inflammation in the hepatic microenvironment creates a positive feedback loop of myofibroblast-mediated fibrosis and stimulation of proinflammatory cytokines results in bridging fibrosis between portal tracts or between portal tracts and central veins. It is likely that the microenvironment within the wave front of fibrosis is less immunosuppressant and more conducive to inflammation than the adjacent hepatic parenchyma. This could explain the phenomenon of bridging fibrosis and, ultimately, its progression to cirrhosis.

A Working Model of AIH Pathogenesis

A working model of the immunopathogenesis of AIH can be constructed that explains the published observations regarding susceptibility, triggering events, autoreactivity, failure of immunoregulation, perpetuation of CD4 Th1, CD8 CTL, and B effector cell responses to autoantigens and cytokine-mediated fibrogenesis required for progression to cirrhosis. This model emphasizes the critical importance of temporal relationships among these events.

Genetic Susceptibility

Since generation of peptide autoantigens for presentation by a restricted number of HLA class I and II molecules on APCs to a restricted repertoire of autoantigen-specific TCRs, immunogenetics involving HLA and non-HLA loci is a key requirement for AIH. Multiple other genetic influences, especially SNPs involved in innate immune responses, likely contribute to the probability of initiating an immune response against an autoantigen.

Evolution of a Permissive Immune Repertoire

While immunogenetics confers the HLA alleles necessary to recognize the putative autoantigen(s) in type 1 AIH and the CYP2D6 autoantigens recognized in type 2 AIH, the immune repertoire, including natural and inducible Tregs, determines whether presentation of autoantigenic peptides by HLA class I and II molecules results in activation of an immune response or anergy. Crucial components of the immune repertoire include (1) the number of functional autoreactive CD4 and CD8 T cells that escaped elimination during negative selection in the thymus; (2) the number of functional natural Tregs with the same autoantigen specificities as the autoreactive

CD4 and CD8 T cells; (3) the number of autoreactive B cells that escaped negative selection in the thymus; (4) the capacity for immunoregulatory expression of suppressive co-stimulatory CTLA-4 and PD-1; and (5) the susceptibility of activated CD4 and CD8 and Treg cells to apoptosis. Since age plays a crucial role in the cumulative generation of an immune repertoire, the status and reactivity of the repertoire most likely differs significantly in childhood compared to adulthood.

Environmental Triggers and Permissive Hepatic Microenvironment

Hepatic viral infections or injurious exposures to xenobiotics or drugs are likely causes of hepatocellular stress, apoptosis, and/or necrosis that provide "danger signals" for initiation of innate and adaptive immune responses. However, the majority of adaptive immune responses in a normal liver are generated in a microenvironment of immunosuppressive IL-10, which biases T cells toward tolerance. Environmental triggers would be more conducive of AIH if they coincided with a period when the cytokine microenvironment favored CD4 Th1 and CD8 T cell activation. Since Kupffer cells respond to LPS and phagocytosed proteins and particles by secretion of proinflammatory cytokines IL-12, IL-18, and TNFα(alpha) and lesser amounts of immunosuppressive cytokine IL-10, their status is a likely determinant of whether a triggering event does or does not occur in a proinflammatory milieu. Production of INFγ(gamma) by intrahepatic NK cells stimulated by hepatic cell injury and by activation of CD4 T cells could help overcome the effects of IL-10 and promote further Kupffer cell secretion of IL-12 and IL-18 required for CD4 Th1 and CD8 T cell effector functions. Differentiated, hepatic-antigen specific CD4 and CD8 effector T cells activated by mDCs in regional lymph nodes would traffic back to the portal tracts and provide positive feedback for a proinflammatory microenvironment.

Presentation of Hepatocyte Autoantigens or Molecular Mimics

Apoptotic bodies or blebs contain high concentrations of nuclear, organelle, and cytoplasmic autoantigens. Thus, receptor-mediated uptake of apoptotic bodies from hepatocytes by professional APCs would be expected to present hepatocyte-specific autoantigenic peptides in HLA class II molecules to CD4 Th0 cells. In addition, autoantigenic peptides would also be cross-presented in HLA class I molecules to naïve CD8 T cells. Alternatively, viral or xenobiotic triggers capable of causing sufficient hepatocyte stress or injury to generate the necessary "danger signals" for an innate immune cellular and cytokine response might have aa sequences or a conformational structure mimicking hepatic autoantigenic peptides. In this case, adaptive immune responses to the mimics would result in cross-reactions of effector CD4 Th and CD8 CTLs with autoantigens.

Activation of Autoreactive T Cells and B Cells

While activation of autoreactive CD4 and CD8 T cells within the hepatic parenchyma by Kupffer cells, LSECs, stellate cells, or hepatocytes could be short-lived and dysfunctional, the generation of autoreactive CD4 and CD8 T cells in regional lymph nodes by mDCs from the liver would be expected to result in trafficking of differentiated, autoreactive effector T cells into the portal tracts. Following initiation of the immune response against autoantigens or mimics, epitope determinate spreading would result in activation of additional T cell clones by a restricted number of additional autoantigens. This would be fully consistent with the observation of both major and minor autoantigenic epitopes in CYP2D6 and the oligoclonality of TCRs observed in patients with AIH.

The type and magnitude of the innate immune response to initial hepatocyte injury (Event 3) and the positive feedback of cytokines from lymph-node activated CD4 and CD8 T cells would dictate the dynamic balance among CD4 Th1, Th2, Th17, or inducible regulatory Tr1 and Th3 lineages toward CD4 Th1. This is most consistent with the intense immunopathology of AIH. Persistence of a population of CD4 Th2 cells would be predicted as a source of cytokines and chemokines for recruitment of B cells into the portal inflammatory infiltrates and for their terminal differentiation into plasma cells. CD4 Th1 predominance would also favor a robust CD8 CTL response against hepatocytes expressing autoantigens in class I HLA molecules. Finally, cytokines produced by CD4 Th1 and Th2 cells would provide continued help for secretion of IgG by activated B and plasma cells.

Production of Autoantibodies

Necrosis of hepatocytes would provide whole autoantigenic peptides to be bound and internalized by B cells bearing autoantigenic epitope-specific immunoglobulin receptors that had escaped negative selection. These B cells would then process and present autoantigenic peptide epitopes to CD4 and CD8 T cells. Presentation of peptide autoantigens to CD4 Th1 and Th2 cells would result in their activation and secretion of cytokines that promote clonal B cell expansion and secretion of autoantibodies.

Failure of Immunoregulation

The fact that AIH is a chronic disease without spontaneous remissions is testimony to the failure of cumulative immunoregulatory mechanisms. The inability to prevent activation of T and B cells to hepatic autoantigens or molecular mimics suggests that patients developing AIH have at least two primary immunoregulatory defects: (1) deficient quantities of functional thymic-derived natural Tregs with hepatic autoantigen specificity; (2) failure of thymic negative selection to eliminate B cells

expressing receptors for hepatic autoantigen epitopes. Perpetuation of chronic inflammation in AIH may also involve defective expression of or response to suppressive costimulatory CTLA-4 and PD-1 and resistance of effector T and B cells.

Intensification of Immunopathology: Bias Toward CD4 Th1, CD8 CTL, and Activated Effector Mechanisms

The immunopathology observed in liver biopsies is indicative of a proinflammatory milieu containing IL-1β, IL-6, IFNγ(gamma), and TNFα(alpha) favoring a predominance of CD4 Th1 and, possibly, TH17 cells over CD4 Th2 cells. Skewing of CD4 Th1 would promote CD8 CTL effector cells, which is consistent with the composition of the portal tract inflammatory infiltrates and the enrichment of CD8 CTLs in the infiltrates at sites of interface hepatitis. Although a role for Th17 cells has not been established in AIH, they could contribute to the intensity of inflammation and perpetuation of dysfunctional immunoregulation. A proinflammatory milieu would also explain the increased numbers of activated macrophages observed in the portal tracts and periportal infiltrates. Variability in the magnitude of the CD4 Th1 versus CD4 Th2 cytokine balance could explain why the numbers of plasma cells observed in the portal tracts or periportal infiltrates are so variable.

Regulation of Hepatocyte Cytolysis

Since all hepatocytes theoretically could express autoantigens recognized by autoreactive CD8 CTLs, global hepatocyte cytolysis could be life threatening. Indeed, this is the case in the minority of patients who present with acute liver failure due to AIH. The majority of patients, however, have a more indolent disease, which may reflect the net effects of Kupffer cell-mediated immunosuppression and apoptosis of liver-infiltrating CD4 and CD8 T cells. Kupffer cells immunosuppress activated CD4 and CD8 T cells by secreting IL-10 and expressing PD-L1/2 (CD274/273) to provide an inhibitory signal to T cells expressing PD-1 (CD27). Kupffer cells also trap T cells and induce apoptosis by either the FasL (CD178) or TRAIL mechanisms. Concurrent loss of natural Tregs and PD-1 inhibitory signaling resulted in lethal intensification of AIH in mice, indicating the importance of control of effector T cells in the hepatic microenvironment [117].

Evolution of Effector Cells and Cytokines

Despite deficits in immunoregulation and failure of appropriate apoptotic elimination of autoreactive CD4 T cells and CD8 CTLs in AIH, it is very likely that chronically inflamed livers undergo changes in the composition and proportion of effector cell populations and cytokines. These changes likely involve relative increases in

antigen-nonspecific effector cells and decreases in CD4 and CD8 antigen-specific T cells. γδ (gamma delta)T effector cells have been cloned from liver-infiltrating inflammatory cells and likely induce apoptosis of hepatocytes exhibiting stress molecules, while providing a perpetual source of proinflammatory IFNγ(gamma) and TNFα (alpha) to subvert the normal immunosuppressive microenvironment of the hepatic sinusoids. In a microenvironment of proinflammatory IFNγ(gamma) and TNFα(alpha) and activated myofibroblasts expressing CD1d, it is also plausible that NKT cells become activated in the periportal sinusoids and secrete additional IFNγ, IL-2, IL-4, TNFα(alpha), G-M-CSF, and chemokines. These in turn would facilitate recruitment and retention of CD8 CTLs without hepatic autoantigen specificity. This speculation is supported by an experimental model in which CD8 CTLs with specificity for nonhepatic antigens migrated to the liver and caused apoptosis of hepatocytes by engaging their Fas (CD95) molecules [118].

Positive Feedback for Fibrogenesis Resulting in Accelerated Bridging Fibrosis and Progression to Cirrhosis

Inflammatory T cells and activated macrophages within the wave front of fibrosis extending from the portal tracts into the hepatic parenchyma would be protected from inhibition provided by immunosuppressant IL-10 and inhibitory PD-L1/2 (CD274/273) and result in intensified inflammation and hepatocyte cytolysis. This in turn would provide the requisite proinflammatory cytokines, reactive oxygen species, and DAMPs to accelerate myofibroblast formation and replication. A positive feedback loop of myofibroblast-mediated fibrosis would result in rapid extension of bridges of fibrosis between portal tracts and between portal tracts and central veins. Continued bisection of the hepatic architecture by bridging fibrosis would ultimately trap hepatocytes and stimulate nodular regeneration characteristic of cirrhosis.

Chapter Summary

The liver is an immune organ, whose microenvironment influences both innate and adaptive immune responses. Genetic factors, both immune and nonimmune, influence susceptibility and resistance to AIH. Environmental triggering events appear to initiate AIH in susceptible persons. Susceptibility requires an immune repertoire capable of responding to hepatic autoantigens or molecular mimics.

Failure of immunoregulatory control of the autoimmune response, especially by T regulatory cells, leads to the development of cytotoxic effector mechanisms and chronic hepatic inflammation.

Hepatic necroinflammation and cytokines induce progressive fibrosis leading to cirrhosis in the absence of immunosuppressive therapy.

Key Messages

1. The liver is an immune organ, whose microenvironment influences both innate and adaptive immune responses.
2. Genetic factors, both immune and nonimmune, influence susceptibility and resistance to AIH.
3. Environmental triggering events appear to initiate AIH in susceptible persons.
4. Susceptibility requires an immune repertoire capable of responding to hepatic autoantigens or molecular mimics.
5. Failure of immunoregulatory control of the autoimmune response, especially by T regulatory cells, leads to the development of cytotoxic effector mechanisms and chronic hepatic inflammation.
6. Hepatic necroinflammation and cytokines induce progressive fibrosis leading to cirrhosis in the absence of immunosuppressive therapy.

Appendix

Immune Responses: A Primer

The following emphasizes general principles pertinent to understanding not only the immunopathogenesis of AIH but also the pathogenesis of other necroinflammatory hepatobiliary diseases [10, 11]. The functions of the human immune response are to (1) recognize, (2) respond, (3) regulate, and (4) remember. These functions require coordinated responses from both limbs of the immune response: the innate or immediate response limb and the adaptive or antigen-specific response of T cells and immunoglobulins (Ig) produced by B cells [119–121].

Innate Immunity

This primal limb immediately reacts against microbial pathogens and cells altered by stress, infection, or neoplasia. Innate immunity is mediated by neutrophils, macrophages, dendritic cells (DCs), natural killer (NK), natural killer T (NKT) cells, antimicrobial proteins, and complement proteins [119, 122, 123]. The innate immune system plays a critical role in the regulation of adaptive immunity and in the development of autoimmune diseases [124, 125]. Importantly, the liver is now recognized to be a preeminent innate immune organ that also plays unique roles in adaptive immunity due to its cellular composition and microenvironment [6–10, 14].

Pattern Recognition Receptors (PRRs) and Pathogen-Associated Molecular Patterns (PAMPs)

Macrophages, including Kupffer cells in the liver sinusoids, and DCs constitutively express evolutionarily conserved PRRs for equally conserved microbial PAMPs. Examples of microbial PAMPs include (1) lipopolysaccharide (LPS, or endotoxin) from cell walls of all Gram-negative bacteria; (2) lipotechoic acid from cell walls of all Gram-positive bacteria; (3) peptidoglycans, essential components of cell walls of all bacteria; (4) unmethylated, bacterial CpG dinucleotides; and (5) single- and double-stranded viral RNA. A family of PRRs, called "toll-like receptors" (TLRs), react with extracellular and intracellular microbial PAMPs [10]. Cytoplasmic PRRs called nucleotide-binding oligomerization domain (NOD) proteins react with intracellular bacterial PAMPs [126]. Other PRRs act as receptors for C′-coated pathogens or cells [123], and still others mediate phagocytosis of apoptotic bodies [58].

Damage-Associated Molecular Patterns

Damage-Associated Molecular Patterns (DAMPs) are nonmicrobial, endogenous molecules released from injured or necrotic (as opposed to apoptotic) cells that also engage and signal through TLRs, as well as intracellular inflammasomes [127, 128]. Inflammasomes are cytoplasmic multiprotein complexes that act as molecular scaffolds for the activation of caspases required to generate proinflammatory cytokines IL-1β and IL-18 [129]. Whereas initiation of immune responses have been attributed historically to microbes, it is now clear that DAMPs and inflammasomes activated by endogenous stimuli play seminal roles in coordination of initial immune responses to "danger signals" and genetic predisposition to autoinflammatory diseases [130]. The inflammatory response to DAMPs is central to the Danger Signal Hypothesis of adaptive immune responses, which postulates that immune reactions are triggered by APCs responding to a microenvironment of cell injury comprising reactive oxygen species, stress proteins, and necrotic debris [131]. These mechanisms are involved in the persistence of necroinflammation in sterile environments.

Proinflammatory and Immunosuppressive Cytokines

Activation of DCs and macrophages (including Kupffer cells) by PAMPs and/or DAMPs stimulates phagocytosis and generates production of chemokines and proinflammatory cytokines IL-1β(beta), IL-6, IL-12, IL-18 and TNFα and immunosuppressant cytokine IL-10 [11, 93, 119]. Bacterial peptidoglycans that enter the cell cytoplasm react with NOD proteins to induce immunosuppressive IL-10, which antagonizes both the production and function of proinflammatory cytokines [10]. Thus, a dynamic balance between production of cytokines promoting and inhibiting inflammation ensues.

NK Cells

NK cells lack T cell receptors (TCRs) for antigen recognition but have killer inhibitory receptors that prevent them from killing normal cells [16]. The major histocompatibility complex (MHC in all mammals and designated as HLA in human beings) class I genes, designated MICA or MICB (MHC class I chain-related genes A and B) encode highly polymorphic ligands that are expressed by cells damaged by oxidative stress, infection, or neoplasia. Natural killer group 2 member D receptors (NKG2D; found on NK cells, as well as NKT cells, macrophages, γδ(gamma delta) T cells, and CD8 T cells) bind to MICA and MICB ligands and induce apoptosis [16, 17]. Activated NK cells also secrete interferon-gamma (IFNγ), contributing to a proinflammatory milieu [132]. NK cells induce apoptosis by releasing perforin to open pores that target cell membrane and granzymes (serine proteases that enter the membrane pores and activate intracellular cysteine proteases resulting in a caspase cascade causing apoptosis). NK cells, along with macrophages and NKT cells, also express Fc receptors (FcR) for the Fc portions of antibodies activated by binding to cell surface antigens. FcR binding induces NK cell cytolysis of the target cell, a process called antibody-dependent cellular cytotoxicity (ADCC) [16].

NKT Cells

NKT cells exhibit properties of both NK cells and T cells [133]. The most common NKT cells have αβ(alpha beta) TCRs, which have invariant specificity for lipids and glycolipids presented by CD1d molecules on host cells. Activated NKT cells secrete large amounts of cytokines and chemokines, including IFNγ(gamma), interleukin-2 (IL-2, the primary mitogen for proliferation of activated T cells), IL-4, tumor necrosis factor alpha (TNFα(alpha)), granulocyte-macrophage-colony stimulating factor (G-M-CSF), and chemokines (chemoattractant cytokines). The magnitude and duration of NKT cell secretion of these cytokines can greatly influence the type of adaptive immune responses generated in the vicinity.

Adaptive Immunity

This represents the response of T cells and B cells to specific foreign or autoantigens (Table 2.2 and Fig. 2.7). The response of T cells is often called cell-mediated immunity and that of immunoglobulins (Ig) produced by B cells is referred to as humoral immunity. Normally, DCs and B cells act as APCs in lymphoid organs, and activated T and B cells subsequently enter the circulation and migrate through the tight junctions of endothelial cells to enter tissues or organs to mediate effector cell functions. In contrast, the liver is an active immunological organ with dynamic

interplay between innate and adaptive immunity, which included the ability to directly activate CD4 and CD8 T cells in the liver and to generate DCs that have phagocytosed hepatic antigens to activate CD4 and CD8 T cells in lymph nodes.

T Cell Receptors and HLA Class I, II, and III Molecules

T cells express TCRs that interact with peptide antigens presented in the antigen-binding grooves of HLA class I and II major molecules on professional APCs [19, 120, 121]. Professional APCs include activated macrophages, including Kupffer cells, DCs, activated B cells and in the liver, both LSECs and cytokine-activated hepatocytes [7, 8]. CD4 TCRs react exclusively with exogenous antigenic peptides presented in the antigen-binding grooves of HLA class II molecules, while CD8 TCRs react to endogenous (including viral) antigenic peptides presented in the antigen-binding grooves of HLA class I molecules [134]. Most T cells have TCRs composed of α(alpha) and β(beta) chains and are called αβ (alpha beta)T cells. A minority of T cells have TCRs composed of γ(gamma) and δ(delta) chains and these γδ(gamma delta) T cells bridge innate and adaptive immunity by recognizing antigenic molecules without HLA restriction [135, 136].

All TCRs contain an amino acid (aa) sequence in their variable (V) regions called a complementarity determining region (CDR). It is the aa sequence of CDR that dictates the TCR specificity for peptide antigens presented by HLA class II and class II molecules. Similarly, the aa sequences of the antigen-binding groove and walls of HLA class I and II molecules determine which antigenic peptides can be bound and presented to TCRs. Since these sequences are encoded by highly polymorphic alleles, the capacity to present autoantigens to T cells is strongly related to the genetics of HLA genotypes [56]. In addition, only a restricted number of autoreactive T cells would be expected to have CDRs capable of binding autoantigens.

The class III HLA region also encodes proteins important for the innate immune response, C′ proteins 4 and 2, MICA, MICB, heat shock proteins, and TNFα(alpha) [137]. Having the null allele for C′4 (C4AQ0) is strongly associated with autoimmune diseases [95].

Selection of the Immune Repertoire and Natural T Regulatory Cells

Immature T cells bearing both CD4 and CD8 co-receptors are exposed to a wide variety of autoantigens in the thymus. TCRs that bind either too weakly or too strongly to autoantigen–HLA complexes are deleted [138]. Thus, surviving T cells have intermediate capacities to react with autoantigen–HLA complexes. This confers a potential risk for autoimmune reactions in everyone. Surviving T cells then differentiate into

either CD4 or CD8 T cells based on whether their TCR recognizes antigen presented by class II HLA (CD4) or class II HLA (CD8). Prior to the final conversion from CD4 and CD8 positivity to CD4 T cells, a variable proportion of the T cells begin to express repressor forkhead winged helix transcription factor box (FoxP3) and later differentiate into natural CD4 T regulatory (Treg) cells with the phenotype CD4+, CD25+, FoxP3+ [139]. CD25 is the receptor for the α(alpha)-chain of the T cell mitogenic cytokine IL-2. FoxP3 expression, the key determinant of natural Tregs, is subject to epigenetic control, which allows altered gene programs to be inherited by progeny cells [140]. Whether epigenetics contributes to dysfunction of Tregs associated with AIH is unknown. In theory, each TCR is expressed by both T cells capable of becoming effector cells as well as natural Tregs capable of suppressing each antigen-specific activated effector cell.

From birth onward, the interplay between innate and adaptive immune responses to environmental stimuli of PAMPs and DAMPs [124] molds unique immune repertoires, even in monozygotic twins. The most robust immune repertoires result from multiple microbial exposures early in life and are associated with protection from autoimmunity and allergy [95]. In contrast, reduced microbial exposures early in life have been associated with evolution of an immune repertoire with higher risk of both autoimmunity and allergy [141].

Costimulation

Positive costimulation results in functional T cell activation results and from the binding of T cell receptors CD28 or CD152 (aka CD40 ligand, CD40L) to APC costimulatory molecules CD80/86 and CD40, respectively. Costimulated functional T cells subsequently express the negative costimulatory molecule cytotoxic T lymphocyte antigen 4, CTLA-4 (CD152), which competes for binding to CD80/86 with a 20-fold higher avidity than CD28 and attenuates T cell activation. In addition to CTLA-4, costimulated T cells subsequently express a second negative costimulation molecule, programmed cell death-1 (PD-1, CD27), conferring an exhausted, hypofunctional T cell phenotype subject to functional inactivation after engaging with PD-ligands1/2 (PD-L1/2, CD274/273).

Dynamic Family of CD4 T Cells

Differentiation into specific subsets is controlled by lineage-specific transcription factors, which are under epigenetic control [142]. Thus, programs of gene expression altered by epigenetics can be inherited by progeny cells. Th0 conversion to Th1 is stimulated by exogenous proinflammatory cytokines and LPS [21, 143]. Th1 cells secrete cytokines IL-2 (the most potent mitogen for proliferation of CD4 and CD8 T cells), IFNγ(gamma), and TNFβ(beta). In contrast, Th0 to Th2 conversion is

induced by exogenous IL-4 and Th2 cells secrete IL-4, 5, 6, 10, and 13. Th0–Th17 conversion is stimulated in a microenvironment containing a combination of TGFβ, IL-1β, and IL-23. Th17 cells express IL-23 receptors and secrete IL-17, IL-21, and IL-22. Activation by a tolerogenic subset of pDCs converts Th0 to inducible Treg cells that secrete either immunosuppressive IL-10 (Tr1 cells) or TGFβ(beta) (Th3 cells) [144, 145].

The mutually exclusive cytokines secreted by Th1 and Th2 subsets create a dynamic Th1/Th2 balance by inhibiting both the proliferation and cytokine secretion of the other subset. Th1 cytokines promote proliferation of CD8 cytotoxic T lymphocytes (CTLs), Th2, Th17, and inducible Treg cells. In contrast, Th2 cytokines activate eosinophils and mast cells. Both Th1 and Th2 cells stimulate B cells to secrete antibodies: Th1 cells induce C'-fixing IgG2a antibodies, while Th2 cells induce IgG1 and IgE. Th1 cells are preferentially produced in microenvironments with LPS and active innate immune production of IL-12, IL-18, and INFγ(gamma). The Th1 cytokine IFNγ(gamma) also increases secretion of IL-12 by DCs and macrophages, creating a positive feedback loop promoting Th1 dominance.

Th17 cells augment inflammation and tissue damage in the autoimmune diseases, multiple sclerosis, type 1 diabetes and primary biliary cirrhosis, as well as in immune-mediated inflammatory disorders, such as rheumatoid arthritis, psoriasis, and inflammatory bowel disease [85, 86]. Secretion of IL-17 recruits neutrophils for clearance of pathogens, while IL-21 and IL-22 stimulate epithelial cell production of antimicrobial proteins and inflammatory mediators. Thus, Th17 cytokines generate intense inflammation and tissue injury. Both IFNγ and IL-4, the primary cytokines produced by Th1 and Th2 cells, downregulate Th17 differentiation. Thus, a dynamic balance is established among Th1, Th2, and Th17 cells within sites of inflammation.

Inducible Treg cells generated by antigen-specific activation of CD4 T cells in the liver play immunoregulatory roles in animal models [144]. However, their role in humans remains debated [145].

CD8 T Cells

Activated CD8 T cells are predominantly antigen-specific cytotoxic T lymphocytes (CTLs) [120, 121]. Naïve CD8 T cells are activated by TCRs reacting with specific peptide antigens presented in the antigen-binding grooves of class I HLA molecules of professional APCs. Since HLA class I molecules consistently contain endogenous, processed, self-proteins, the HLA class I antigen-binding grooves are always occupied. Cells infected with a virus also process and present viral antigenic peptides in HLA class I molecules. Professional APCs, including DCs, activated macrophages, Kupffer cells, LSECs, and B cells, can also phagocytose *exogenous* antigens and present their processed peptides to CD8 T cells in HLA class I molecules. This phenomenon of cross-presentation is an important mechanism of CD8 T cell activation in the liver [7].

With appropriate positive costimulation and IL-2 and growth factors produced by CD4 Th1 cells, CD8 T cell clones proliferate and differentiate into antigen-specific CTLs. A CD8 CTL does not require further costimulation to cause apoptosis of target cells by either the perforin-granzyme or the Fas-mediated mechanisms. While the perforin-granzyme mechanism of cytotoxicity predominates outside the liver, hepatocyte expression of inhibitory serpins limits this mechanism in the liver. Thus, CTL FasL (CD178) binding to target cell Fas (CD95) plays a dominant role in the apoptosis of Fas-expressing hepatocytes.

Recently, CD8 T cells with innate immune functions, non-antigen-specific cytotoxicity and suppressor Treg functions have also been identified [84, 146, 147]. These non-antigen-specific, non-cytotoxic, cytokine-secreting innate CD8 T cells have been detected in chronic inflammatory infiltrates. These observations indicate that caution is necessary in assigning a cytotoxic role to CD8 T cells identified by phenotype in sites of inflammation.

γ(Gamma)/δ(Delta) T Cells

Approximately 2–3% of T cells express a γδ (gamma delta) TCR, and they are most abundant in gut mucosa, skin, genitourinary tract, and liver. Normally γδ(gamma delta) T cells comprise 15–25% of T cells in the liver [7, 8]. γδ(gamma delta) T cells bridge innate and adaptive immune responses by being non-HLA restricted and capable of recognizing bacteria and fungi, nonpeptide microbial metabolites, and both stress-related and neoplastic cellular proteins [135, 136]. They kill by release of perforin-granzyme and secrete proinflammatory IFNγ(Gamma) and TNFα(Alpha). Recently, γδ(Gamma delta) T cells were noted to be involved in the pathogenesis of the autoimmune disease, multiple sclerosis [91], and their isolation from liver infiltrating T cells in children with AIH suggests a pathogenetic role [148].

B Cell Activation and Functions

The dual functions of B cells are to produce antigen-specific antibodies and to serve as professional APCs capable of providing the costimulatory signals required for CD4 and CD8 T cell activation [149]. Naïve B cells express immunoglobulin (Ig) on their cell surfaces that serve as receptors for an antigenic aa sequence or epitope on unprocessed foreign or autoantigens. The epitope specificity of each unique Ig is generated by recombination of gene segments that produce an Ig repertoire capable of binding a near infinite variety of epitopes. Thus, the Ig of each B cell is analogous to the TCR of each T cell, and, like TCRs, the Ig repertoire is selected against autoantigens and B cells with autoreactivity are deleted.

Binding of a specific antigen epitope to the B cell Ig initiates two events: (1) phagocytosis and processing of the entire antigen for presentation as peptides to CD4

and CD8 TCRs; (2) secretion of epitope-specific antibodies. When the antigen-HLA class II molecule on the B cell activates the TCR of a CD4 T cell, the subsequent Th1 and Th2 cells produce cytokines necessary for the B cell to secrete a soluble form of its surface Ig receptor, an epitope-specific antibody. Since whole antigens contain many peptides, the epitopes recognized by antibodies usually differ from those activating the TCRs of CD4 and CD8 T cells. Thus, it is possible to have autoantibodies and T cell reactions against different epitopes of the same macro-antigen. Conversely, T cells may react to autoantigens in the absence of autoantibody production against overlapping B cell epitopes.

Three other features of B cell responses are important in the coordination of innate and adaptive immune responses and disease pathogenesis. First, certain types of IgG and all IgM antibodies can activate C′ after they bind to their antigenic epitopes [150]. Thus, binding of these antibodies to an antigen on the surface of a cell or a pathogen causes C′-mediated lysis and can injure innocent bystander cells. In the liver, such collateral damage may provide a "danger signal" for additional inflammation [131]. Second, IgG antibodies bound to antigenic epitopes become recognizable by a family of Fc receptors on NK cells, macrophages, DCs, neutrophils, and mast cells. When Fc receptors of NK cells or macrophages engage IgG bound to a cell surface antigen, they kill the cell through ADCC. Fc receptors on macrophages also trigger production of proinflammatory cytokines. Third, binding of an antigen–antibody complex to the Fc receptor on DCs or macrophages (FcγRI or CD64) leads to phagocytosis and peptide antigen presentation that enhances the generation of effector CD8 T cells [81].

Generation of T and B Effector Cell Responses

The effector functions of T cells expressing PD-1 (CD27) can be functionally inactivated in the liver after engaging PD-L1/2 (CD274/273) expressed on Kupffer cells and stellate cells [7].

Immunoregulation of Immune Responses

The ability to regulate and/or terminate immune responses is a key requirement for immune responses and maintenance of self-tolerance. Inhibitory costimulation through T cell CD152 (CTLA-4) and PD-1 is important in extinguishing immune responses to exogenous antigens. In addition, natural Treg [139] and inducible Treg cells [144, 145] play pivotal roles in terminating T cell-mediated reactions and suppressing autoreactive T cell clones that escape deletion in the thymus. Recently, CD8 Tregs have also been identified and γδT cells may also mediate immunosuppression [146].

T Regulatory Cells

Natural Tregs are CD4+CD25+FoxP3+ and following selection as autoantigen reactive T cells in the thymus, they migrate to peripheral tissues [139]. As noted earlier, the expression of FoxP3 in natural Tregs is under epigenetic control, which might influence peripheral function of natural Tregs in AIH [140]. Inducible Tregs, designated as T regulatory 1 (Tr1) cells secreting IL-10 and T helper cell 3 (Th3) cells secreting TGFβ(beta), are generated from naïve CD4 Th0 cells activated by a subset of tolerogenic DCs that induce production of IL-10 and tolerogenic costimulatory molecules [144, 145]. IL-10 inhibits production of proinflammatory TNFα(alpha) and IL-12, while TGFβ(beta) inhibits Th1 responses and CD8 CTLs through its effects on the transcription factors and cytokines. In addition, non-antigen-specific CD8 Tregs secreting either IL-10 or TGFβ(beta) or γδ(gamma delta)T cells secreting both IL-10 and TGFβ(beta) can suppress experimental murine autoimmune diabetes and antitumor activities of CD8 CTLs and NK cells [146]. Generation of non-antigen-specific CD8 Tregs is favored by a milieu containing IL-10, a principal cytokine in the hepatic lobule [8]. Since NKT cells and CD4 Th2 cells also secrete IL-10, they likely contribute to immunosuppressive regulation of cellular immunity. Since CD4 Tregs control the magnitude and duration of a cellular immune response, protracted immunopathology in AIH is indicative of inadequate Treg cell function.

Regulatory Dendritic Cells

Activation of mature mDCs leads to processing and presentation of peptide antigens and secretion of proinflammatory cytokines required to generate an adaptive T cell response. However, the hepatic microenvironment disproportionately contains immature mDCs and pDCs, which are poor stimulators of naïve T cells [7, 8, 13]. pDCs produce large amounts of IFNα(alpha)/β(beta)/γ(gamma) and proinflammatory cytokines TNFα(alpha) and IL-6 involved in autoimmune diseases [151]. A subset of hepatic DCs in mice, referred to a liver regulatory DCs (LRDCs), can inhibit CD4 T cell proliferation through expression of CD274 (PD-L-1) and secretion of prostaglandin E2 and IFNγ [152]. Infused LRDCs effectively inhibited AIH in a murine model, indicating a capacity for intrahepatic homing and immunoregulation. No human counterparts of LRDCs have been reported.

Th17 Cells and Immunoregulation

The interplay between natural Tregs and Th17 cells has been only partially defined, but it appears to influence immunoregulation [85, 153, 154]. For example, IL-2, the cytokine required for Treg proliferation and survival, suppresses Th17 differentiation.

Conversely, the presence of proinflammatory IL-1β(beta) can negate IL-2 suppression of Th17. Of greater importance are the observations that Tregs can convert to Th17 cells in sites of inflammation and that retinoic acid from gut DCs can abrogate inflammation by suppressing Th17 cells and increasing Treg cells. In contrast to the general view that Th17 cells only promote inflammation, natural Th17 cells selected in the mouse thymus actually downregulate peripheral inflammation [88]. These thymic Th17 cells migrated spontaneously to the liver, gut, and lung. In the liver, Th17 secretion of IL-22 prevented experimental hepatitis caused by galactosamine and LPS. Thus, specific populations of Th17 cells with hepatotrophism and capacity to suppress hepatic inflammation may be selected by exposure to self-antigens in the thymus.

Liver as an Organ of Adaptive Immunity

In addition to being a primary organ for innate immunity, the liver also plays important roles in adaptive immunity. Importantly, the portal tracts represent a distinct "lymphoid" compartment into which activated T cells are recruited by resident APCs [73]. The microenvironment of the portal tracts supports the accumulation of functional T cells, B cells, DCs, and macrophages and may explain why portal inflammatory infiltrates are observed universally in chronic, inflammatory liver diseases [7]. Along with abundant mDCs, pDCs, Kupffer, NK, and NKT cells involved in innate immunity, the normal liver also contains enriched populations of CD8 αβ(alpha beta) T cells, activated CD4 and CD8 T cells, γδT cells, memory T cells, and B cells [7, 8]. The percentage of highly activated hepatic T cells is higher than in peripheral blood, but naïve T cells and B cells are underrepresented in the liver.

Multiple hepatic cells serve as APCs for T cell activation, including several hepatic subsets of DCs, hepatocytes, LSECs, and stellate cells [7, 8]. The architecture of the hepatic sinusoids permits T cells to make direct contact with not only Kupffer cells, LSECs, and a variety of DCs but also with hepatocytes and stellate cells through the fenestrations of the LSECs. Hepatic DCs have immature phenotypes consistent with the fact that they are less immunogenic than DCs in other tissues. However, hepatic DCs are more phagocytic and produce more cytokines than DCs in lymphoid tissues. Normally, CD4 T cell activation by hepatic APCs preferentially generates Th2 cells secreting immunosuppressive IL-10. It is unclear whether the hepatic microenvironment does or does not favor differentiation of inducible Tr1 and Th3 regulatory cells. In mice, the quantities of natural Tregs are decreased compared to those in lymph nodes, but CD8 T cells in the liver cause a rapid recruitment of natural Tregs. CD8 T cell activation by antigens presented solely by hepatocytes has been conclusively demonstrated, but it normally results in T cell inactivation or apoptosis. Stellate cells can also present endogenous antigens to T cells, resulting in an immunosuppressive response. In contrast, LSECs cross-present exogenous antigens in HLA class I molecules to activate CD8 T cells into CTLs. Kupffer cells expressing FasL and death receptors 4 and 5 (DR4, DR5 that

activate TNF-apoptosis-inducing ligand, [TRAIL]) trap senescent CD8 T cells from extrahepatic or intrahepatic sites and induce apoptosis by engaging Fas (CD95) [7]. CD4 or CD8 T cells that recognize antigens in the liver are exposed to both IL-10 and to inhibitory PD-L1/2 (CD273) expressed on both Kupffer and stellate cells.

Adaptive immune responses against viral, neoplastic, or autoantigens in the liver can occur either directly in the liver or in regional lymph nodes [7]. The latter involves migration of mDCs that have phagocytosed antigens in the liver to regional lymph nodes where they present antigenic peptides to naïve CD4 and CD8 T cells. Direct T cell activation in the liver favors tolerance unless counteracted by a distinct proinflammatory cytokine milieu. In contrast, effector CD4 and CD 8 T cells activated by hepatic DCs in lymphoid tissues enter the circulation and in the portal tracts after transendothelial migration across the endothelial cells of the portal veins. These activated cells exhibit effector functions in the proximal sinusoids at the interface of the portal tracts and hepatocytes [15]. However, the magnitude of their proinflammatory and cytotoxic functions is dictated by the sinusoidal milieu containing immunosuppressive IL-10 and by the expression of inhibitory PD-L1/2 (CD274/273) by Kupffer cells and stellate cells [155].

B cells normally comprise <10% of intrahepatic lymphocytes, and the majority of these B1 cells differ from splenic B2 cells by expressing CD5, characteristic of a subtype of innate B cells [7, 8]. B1 cells appear to connect the innate and adaptive limbs of immunity by being activated in a Th-independent manner and producing low affinity IgM antibodies against glycoproteins, called natural antibodies. Nonhepatic CD5+ B cells also secrete IL-10, but it is unknown if their intrahepatic counterparts may also contribute to an immunosuppressive cytokine milieu.

Alternatives to an Autoimmune Pathogenesis

Since AIH is a necroinflammatory disease of unknown cause, it is important to consider the possibility of alternative mechanisms of pathogenesis. This requires scrutiny of AIH from different perspectives of pathogenesis and suggests testable hypotheses. The most plausible alternative to an autoimmune pathogenesis in AIH is an immunological response to an undefined, noncytopathic hepatotrophic virus whose mode of transmission and latency obscures its infectious nature. Consider a scenario in which HCV infections were not transmitted by blood transfusions and, therefore, an infectious etiology was unsuspected. Prior to development of diagnostic tests for HCV infection, patients with chronic hepatitis C were often misdiagnosed as AIH, especially those who had developed type 1 or type 2 autoantibodies through molecular mimicry. If a hypothetical virus did not replicate in response to corticosteroid treatment, as does HCV, it might even appear responsive to immunosuppressive therapy. Recrudescence, which occurs after withdrawal of immunosuppression in AIH, would also be expected in chronic viral hepatitis. Finally, the restricted number of viral antigenic epitopes for T cell and B cells would also generate an oligoclonal response. However, a viral etiology would not be expected to have HLA associations

with susceptibility or resistance unless HLA dictated a difference in the probability that the infection would become chronic. An alternative to an autoimmune pathogenesis of AIH is that the disease is an immune-mediated inflammatory disorder (IMID), similar to inflammatory bowel diseases, rheumatoid arthritis, and psoriasis (Table 2.1). In contrast to autoimmune diseases, IMIDs are not induced by immune responses to specific autoantigen(s) but, instead, are characterized by dysregulated interplay between innate and adaptive immune responses to environmental antigens, resulting in sustained tissue/organ-specific damage caused by inflammation and proinflammatory cytokines, such as IL-12, IL-6, and TNFα(alpha). The tissue or organ specificity of inflammation results from the generation of chemokines and adhesion molecules that chemoattract and activate T cells, macrophages, and B cells. Autoantibodies are generated as epiphenomena and HLA associations reflect a capacity for dysregulated inflammatory responses. In this scenario, prominent roles for proinflammatory Th17 cells and γδ (gamma delta)T cells would be expected. Apoptosis of hepatocytes could potentially induce autoreactive CD4 T cells to provide help for autoantibody production by autoreactive B cells. Therapy with corticosteroids and/or azathioprine would be anticipated to significantly reduce inflammation and tissue damage. However, this scenario does not adequately explain the female predilection of AIH. Finally, the capacity of the hepatic microenvironment to serve as a lymphoid organ suggests that it could congregate CD8 T cells chronically generated against nonhepatic autoantigens in extrahepatic sites that could mediate chronic interface hepatitis and fibrosis as an innocent bystander phenomenon [158].

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Chapter 3
Clinical Presentation

E. Jenny Heathcote

Keywords Asymptomatic autoimmune hepatitis • Advanced liver disease • Fulminant hepatic failure • Liver transplant • Immunosuppressive therapy

Introduction

Waldenstrom was the first to describe what later became recognized as Autoimmune Hepatitis (AIH) [1]. No particular ethnicity precludes the development of AIH although its presentation may vary across the world. AIH may present at any age, from infancy through to the elderly. Although more common in women this disease must nevertheless be considered in the differential diagnosis of "hepatitis" in men. When AIH was first recognized as a specific entity it was assumed that the disease was always symptomatic. Manifestations of advanced liver disease were usual at first presentation. The recent introduction of routine screening blood tests indicates that some individuals may have "asymptomatic" AIH. At the other end of the spectrum, the dramatic improvement in the understanding and management of Fulminant Hepatic Failure (FHF) has allowed the recognition of AIH as one of its many causes. Liver transplant saves the lives of most of those who need it, although recurrent AIH in the new liver may develop.

It is important to recognize the change in demography over the last 40 years as the patients who were entered into any one of the three "classic" randomized control trials of immunosuppressive therapy (IST) for AIH [2–4] in the late 1960s do not necessarily represent the patient population seen in the physician's office or emergency

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room today. Thus, it is wrong to assume that the therapies shown to be lifesaving in those with advanced liver disease benefit all those given a diagnosis of AIH today, particularly those at the two extremes alluded to above.

The Many Faces of Autoimmune Hepatitis

The presentation of patients with liver disease subsequently shown to be due to AIH varies widely. Thus, AIH needs to be in the differential diagnosis of any acute (including fulminant) or chronic liver disease associated with a "hepatitis" (rarely with additional "cholestasis"). Recently, asymptomatic cases of AIH have been reported. An individual who presents de novo with hepatic decompensation or a background of inactive cirrhosis may have a "burned out" AIH. Occasionally another autoimmune liver disease may "overlap" with AIH (i.e., PBC or PSC) (see Chap. 12).

Acute AIH

Recurrent episodes of transient rises in serum transaminase levels are most often ascribed to a "viral" infection, a drug reaction – or left unexplained! There are no symptoms specific to acute AIH but as is usual with any hepatitis, fatigue is common and there may be an accompanying arthralgia or even a serum sickness-like syndrome. Abdominal discomfort is frequent but fever is unusual. As AIH is relatively rare in comparison to other causes of an acute hepatitis there are no long-term follow-up studies of patients who present acutely. Whether this disease always becomes chronic or may resolve for good remains unknown. There are many mimics of AIH (e.g., a toxic hepatitis secondary to new medication or herbal remedy, Wilson disease). A preceding viral infection is sometimes obtained from the history (e.g. acute hepatitis A or C, measles, and EBV/CMV [5, 6]). If the history taking and/or work-up at the time of first presentation was incomplete it may be impossible to be sure that a prior "hepatitis" was viral or drug induced.

In a series of 86 Italian patients given a diagnosis of AIH 26% presented acutely, and it was the negative viral serology, the higher γ(gamma) globulin (26.9 versus 13.4 g/L) and the AST/ALT ratio (1.20 versus 0.61) which most helped to distinguish cases of acute viral hepatitis from acute AIH [7]. Those with an acute presentation are more likely to be jaundiced, and have high serum transaminase values. There may be no differences in levels of IgG, severity of liver disease on histology in terms of both activity and fibrosis which distinguish those who present acutely from those with a more indolent (chronic) presentation.

A Japanese series of 53 cases of acute AIH were compared with 123 who were diagnosed with chronic AIH [8], the former were younger (mean age 37 years compared to 56 years). In this series an acute presentation was defined as a sudden onset

Table 3.1 Simplified Diagnostic Criteria for Autoimmune Hepatitis

Variable	Cutoff	Points
ANA or SMA	≥1:40	1
ANA or SMA	≥1:80	
or LKM	≥1:40	2*
or SLA	Positive	
lgG	>Upper normal limit	1
	>1.10 times upper normal limit	2
Liver histology (evidence of hepatitis is a necessary condition)	Compatible with AIH	1
	Typical AIH	2
Absence of viral hepatitis	yes	2
		≥6: probable AIH
		≥7: definite AIH

Adapted from Hennes EM. Hepatology 2008;48:169–76
*Addition of points achieved for all autoantibodies (maximum, 2 points).

of jaundice and/or fatigue and/or anorexia with a serum bilirubin ≥5 mg/dl and/or serum ALT ≥tenfold ULN. These authors found that serum IgG values of <2,000 mg/dl and undetectable ANA were significantly more likely than reported for the more classical (chronic) presentation. Retesting a few weeks later may reveal an increase in IgG and the appearance of ANA/SMA. In common with other reports of acute onset AIH, zone 3 necrosis seen on liver biopsy was present in half of those with the acute onset; a manifestation of AIH rarely observed in the liver biopsies of those with a chronic presentation.

Confirmation of Diagnosis of AIH

To make a confident diagnosis of AIH, other diseases presenting similarly need to be sought. The International Autoimmune Hepatitis Group (IAHG) developed a complex scoring system [9] which has recently been considerably modified (Table 3.1). This simpler system [10] has been evaluated in well-characterized cases of AIH [11] but more extensive validation in "control" populations and in those with acute and fulminant AIH are needed. All these scores give a range for both a "probable" or "definite" diagnosis. In a patient in whom a liver biopsy is not feasible a "probable" diagnosis is suggested by high titer autoantibodies and an elevated IgG in serum. Unlike the earlier scoring systems "atypical" features such as an elevated alkaline phosphatase (ALP) or detectable AMA do not require one to subtract from the overall score: neither does this new score require specific values for ALT or ALP. This latest scoring system reflects the greater appreciation for the wide range of manifestations of this disease (e.g., AMA+ve AIH or asymptomatic inactive AIH).

Table 3.2 Untreated versus treated demography asymptomatic AIH

	Treated ($n=15$)	Untreated ($n=16$)
Mean age at presentation (years)	46.5 ± 12.57 (23–68)	51.2 ± 12.77 (31–5)
Mean follow-up (years)	5.18 ± 4.8(0.8–16.9)	3.11 ± 4.7 (0.3–19.6)
AST (<40 IU/L)	275 ± 375	234 ± 343
Bilirubin (<23 mM)	25 ± 29	15 ± 9.9
Albumin (35–50 g/L)	41 ± 5	43 ± 5
IgG (5–13 g/L)	21.3 ± 10.6	18.0 ± 8.1
Cirrhotic at presentation	1 (6.7%)	7 (43.8%)
Mean AIH biopsy score	2.61 ± 1.88	1.56 ± 1.67%
Endpoints	1 (6.7%)	2 (12.5%)
Transplant	1 (6.7%)	0
Liver death	0	0
All death	0	2 (12.5%)

Adapted from Feld JJ, Dinh H, et al. Hepatology. 2005;42:53–62
*$p<0.05$ between the two values

Chronic Autoimmune Hepatitis

Individuals with chronic AIH often give a history of being told they have a persistent or a relapsing "hepatitis." Specific symptoms may or may not be present. The nonspecific and intermittent nature of this disease means that chronic AIH often goes unrecognized from months to years following initial presentation. Negative viral serology and at least a ≥1.2 × ULN for γ(gamma) globulin and high titer ANA/SMA are the laboratory features which could allow a "probable" diagnosis of AIH without a liver biopsy [10].

It is quite possible for an asymptomatic individual found to have a "transaminitis" to already have hematological evidence of liver failure (e.g., an elevated INR) yet be living a full and active life. When youngsters present in this fashion, it may be difficult to convince them that treatment (with all its untoward side effects) will be lifesaving. Despite lack of symptoms referable to their liver, a history in women of secondary amenorrhea may be obtained and physical examination may reveal spider nevi and/or a small liver with splenomegaly.

In a case series where patients with asymptomatic AIH were compared to those who presented with symptoms, cirrhosis (mostly inactive) was evident in 25% of the asymptomatic patients and in 36% of those were symptomatic [12]. The survival of the symptomatic (all treated) and the asymptomatic group, half of whom were not treated was no different after 10 years of follow-up. The 10-year survival was 83% in those who had symptomatic disease and 80% in those without symptoms present at diagnosis (NS). The mean age at presentation was older in the asymptomatic group – 48.5 years versus 41.6 years for the symptomatic. Two of the three asymptomatic patients died of myocardial infarction. A background cirrhosis at the time of diagnosis regardless of the presence or absence of symptoms was associated with a significantly reduced 10-year survival being 61.9% in cirrhotics versus 94% in noncirrhotics (Table 3.2).

Individuals may present de novo with both the symptoms and signs of hepatic decompensation (e.g. ascites, variceal hemorrhage, or chronic hepatic encephalopathy) but with few or no serologic markers of AIH. In such cases liver biopsy generally shows an inactive cirrhosis. Such individuals are rarely given the correct diagnosis. The term "cryptogenic AIH" has been applied to subjects who have a chronic hepatitis but no detectable autoantibodies [13] (not to be confused with the end result of nonalcoholic fatty liver disease). However, despite the lack of detectable autoantibodies, serum IgG levels were noted to be elevated in support of an autoimmune origin to their cirrhosis.

Fulminant AIH

Although an unusual presentation of AIH is one of the many potential causes of FHF, it is reported to be present in 8% cases with FHF [14] (Table 3.3). In the younger (predominantly but not always in children) population it is vital that a diagnosis of Wilson disease be excluded [15] where typically jaundice is mostly due to an unconjugated hyperbilirubinemia secondary to hemolysis. The INR may be high and the ALT only moderately elevated. Subnormal values (corrected for age) of ALP may be the diagnostic "give away" in those with Wilson disease presenting acutely. Ocular examination showing Kaiser–Fleischer rings will often rapidly clinch the diagnosis.

Table 3.3 Survival rate of serious[a] acute liver failure according to etiology

Etiology	Total	LDLT	Survival rate without LDLT	Survival rate with LDLT	Total survival rate
HAV	1	0	0/1 (0)	–	0/1 (0)
Acute HBV	1	0	0/1 (0)	–	0/1 (0)
SAE	6	0	1/6 (17)	–	1/6 (17)
HEV	1	0	0/1 (0)	–	0/1 (0)
Drugs	1	1	–	0/1 (0)	0/1 (0)
Alcohol	1	0	0/1 (0)	–	0/1 (0)
AIH	4	1	2/3 (67)	1/1 (100)	3/4 (75)
Cryptogenic hepatitis	12	3	0/9 (0)	3/3 (100)*	3/12 (25)
Total	27	5	3/22 (14)	4/5 (80)**	7/27 (26)

*$P = 0.004$ versus without LDLT

**$P = 0.0089$ versus without LDLT

[a]Estimated to die by Muto's formula (log it[λ] = 0.0649 × prothrombin time + 0.0357 × age − 2.81 × direct/indirect bilirubin + 0.703 × log total bilirubin + 1.04 × [O–C][O–C]; acute form = 0, subacute form = 1.0, death rate (p) = $1/1 + e^{-\lambda}$)

When limited to patients with serious acute liver failure (ALF), the prognosis of cryptogenic cases with LDLT was significantly better than that of patients without LDLT. *AIH* autoimmune hepatitis, *HAV* hepatitis A virus, *HBV* hepatitis B virus, *HEV* hepatitis E virus, *LDLT* living donor liver transplantation, *SAE* severe acute exacerbation of chronic hepatitis B infection

Adapted from Takahashi SJ. Gastroenterol Hepatol. 2008;23:1216–22

Table 3.4 Comparison of response to therapy of AIH patients with acute and chronic presentation

	Acute AIH ($n=10$)	Chronic AIH ($n=20$)	P value
Clinical outcome			
Complete response	4	16	0.0433
Died	2	0	0.0449
OLT	3	2	0.10
Listed for OLT	1	2	

Adapted from Kessler WR. Clin Gastroenterol Hepatol. 2004;2:625–31

As background liver disease in patients with AIH presenting with FHF may be acute or chronic, a liver biopsy (transjugular) is needed to demonstrate which is the case; zone 3 necrosis may be the clue to a diagnosis of acute AIH. Liver transplant is very often the optimal treatment for those with acute fulminant AIH [16] (Table 3.4). For those with evidence of chronic disease at presentation, introduction of IST may be considered as initial treatment and in some this treatment prevents the need for liver transplantation. However, high dose IST poses a problem in such patients because it promotes both bacterial sepsis and disseminated fungal infection, particularly the latter may preclude a subsequent liver transplant should this be deemed necessary. Thus, potentially untoward consequences of instituting IST in FHF due to a chronic AIH need to be considered carefully prior to their introduction.

A further study of 14 patients with AIH who presented with FHF reported that seven responded to IST and did not require liver transplant and six of seven nonresponders underwent liver transplantation. The stability of the markers of liver function distinguished the stable from unstable FHF [17]. In IST responders their pretreatment MELD score was ≤28 and they were more likely to be cirrhotic with a stable bilirubin value even in the face of a coagulopathy. In this series a response to steroid therapy was observed within 3–4 days of their introduction and precluded the need for transplant. In nonresponders their bilirubin and INR levels rose and review of their liver biopsy indicated submassive hepatic necrosis. All these studies emphasize the need for an immediate liver biopsy in cases of FHF to establish whether the liver disease is acute or acute on chronic, while the results of serologic testing are pending thus allowing the rapid introduction of optimal therapy.

Recurrence of AIH Postliver Transplantation

Recurrence of AIH is reported to occur in the liver allograft in 23% after a median interval of 26.4 months and this figure may increase to 41% at 10 years of follow-up [18].

Moderate to severe inflammation and very high levels of IgG before transplantation may be risk factors for recurrence postliver transplant [19]. It has been hypothesized that this observation indicates that it is the host that is susceptible (not the donor liver) as it is the host who appears unable to suppress their immune reactivity

despite IST prior to transplant. Introduction of higher dose IST will usually induce a remission but on occasion retransplant is necessary.

In a systematic review of recurrence of autoimmune liver disease following liver transplant, recurrent AIH was found in 22% [20]. Twenty-five publications on recurrence of autoimmune liver disease postliver transplant were identified, 13 of which were suitable for inclusion in their systematic review. Recurrence of their prior autoimmune liver disease was diagnosed in 94 of 414 transplant recipients after a follow-up of 2 years±, yet the patients all remained asymptomatic. They were initially identified biochemically but a conclusive diagnosis was made on liver biopsy: periportal hepatitis ± a lobular hepatitis was identified in those who reactivated their prior AIH. As typical pretransplant markers of AIH may persist posttransplant (↑IgG, ↑AST/ALT and detectable autoantibodies), the method most likely to facilitate an accurate diagnosis of recurrent AIH posttransplant is liver biopsy.

Overlapping Autoimmune Hepatitis and Sclerosing Cholangitis

The overlap of AIH with a cholangitis has been best defined in a study of children diagnosed with AIH who as part of a prospective study underwent routine ERCP at first presentation. Biliary changes on ERCP were found in half [21]. An earlier retrospective study reported similar findings but with the primary liver disease being PSC. These children who were known to have a sclerosing cholangitis with both biochemical and histological features of cholestatic liver disease also had the serologic and histologic features of an AIH [22].

The prevalence of an overlap between AIH and PSC in adults appears to be much less common although there are many isolated case reports. There are only two (albeit retrospective) studies which report MRC findings in adult patients with a diagnosis of AIH: the results differ! The first study reported that 10% of patients with a prior diagnosis of AIH (often longstanding) were found to have biliary features typical of PSC [23]. In another more recent study, these observations were not confirmed and the authors drew attention to the fact that MRC may be misread as showing a pattern similar to PSC in the periphery of the liver in any patient with cirrhosis [24]. Nevertheless, overlaps certainly occur and small studies and case reports suggest that the outcome (in terms of the need for liver transplant) may be greater in those with this overlap although this observation may just be a consequence of reporting bias. A small Italian study indicated the survival of AIH versus AIH + PSC was better than for PSC alone [25] but this was not the case in another report [26]. It is noteworthy that the clinical presentation of those with AIH/PSC cannot be distinguished from AIH alone. Pruritus is rarely a predominant symptom in AIH except in some after estrogen therapy is prescribed. One case series of six patients with AIH, three of whom underwent an ERCP at the time of initial diagnosis (all normal) were subsequently shown to have typical cholangiographic features after their liver disease initially controlled with prednisone failed [27].

Overlapping Autoimmune Hepatitis and Primary Biliary Cirrhosis

There are two circumstances when an overlap of AIH and PBC may be suspected. There are a number of case reports of individuals given a clear-cut diagnosis of PBC (cholestatic biochemistry, positive AMA, and classical findings on liver biopsy) with a good biochemical response to UDCA who subsequently show a change in their biochemical pattern of disease more in keeping with a hepatitis with loss of AMA and appearance of ANA [28]. Such cases, albeit rare, clearly indicate that patients given a diagnosis of PBC may subsequently alter the pattern of their presumed autoimmune liver disease to that of AIH. Good response to IST is as is expected for AIH [29]. There are to date no well-described case reports of the reverse (i.e., individuals with AIH who subsequently change in the pattern of their disease to primary biliary cirrhosis). Reference is made to a few such individuals in one series of patients with PBC thought also to have AIH but no clinical description of their biochemical/histologic profile was reported [30]. Transient appearance of AMA may be detected in patients with AIH [31] or with FHF of any cause [32].

The Mayo Clinic has reported that features of AIH may be present in about 12% of all those given a diagnosis of PBC when the International Autoimmune Hepatic Score (IAIH) was applied. The survival of individuals given a primary diagnosis of PBC yet with a positive IAIH score was worse, particularly in terms of the complications of portal hypertension [33].

There are also reports of patients with clear-cut AIH who nevertheless test positive for antimitochondrial antibodies but without any clinical, biochemical, or histological evidence of the small duct disease typical of PBC [34]. In a series 15 such individuals who were followed for up to 26 years, no evidence of PBC was observed at any time despite persistent detection of AMA.

Clinical Presentation AIH According to Ethnicity

African Americans/Blacks

Particularly relevant to a multicultural society (so often present in the West) is to appreciate that the clinical presentation of AIH varies widely according to ethnicity. In a study from the USA, African Americans were more often symptomatic at presentation (84%) versus 64% nonblacks in the USA [35]. In this series those with an acute presentation were more likely to be black (76%) than not (32%), and blacks had lower levels of albumin and higher INR values even though their disease appeared to be at a similar histologic stage to nonblacks. Blacks presented younger age (42 years) than nonblacks (45 years). In this study cirrhosis was a predictor of poor outcome and noncompliance with IST was higher in those with a bad outcome (Table 3.5).

Table 3.5 AIH in African Americans versus Caucasians: clinical, biochemical, and outcome data

	Blacks (37)	Non-Blacks (64)	*P* value
Cirrhosis	57%	38%	0.061
Liver failure	38%	9%	0.001
Remission with IST	76%	90%	0.016
Referred liver transplant	51%	23%	0.009
Mortality	24%	6%	

Adapted from Verma S. Hepatology. 2007;46:1828–35

In another study from the USA, 27 African Americans with AIH were compared with 24 nonblacks who presented to the same institution with AIH. African Americans were found to have significantly higher INR values at presentation likely because 85% were found to be cirrhotic: whereas cirrhosis was only present in 38% of Caucasians [36]. Response to IST was similar but higher doses of IST were needed to maintain African Americans in remission – this could be a consequence of their higher rate of background cirrhosis.

There is little data on AIH in blacks outside of the USA. In a small study from the UK [37] which looked at the pattern of disease in 12 non-European, non-Caucasoid patients, half were African in origin. Their ages ranged from 12 to 39, their total serum bilirubin values at first presentation ranged from 22 to 400 μ(mu)mol/L and all had elevated ALP levels ranging from 850 to 230 iu/L (NR <130 iu/mL) simultaneously levels of IgG were high, ranging from 25.3 to 70.7 g/L (NR <18). All but one of the six African patients tested positive for ANA and/or SMA. In this series of 12 patients three had histologic evidence of biliary changes, two were African. None tested positive for AMA and all had normal cholangiograms (only one of whom had a normal bilirubin at presentation). Only one of these six African patients had a good response to IST but none required a liver transplant. Need for liver transplant was limited to three of the four Asians in this case series.

In another case series also from the UK [38] the authors describe the presentation of six Somalian males with type 1 AIH (all ANA/SMA+ve) – four of whom had cholestatic changes on liver biopsy. Only one had a complete response to IST, two failed to respond at all. Of these six patients, two-thirds despite their acute presentation already had advanced liver disease. These three patients all had pericholangitis, ductopenia, cholestasis, and ductular proliferation on liver biopsy yet all three had a normal MRC or ERCP. Unfortunately, the authors did not report the long-term outcome of these Somalian men with a cholestatic form of AIH. In a small study of 37 patients presenting with FHF from the Sudan, 3 (8%) were thought to have AIH only one of whom survived (no liver transplant program was available) [39].

South America

Czaja and colleagues from Brazil reported on the clinical manifestation of 115 Brazilians given a diagnosis of AIH and compared them to 161 cases in the USA [40]. In the Brazilian patients fewer had associated autoimmune diseases (17%) versus

38% in those from the USA. The Brazilian patients were younger at presentation and more often male. At presentation the Brazilian patients had both higher ALT values and gamma globulin levels. Recruitment patterns were very different in the two countries and this likely introduced bias into the study.

North American Indigenous Peoples

In a comprehensive study conducted in Alaska [41], the prevalence (36/100,000) of definite AIH was much higher than that reported in Sweden (10.7 per 100,000) [42]. In Alaska, 40% of AIH presented acutely and the other 60% were mostly identified at the time of screening blood tests. Although the age range at diagnosis ranged from 15 to 82 years they tended to be younger than reports from Norway [42]. In another report of AIH in Indigenous people in Canada, age at presentation was similar between First Nations People and those who were not. When 33 First Nations People given a diagnosis of AIH and were compared to non-FNP there were no distinguishing features on presentation although both the grade and stage of liver disease on liver biopsy was found to be 3–4 in 10/17 (58%) FNP compared to 24/65 (34%) non-FNP – suggesting that more severe disease was present at first diagnosis in FNP – this observation could be due to referral bias as many FNP live in rural areas which are hard to access [43].

South Asia

AIH has in the past been considered rare in the Indian subcontinent but a report [44] from New Delhi indicated that of 1,358 patients with chronic liver disease 50 (3.43%) (1:3 males to females) were given a diagnosis of AIH. Almost all were described as being asymptomatic at presentation yet 83% were jaundiced! The mean values for bilirubin were 4.6 ± 4.9 mg/dl, AST 301 ± 267 iu/l, albumin 2.9 ± 0.6 g/l, and γ(gamma)globulin 4.3 ± 0.8 g/l. At presentation 66% had splenomegaly and an active cirrhosis was present at initial referral in 76%. There was a 25% mortality over a mean follow-up period of 15.7 months. We are not told who received treatment or who maintained treatment. The fact that three-fourths presented for first time with severe disease suggests that access to care may also be a factor leading to more advanced disease in this patient population.

In a subsequent report from a different centre in India [45] only 1.5% of their population of 2,401 patients given a diagnosis of chronic liver disease were thought to have AIH and only 34% were cirrhotic at the time of initial presentation – 79% of their patients had “definite” AIH according to the IAIH score. Mean duration of symptoms prior to diagnosis was 20.3 months (0.2–72). Age at presentation ranged

from 6 to 68 years with a peak seen at 30 years. In this case series, 39% had associated autoimmune disease – diabetes, thyroiditis, and vitiligo being the most common. Of the 30 treated with corticosteroids ± azathioprine a clinical and biochemical response was seen in 70.8%. To their knowledge only one patient on immunosuppressive medications developed acute liver failure and died.

South East Asians

In a small series reported from Taiwan [46], 22 Chinese patients (M:F 1:2) 11 had a "definite" diagnosis and 11 "probable" according to the IAIH score – only five were cirrhotic at the time of presentation (22%) despite a relatively long duration of symptoms prior to presentation, particularly in the women (91 ± 217 months). More than half of these Taiwanese patients had superimposed cholestasis (all AMA−ve) on a background of AIH in that either their serum levels for ALP >×2ULN or they had evidence of a cholangiopathy on liver biopsy (no cholangiography performed). Nevertheless, all these patients responded well to IST (87.5%).

Age at Clinical Presentation of AIH

In a large (205 cases) series with AIH, a retrospective review of factors that distinguished patients given a diagnosis over the age of 60 years was compared to those diagnosed under 30 years [47]. There was really only one overt difference separating age at presentation, HLA typing. In those over 60 years, DR3+/DR4− was present in 23% (58% ≤30 years) and the findings for DR4+/DR3− were the reverse 47% in those >60 years versus 13% in those ≤30 years old. IAHG scores were similar. In terms of treatment response, the remission rates, relapse rates, and sustained remission rates were similar for these two age categories, but treatment failure in those >60 years was only 5%, significantly less than the 24% for those aged 30. This difference in failure to respond to therapy may be in part related to the distribution of HLA haplotypes although compliance with therapy may well have been another factor influencing outcome (Table 3.6). Not surprisingly, liver transplant rates were higher in the young group (24%) whereas only 5% of those ≥60 years were accepted for a liver transplant.

Most reports on AIH in the elderly indicate that cirrhosis at first diagnosis is more likely, suggesting that their diagnosis has been delayed. This may be because the general perception is (from studies published many years ago) that AIH is a disease of young women. Now it is evident that AIH may present at any age and thus should always be included in the differential diagnosis of someone with a

Table 3.6 Clinical and HLA Findings in the Young and Elderly Age Groups at Presentation

Clinical Features	Patients ≥ 60 years (N = 47)	Patients ≤ 30 years (N = 31)
Age (years)	69 ± 1[a]	25 ± 1[a]
Female	43 (91)	25 (81)
Concurrent immune diseases	22 (47)	8 (26)
Duration of symploms at accession (mo)	34 ± 7	32 ± 6
Symptoms ≤1 month duration	4 (8)	0 (0)
Symptoms ≤ 6 month duration	16 (34)	4 (13)
AST (nl, ≤31 U/L)	355 ± 48	510 ± 4
Bilirubin (nl, ≤1.1 mg/dL)	3.2 ± 0.6	3.7 ± 1
γ-globulin (nl, 0.7–1.1 g/dL)	3.1 ± 0.2	3 ± 0.2
Immunoglobulin G (nl, 600–1500 mg/dL)	2812 ± 218	2630 ± 239
Cirrhosis at accession	15/46 (33)[d]	3/30 (10)[d]
DR3+/DR4–	11 (23)[c]	18 (58)[c]
DR4+/DR3–	22 (47)[b]	4 (13)[b]
DR3+-DR4+	7 (15)	6 (19)
IAHG Score	18.9 ± 0.3	18.4 ± 0.3

Adapted from Czaja AJ and Carpenter HA. Hepatology 2006;43:532–538
Note: Numbers in parentheses are percentages. *AST* serum aspartate aminotransferase level; *IAHG* International Autoimmune Hepatitis Group.
Significantly different from each other at level of [a]$P < .0001$, [a]$P = .003$, [c]$P = .004$, and [d]$P = .03$.

"transaminitis." If patients such as these do not undergo testing for IgG their correct diagnosis may be missed. Auto-antibody testing particularly ANA is less useful as with age the prevalence of ANA in the general population increases.

In another study from Italy [48], the pattern of disease in their patients >65 years given a diagnosis of AIH confirmed the higher prevalence of HLA – DR4 (45% versus 18% in those <65 years) and they were more often asymptomatic. In this case series, the pattern on liver histology was not more severe in the elderly. This review also highlights the importance of considering a diagnosis of AIH in an older even in an asymptomatic patient identified only by the incidental finding of elevated liver enzymes (AST/ALT).

At the other end of the disease spectrum (i.e. adolescent and early adulthood), differences are observed in the distribution of HLA pattern in youngsters versus that of adults [49, 50]. In this study from Japan [49], the authors compared clinical biochemical and histologic findings in 15 youngsters (all <30 years) with 79 patients given a diagnosis of AIH between the ages of 40 and 50 years. The only significant differences they observed was again in the distribution of HLA – DR4 (27% in children, 77% in adults) and presentation with an acute hepatitis (27% in children, only 4% in their adult group).

The previously mentioned report by Gregorio et al. [21] indicated that children given a diagnosis of AIH on presentation had an approximately 50% chance they would at ERCP be noted to have an abnormal extrahepatic biliary tree – the findings were not typical for PSC and the authors labeled these children as AIH plus "autoimmune sclerosing cholangitis." Response to treatment was similar but the need for liver transplant appeared to be greater for those with additional biliary changes.

Gender

In a recent report on gender differences in "definite" AIH (extra two points for being female) from the UK, the authors confirmed the usual greater prevalence of AIH in women [51]. Of 238 patients with AIH, there were 51 males. Females had a higher IAHG score than men at presentation (even though four points had to be deducted in seven females who tested AMA+ve because the old AIH score was employed). Relapse off IST was observed more often in men than women (71 versus 55%). Rates of cirrhosis at baseline were more common in men although death from any cause or need for liver transplantation was greater in the women. The author postulated that as estrogens enhance immune activity and androgens reduce it – this may in part relate to the differences in survival between men and women with AIH. This report also showed that independent of gender, cirrhosis at presentation (one-third) carried with it a worse prognosis. But the chance of a liver-related death was similar between males and females. Multivariate analysis indicated that the factors associated with reduced survival in the entire cohort were jaundice, ascites, hematemesis, and grade on index biopsy. Men tended to present at a younger age and to have higher values of GGT.

Chapter Summary

1. AIH can present in many ways from asymptomatic transaminitis to fulminant liver failure.
2. Careful exclusion of drug injury and Wilson disease particularly in young patients is important.
3. AIH is a chronic, relapsing and remitting disease: careful evaluation at baseline, nearly always including liver biopsy, is important before committing patients to therapy.

Useful Tips for Practitioners

1. AIH can present with systemic complaints including arthralgias, acne, and amenorrhea.
2. Immunoglobulins and autoantibodies may become positive later in the clinical course, and if the diagnosis remains in doubt these tests should be repeated.
3. A therapeutic response to treatment should be expected in more than 90% of patients: a failure to respond to treatment should lead to a re-evaluation of the diagnosis.

Common Pitfalls in Practice

1. Budesonide is not appropriate in patients with cirrhosis and is not free of side effects.
2. Lack of compliance with therapy is the most common reason for a poor treatment response.
3. Patients with acute liver failure should be managed in conjunction with a transplant team as steroids (by enhancing the risk of either bacterial or fungal sepsis) can be dangerous.

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Chapter 4
Confirmation of the Diagnosis: Interpreting the Serology

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Keywords Antibody • Autoantibody • Autoantigen • Autoimmunity • Clinician • Disease • Hepatitis • Liver • Primary biliary cirrhosis • Primary sclerosing cholangitis

Abbreviations

AIH	Autoimmune hepatitis
AMA	Antimitochondrial antibody
ANA	Antinuclear antibody
ANCA	Antineutrophil cytoplasmic antibody
ASGPR	Asialoglycoprotein receptor
c-ANCA	Cytoplasmic antineutrophil cytoplasmic antibodies
CYP	Cytochrome
ELISA	Enzyme-linked immunosorbent assay
HEp	Human epithelioma (cells)
IAIHG	International Autoimmune Hepatitis Group
IFL	Indirect immunofluorescence
IgG	Immunoglobulin G
F-actin	Filamentous actin
FTCD	Formiminotransferase cyclodeaminase
LKM1	Liver kidney microsomal type 1
LC1	Liver cytosol type 1
p-ANCA	Perinuclear antineutrophil cytoplasmic antibodies
PBC	Primary biliary cirrhosis

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PSC Primary sclerosing cholangitis
SLA Soluble liver antigen
SMA Smooth muscle antibody
TBB5 Tubulin beta B5

Introduction

Serological testing for autoantibodies plays an important role in the diagnosis and classification of autoimmune hepatitis (AIH) [1–4]. Autoantibody testing can also help to distinguish this disease from autoimmune cholestatic diseases, such as primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC) and facilitate diagnosis of overlapping conditions [1, 2, 5].

This chapter attempts to provide an overview of the autoantibody serology in patients with AIH that will be of value not only to hepatologists but also to those physicians and health care professionals who look after patients with this condition. Before ordering a test, the physician must have a clear indication of the outcome of the test (Fig. 4.1). Worryingly, clinicians tend to rely on the results of autoantibody

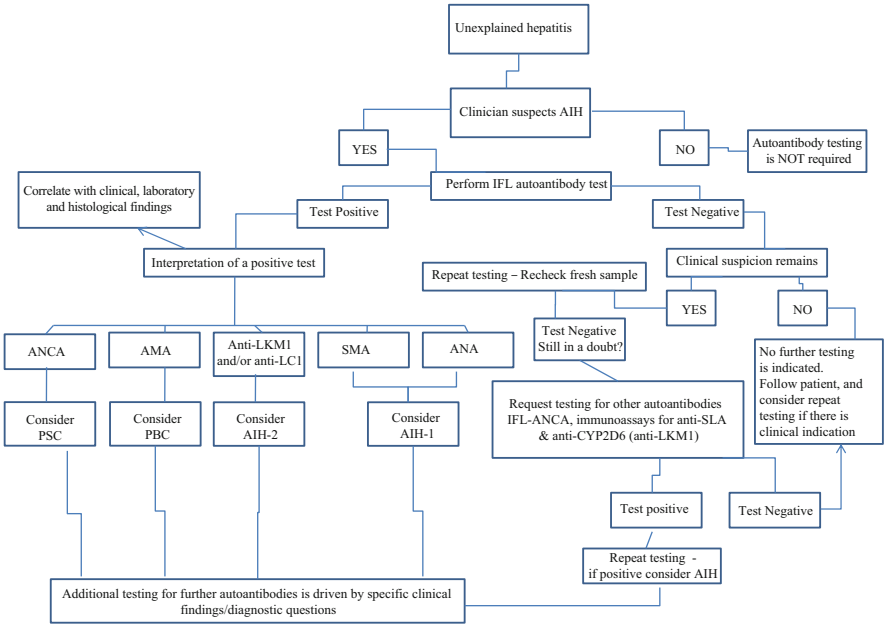

Fig. 4.1 Flow chart for autoantibody testing of individuals with unexplained hepatitis or clinical suspicion of autoimmune liver diseases. *AIH* autoimmune hepatitis, *AMA* antimitochondrial antibody, *ANA* antinuclear antibody, *ANCA* antineutrophil cytoplasmic antibody, *CYP2D6* cytochrome P4502D6, *HEp-2* human epithelioma type 2 (cells), *IFL* indirect immunofluorescence, *F-actin* filamentous actin, *FTCD* formiminotransferase cyclodeaminase, *LKM1* liver kidney microsomal type 1, *LC1* liver cytosol type 1, *PBC* primary biliary cirrhosis, *PSC* primary sclerosing cholangitis, *SLA* soluble liver antigen, *SMA* smooth muscle antibody

testing to make, rather than to confirm, a diagnosis of AIH. The detection of particular autoantibody reactivities and identification of their antigen specificity may have both diagnostic and prognostic significance [1, 2]. However, their main indications for use are to confirm a diagnosis of AIH in patients with suspected disease or to exclude the diagnosis in patients with uncertain clinical and laboratory findings [1, 3, 4]. Autoantibody test results are also used to subclassify patients known to have AIH into serologically distinct groups (and to monitor disease activity or response to treatment over time under certain circumstances) [1, 3, 4]. Hence, detection of AIH-related autoantibodies has assumed an important role in the diagnosis and management of patients with this disease.

Although the results of autoantibody testing are often helpful, they can be misinterpreted. AIH represents a small proportion of patients with liver diseases and as a consequence of that the tests per se have low positive predictive value; in practical terms, this means that a considerable proportion of the cases with detectable autoantibodies will not have AIH but another disease instead. Positive results for testing of antinuclear antibodies (ANA), for example, are seen more frequently in patients with systemic rheumatic diseases [6, 7]. Moreover, ANA can be detected quite commonly in patients with liver diseases unrelated to AIH and in a sizable number of healthy individuals which is increased as a result of aging [1, 2, 7, 8]. As few autoantibody specificities are highly specific for AIH, including anti-liver kidney microsomal type 1 (anti-LKM1) or antibodies against soluble liver antigen (SLA), most autoantibody tests alone are insufficient to establish the diagnosis of AIH [3, 4]. For these reasons, the results of autoantibody testing must always be interpreted in the proper clinical context and in relationship to other laboratory test results [9]. The physician needs to be aware of the indications, sensitivity, specificity, and cost of these tests. A better understanding of the diagnostic and clinical significance of autoantibody reactivities will help the clinician to request the most relevant tests and to interpret correctly the report returned by the laboratory. Misuse of autoantibody test ordering or inappropriate interpretation of the results can result in clinical misjudgment, unwanted therapy, and increased health care costs [10]. Insufficient testing or unnecessary repetition of liver-related autoantibody tests has been noted (local audits and personal communications), and may result in a cascade of inappropriate testing, costing the health care providers or the patients a large amount of money [10, 11].

The recent discovery of new AIH autoantigens and the development of new test assays have led to confusion over which tests to order, when to order them, and how to interpret the test results for the benefit of the patient. An increasing number of physicians believe that the laboratory is responsible to assist the doctor to request the right tests, in the right order and at the right time. Conversely, the immunodiagnostician's view is that it is essential for the clinician to be aware of the tests offered by the laboratory and in a position to request the most appropriate autoantibody tests. The more the physician is aware of the significance of individual autoantibody reactivities and the current laboratory practices, the greater the chance of correct autoantibody testing requests and meaningful clinical interpretation [2].

A diagnosis of AIH is not based on one test alone rather upon clinical history, physical examination, and histological and laboratory investigation [9]. AIH is

characterized by histological features of interface hepatitis, elevated transaminase levels, increase in immunoglobulin G (IgG) levels and circulating autoantibodies [9].

Autoimmune Serological Types of AIH

Given sufficiently sensitive immunofluorescence (IFL) techniques (i.e. expert laboratories), autoantibodies can be detected in >95% of patients with AIH at diagnosis [1, 12] (Table 4.2). Those seronegative at presentation either have antibodies not detectable by IFL, like anti-SLA antibodies, or will develop autoantibodies over time [2, 13]. Autoantibody negative AIH is practically a nonexistent entity.

The detection of diagnostic autoantibodies has allowed a subdivision in serologically diverse groups with distinct clinical features and treatment outcome [4, 14]: ANA and/or smooth muscle antibodies (SMA) define type 1 AIH (AIH-1) whereas anti-LKM1 and/or anti-liver cytosol type 1 (anti-LC1) antibodies characterize patients with type 2 AIH (AIH-2). AIH-1 is the most common form accounting for 80–98% of AIH in developed countries. Usually the two patterns of serology are mutually exclusive, but if the serology is perplexed and includes positive tests for autoantibodies of both types, the disease manifestations resemble those of AIH-2 [15]. The subclassification has significant diagnostic and clinical implications. This is highlighted by the fact that accurate detection of anti-LKM1 antibodies in children with unexplained hepatitis is highly diagnostic of AIH-2, a condition which requires immediate attention and early administration of immunosuppressive therapy [15, 16]. A suggestion for a third type of AIH which is characterized by the presence of anti-SLA antibodies was not widely embraced and has been abandoned as in the great majority anti-SLA antibody positive cases fall within type 1 or type 2 AIH [17–20]. Other forms of AIH, including the de novo appearance of AIH following liver transplantation for nonautoimmune liver diseases and recurrence of AIH in transplanted cases, are infrequent and their serology is indistinguishable from the conventional AIH types [21–23]. A careful history for potential drug-induced liver injury is always important, as serology may also prove positive.

Physicians should be aware that autoantibodies that occur only in AIH do not exist. Autoantibodies that were previously regarded as disease-specific markers, such as the anti-filamentous actin (F-actin) antibodies have been associated with a variety of diseases [24–26]. The presence of ANA, SMA, anti-LKM1, and anti-SLA antibodies in patients who do not have AIH has diluted the strength of this very powerful clinical association. However, a more recent evaluation of these markers on well-defined patient groups demonstrated the autoantibodies to be robust in assisting the diagnosis of AIH in daily clinical practice [3, 12]. Thus, the results of autoantibody testing form part of the “simplified” criteria of the International Autoimmune Hepatitis Group (IAIHG) for the routine diagnosis of AIH [3].

In general, autoantibodies in AIH fluctuate in titer and can disappear and re-appear in a proportion of cases during immunosuppressive treatment [1, 2, 13]. Their presence

per se does not establish a diagnosis nor does it indicate a specific treatment strategy which is not guided by the clinical setting. In the vast majority of newly diagnosed patients, the titer of autoantibodies is 1:80 or higher [3]. Relatively low titers are seen in a proportion of pediatric cases and in patients at drug-induced remission [13, 15]. There is no published evidence to support the use of autoantibody testing for routine use in the follow up of patients with AIH undergoing therapy.

Autoantibody Testing

There are many methods of testing autoantibodies, which can be a cause of error, confusion, or misinterpretation. (Table 4.1) The primary care physician must understand that "no test is perfect and no test is perfectly performed" [27]. Autoantibody test results in blood samples of the same patient's reference serum may vary between different laboratories [2, 10]. Re-testing of the same sample from the same laboratory can produce inconsistent results. The reasons for these discrepancies are numerous. Clinical laboratories use assay kits obtained from different manufacturers and this can lead to significant assay-to-assay variations [10, 24, 25]. Worryingly, an inter-laboratory variation of results has been noted between different laboratories using the same kits and testing the same serum samples [10].

Thus, problems do exist between laboratory reporting, which partly depend on the variety of the screening tests and insufficient autoantibody assay standardization [2, 12]. These concerns have been addressed by the Committee of Autoimmune Serology of the IAIHG [12]. This international panel of experts has already published a series of recommendations for IFL methods used for autoantibody testing in patients with suspected or documented AIH [12]. A prominent issue remains that is with the significant shift from IFL towards commercial enzyme-linked immunosorbent assay (ELISA) and other enzyme immunoassays as screening tests for autoantibody detection [10, 12, 28]. This has raised concerns regarding the accuracy, reliability, and quality of most of these kits that is largely left to the discretion of the manufacturers.

Routine testing for AIH-related autoantibodies has historically relied on IFL (other acronyms used include IFT: immunofluorescence technique, IIFL: indirect immunofluorescence, and IFA: immunofluorescence assay) [4, 12]. While IFL using animal tissue substrates has remained the mainstream method for the testing of a series of liver disease-related autoantibodies including SMA, AMA, and anti-LKM1 antibodies, the routine testing of ANA by IFL is currently based on human epithelial (HEp-2) cells, an epithelial cell line derived from a human laryngeal carcinoma [6, 29]. IFL is based on rodent substrates which usually include liver, kidney, and stomach [1, 2, 12]. Some laboratories are preparing their own tissue substrates but most rely on commercial kits containing rat/mouse stomach/kidney composite blocks as substrates. Of concern is that several clinical laboratories purchase kits based on just employing kidney tissue which clearly limits costs both for the laboratory and the clinics but it is potentially misleading in the diagnostic workup.

Table 4.1 Practical considerations for autoantibody testing in autoimmune hepatitis

Before requesting the autoantibody tests

- None of the tests are perfect and none of them are perfectly performed
- Get to know your laboratory
- Ask your laboratory to explain how they run the testing
- Let them know you would like to be updated from time to time for new tests/assays
- Keep an eye on the literature

Requesting the autoantibody tests

- Avoid unnecessary repeating of tests – check your records or previous results
- Do not forget to send enough serum
- Make sure you give enough clinical information when you ask for a specific autoantibody test
- Minimize the risk of missing something important – asking for autoantibody testing by immunofluorescence on a combination of rodent tissues
- Do not forget: HEp-2 immunofluorescence autoantibody testing is the "the gold standard" technique for rheumatologists but not for hepatologists
- If the laboratory uses only ELISAs for autoantibody testing, the potential for "false negative" results is highly likely
- Be prepared to be challenged for your autoantibody test preferences by the immunodiagnostician
- Most laboratories should be able to perform the assays and issue the reports in 1–5 working days depending on the tests – ask for fast-track testing if necessary

Clinical interpretation of the autoantibody test results – next steps

- Do not assume that results will be consistent between different assays or laboratories
- Always consider the possibility of "false positive" or "false negative" test results
- Be careful with the interpretation of "weak" or "low" or "borderline positive" results
- Check the "small letters" of the report – they give details of immunofluorescent patterns and autoantibody titers/concentrations – ask if necessary
- If the results are not in agreement with your clinical findings, discuss this with the diagnostician – you will be amazed at how much care they may take to address your concerns. They can recheck the sample
- If there is still a doubt about a result, recheck the test on a fresh sample or ask to be repeated using another method (usually molecularly based assay)
- If the laboratory cannot perform additional or specialized tests, ask for testing to other laboratories if necessary
- Do not keep requesting antibodies without proper justification on the ordering forms – the laboratory must understand your concerns
- Do not forget that many autoantibodies associated with AIH can be found in overlapping conditions like primary biliary cirrhosis and primary sclerosing cholangitis
- Ask for repetition of testing for results reporting the presence of anti-LKM1, anti-LC1, anti-CYP2D6 (anti-LKM1), anti-FTCD (anti-LC1), or anti-SLA – these antibodies are relatively infrequent and you must make sure that the tests are "true positive" or "true negative"

While clinicians do not necessarily need to be aware of all the technical aspects of the assays, it is important that they at least know the substrate used for IFL testing. This is important because the titers described with rodent tissues are frequently dissimilar of those described using HEp-2 cells [7]. In adults, significant titers equal or exceed 1:40 dilution by IFL based on rodent tissue substrates whereas on the HEp-2 cells, titers of 1:80 or higher are considered significant, especially for ANA.

In children, titers of 1:20 for ANA or SMA and 1:10 for anti-LKM1 are considered significant in patients with a reasonable suspicion of AIH [3, 12].

In practice, positive sera should be titrated to extinction and the autoantibody titer provides diagnostically relevant information. Within the "simplified" criteria for the diagnosis of AIH issued by the IAIHG which is designed for routine clinical practice, emphasis is placed on the need to report autoantibody titers [3]. Thus, ANA or SMA IFL titers of 1:40 attract 1 point whereas ANA or SMA titers ≥1:80 or anti-LKM1 of 1 ≥ 40 attract 2 points, the sum of both results being limited to 2 points [3]. Other parameters include elevated immunoglobulin G (1 or 2 points depending on the level of increase), histology compatible with or typical of AIH (1 or 2 points), and exclusion of viral hepatitis (2 points) [3]. A reliable diagnosis of probable AIH can be made at a cutoff point greater than 6 points and a definite AIH at 7 points or higher [3] although validation studies which include a wide spectrum of other liver diseases are still needed.

Selection of 1:80 or even 1:160 as a starting screening dilution expands the number of "false-negative" and can delay the early diagnosis and prompt treatment of cases with clinical suspicion of AIH [2]. More troubling is the appreciation that an increasing number of physicians considers that titers of 1:40 or 1:80 often have minimal clinical significance. On the other hand, results (mainly for ANA) reporting titers of 1:40 or 1:80 are commonly misused by inexperienced primary care physicians to screen for AIH using costly or risky diagnostic procedures, e.g., imaging techniques or liver biopsy, for no clear purpose and when the diagnostic suspicion is extremely low.

Diagnostic Relevance of Autoantibodies in AIH

Testing for a panel of autoantibodies relevant to AIH should be requested in all patients with abnormal liver function tests of unknown etiology and/or symptoms and signs of unexplained acute or chronic hepatitis [1–3, 30–32] (Fig. 4.1 and Table 4.2). There are no published data to support a role for autoantibody testing for population screening. These tests should not be used for generic screening purposes in asymptomatic individuals. Autoantibody testing in first-degree relatives of patients with AIH is only encouraged in the appropriate clinical circumstances.

Antinuclear Antibody Testing

The ANA test also known as immunofluorescence ANA (IF-ANA) or fluorescent (F-ANA) test is widely available and is based on the detection by IFL of antibodies directed against a variety of antigens localized to the cell nucleus [6, 7]. This autoantibody is readily detectable as nuclear staining in all the tissues of the rat or murine substrate [7, 12, 33]. On the liver tissue substrate it is possible to identify different

Table 4.2 Primary (open square), secondary (open triangle), and optional (open circle) tests in patients with autoimmune liver diseases

	Tentative diagnosis		
	Autoimmune Hepatitis	Primary Biliary Cirrhosis	Primary Sclerosing Cholangitis
Tests			
IFL (rodent triple tissue)	□	□	
IFL (fixed neutrophils)			□
IFL (HEp-2)	○	△	
Anti-SLA	△		
Anti-CYP2D6 (LKM1)	△		
Anti-FTCD (LC1)	○		
Anti-F-actin	○		
Anti-sp100		△	
Anti-gp210		△	
Anti-MPO			△
Anti-PR3			△

IFL immunofluorescence, *HEp-2* human epithelioma cells, *SLA* soluble liver antigen, *CYP2D6* cytochrome P4502D6, *LKM1* liver kidney microsomal type 1, *FTCD* formiminotransferase cyclodeaminase, *F-actin* filamentous actin, *MPO* myeloperoxidase, *PR3* proteinase 3

nuclear patterns but these are best seen by F-ANA tests based on HEp-2 [2, 12, 33]. The typical fluorescent patterns of ANA give homogenous, speckled, nucleolar, centromere, nuclear-dot, rim-like membranous (nuclear envelope) staining [2, 5, 6, 34]. The latter two facilitate the diagnosis of PBC-specific ANA and are helpful in distinguishing the autoantibody serology of PBC to that of AIH [2, 5, 34]. Antimultiple nuclear dot antibodies react with the nuclear body sp100 protein and anti-rim-like membranous antibodies recognize the nuclear envelope gp210 antigen (reviewed elsewhere [2, 5, 34]).

Results of F-ANA are usually reported as both titer and pattern [7, 12]. As a general rule, titers of 1:160 or higher are more likely to represent true positives and do not need to be repeated [35]. Elevated titers and certain patterns carry significant diagnostic connotations [1, 2, 6]. These titers correspond to autoantibodies of the IgG class as the IFL procedure used antihuman IgG rather than antihuman immunoglobulin as revealing agent [7].

Most reports include a brief description that states whether the ANA test result is negative or positive at the cutoff dilution. Negative F-ANA test results imply lack of noticeable nuclear fluorescence or clinically irrelevant fluorescent patterns at low titers. A result is considered positive when the nuclei display a specific pattern. In case of the co-existence of several patterns those reported are the most prominent. The report usually includes a single titer which corresponds to that of the strongest pattern.

Clinical Interpretation of ANA Testing

The ANA test is mainly used as a tool for the diagnosis of autoimmune rheumatic conditions. This test is positive in more than 95% of patients with systemic lupus erythematosus, 60–90% of patients with systemic sclerosis, and 40–70% of patients with Sjögren's syndrome [6]. ANA is also useful for the diagnosis of patients with idiopathic inflammatory myositis (30–80%), drug-induced SLE, and mixed connective tissue disorders (approximately 100%) [6, 7].

Outside liver diseases, the ANA test result can be positive in a number of other rheumatic and nonrheumatic disorders including rheumatoid arthritis, Raynaud phenomenon, thyroid disease, malignancies, multiple sclerosis, and infectious diseases [6, 7]. The prevalence varies widely depending on the condition, the study population, and the methodological approach [6, 7].

Relatively low positive F-ANA results occur in variable percentages of healthy individuals [35]. Female sex and increasing age tend to be more commonly associated with positive F-ANA based on HEp-2 cells [35].

ANA testing constitutes a major part of the diagnostic testing for autoimmune liver diseases and is present in patients with AIH-1, PBC, PSC, de novo AIH postliver transplant [1, 2, 5, 13, 22]. ANA tests are also positive in patients with viral hepatitides or infections with hepatotrophic viruses, acute liver failure, nonalcoholic steatohepatitis, alcoholic liver disease, and hepatocellular carcinoma [2]. The fact that ANA can be detected in considerable number of patients with various autoimmune and nonautoimmune liver disorders demonstrates why the ANA test alone is a poor test for screening purposes [6, 36]. For example, if in the clinic a patient has a positive ANA test giving a speckled pattern, this result by itself will not be helpful in distinguishing between AIH and autoimmune cholestatic disease or other liver disease that can be associated with ANA positivity [1, 7].

In AIH-1, a positive ANA test is an integral component of the diagnosis [3, 4]. Amongst AIH-1 cases, 30–75% of them have a positive ANA test [1, 13]. ANA alone is present in approximately 10–15% of the cases whereas ANA and SMA co-occur in ~50% of patients with AIH-1. According to internationally accepted diagnostic criteria, patients with a probable or definitive diagnosis of AIH have a positive ANA, SMA, and anti-SLA or anti-LKM1 antibodies [3]. Thus, when the clinician is faced with a patient with the clinical suspicion of AIH, it is customary to order autoantibody testing to assist the diagnosis [12].

The homogenous nuclear pattern is found in most cases (approximately 40–70%), the remainder displaying speckled or nucleolar patterns [1, 13]. Because of recognition of considerable overlap between patterns and diseases and, and even more worryingly in order to simplify reporting, an increasing number of laboratories report autoantibody titer without description of the fluorescent pattern.

In AIH-1, the pattern and titer of F-ANA results are variable and do not necessarily reflect disease activity [1, 13]. That is to say that the titer of ANA does not necessarily increase when the disease progresses and that the autoantibody does not necessarily disappear as a response to immunosuppressive treatment [1, 13, 37].

F-ANA testing requires highly trained and experienced personnel, is time consuming, and cannot be fully automated, resulting in low throughput and increased staff costs. As an alternative, many laboratories screen sera for ANA by ELISA using plates that have absorbed nuclear extract from cell preparations or mixtures of purified native or recombinant antigens [38]. The clinical usefulness of test results of "generic" ANA immunoassay tests has not been validated in patients with AIH-1 [12]. The laboratory should work closely with clinicians who order generic ANA tests. Proper interpretation of test results necessitates an understanding of the assay method and its limitations. For example, if an enzyme immunoassay-based test is negative and the laboratory cannot offer a complementary F-ANA test to substantiate the "true" negativity of the test, the physician should be made aware of this [7, 39].

Other types of commercially available assays for use in clinical laboratories are those measuring reactivity to individual nuclear antigens. There are also "profile" assays which incorporate a panel of diagnostically relevant tests in the same ELISA plate or line/dot immunoassay membrane [6, 7]. These kits are widely used in rheumatic diseases but their diagnostic utility in AIH is unclear and attempts to incorporate such testing into diagnostic algorithms are discouraged [2, 12]. By and large ANA testing by enzyme immunoassays cannot be recommended at present as a replacement for F-ANA testing in cases with suspected AIH by an accredited laboratory [12].

No single AIH-1 specific nuclear antigen has been identified so far [1, 4]. Sera from AIH-1 patients can recognize a variety of nuclear antigens including single-stranded (ss) and double-stranded (ds)DNA, histones, chromatin, centromeres, Ro-SS-A and SS-B, and various other extractable nuclear antigens, with no individual specificity or combination of specificities being characteristic of AIH [40, 41]. Their testing is useful primarily for clinical research purposes. A limited number of early studies have addressed the diagnostic and prognostic significance of antibodies to individual ANA targets [13, 40–42]. Anti-dsDNA antibodies have been reported in 23–64% of patients with AIH-1 depending on the assay used for their detection [42]. A preliminary study has indicated that patients with anti-dsDNA have higher serum levels of IgG and relapse more frequently during corticosteroid treatment compared to patients without anti-dsDNA antibody positivity [42]. However, the results of ELISA do not correlate well with those of IFL using *Crithidia luciliae* or the Farr immunoprecipitation assay which are required for their proper detection [42, 43]. Antibodies against histones appear only in patients with F-ANA positive test and occur more frequently in younger patients with higher aminotransferase levels than those lacking this antibody [13, 44]. Antibodies to chromatin are present in approximately 40% of patients with AIH-1 [40]. They occur more frequently in male than female patients and are associated with higher levels of serum IgG. A significant proportion of antichromatin antibody seropositive patients (~40%) loose this antibody marker during corticosteroid therapy [40]. These antibodies appear to be more frequent in patients with an active disease and in patients who relapse after treatment [40]. Notwithstanding these findings, the clinical value of these antibodies has not been established. There is no systematic review that addresses the prognostic significance of individual ANA reactivities and their routine testing is discouraged at this time [12].

Antismooth Muscle Antibody Testing

Serum samples from AIH-1 cases with antismooth muscle antibodies (anti-SMA or SMA) typically stain the wall of arterial blood vessels (V), the mesangium of renal glomeruli (G), and fibers surrounding the kidney tubules (T) giving the VG or VGT patterns [12, 45]. SMA also stains the smooth muscle of the gastric mucosa. The use of vinblastine-arrested cultured fibroblasts as a substrate for IFL has also revealed different fluorescent patterns targeting mainly actin, tubulin, or intermediate filaments [46]. The VGT pattern corresponds to the microfilament (MF) staining of isolated fibroblasts and represents a cable pattern across the cell also known as "antiactin" [47]. These antibodies give a characteristic cytoskeletal pattern on vascular smooth muscle (VSM) 47 cells from rat embryonic thoracic aorta [24, 48]. It appears that the characteristic microfilament pattern on VSM47 correlates strongly with that of VGT on rodent tissues and is easier to read. It is also highly specific for AIH-1, being present only in a minor proportion of pathological controls with positive SMA antibody positive tests [24, 48]. If these findings are confirmed on larger number of patients and in other laboratories, VSM47 cells can be proposed as a complementary substrate for the detection of AIH-1 specific SMA [24, 48].

Very few laboratories describe the SMA pattern of IFL on rodent tissue. Most clinicians are unfamiliar with the classifications of SMA and the relevant terminology. The clinician must be aware that autoantibody tests reporting a VGT or "antimicrofilament" or "antiactin" SMA positive tests describe in practice the same autoantibody pattern. Efforts to achieve global harmonization of SMA autoantibody testing and results reporting are underway by the IAIHG. Ideally, a positive SMA test should be reported both as a particular staining pattern and as a titer. In the clinic, it is the presence of the antiactin pattern that the physician needs to know. In practice, the SMA test result is usually reported as positive or negative without a description of the pattern or the titer.

Clinical Interpretation of SMA Testing

SMA positive tests giving the "antiactin" IFL pattern have long been regarded highly specific for AIH-1, though some 3–40% of patients with this form of the disease test negative [1, 49]. The diagnostic utility of the SMA test largely depends on the clinical setting. As for F-ANA, the SMA test performed by IFL has been used for the assessment of patients with clinical and/or laboratory suspicion of AIH and has been incorporated in the diagnostic criteria for this disease by the IAIHG [3, 4]. While the ANA test is positive in AIH and a group of diverse inflammatory disorders, SMAs at high titer are mainly found in patients with AIH-1. Unlike ANA, these antibodies are infrequently present in patients with SLE or other autoimmune rheumatic diseases [46]. Of note, the anti-SMA test needs not to be confused with the SLE-specific anti-Sm antibody test. SMA positive tests are reported in a variety

of liver and nonliver diseases at varying frequencies but their titers and patterns differ from those seen in AIH [8, 46, 50, 51]. SMA have been reported in 20–50% of cases with chronic hepatitis B and hepatitis C viral infection [8, 50–54]. SMA seropositivity has been reported in ~50% of PBC patients without evidence of coexistent AIH [49, 55]. SMA tests in these groups of patients show relatively low titers and a fluorescence staining mainly of the V pattern. In non-liver-related conditions, SMA can be present in patients with malignant diseases and patients infected with cytomegalovirus. Unlike ANA, the incidence of SMA does not increase with age and is not higher in females compared to males [56].

SMA titres tend to become lower and may even disappear during immunosuppressive treatment in a significant proportion of patients with AIH-1 [13]. Their titer at diagnosis and their behavior over time do not predict disease outcome [13]. A longitudinal study of pediatric cases with AIH has reported a correlation of SMA titers with AST levels suggesting that the titers of these autoantibodies can be barometers of disease activity [37].

Attempts to identify a single, AIH-1 specific target of SMA have failed so far [49, 57]. Most of the studies have provided data suggesting that actin in its polymerized filamentous (F-actin) rather than its monomeric G actin form is a major target of SMA [46, 58–60]. However, there is no molecular proof as yet that F-actin is indeed the only autoantigen associated with the AIH-specific VGT/MF pattern [25].

In recent years, commercial ELISA and dot/line immunoassays have been developed to detect the presence of anti-F-actin antibodies [24–26, 51]. The introduction of these assays has generated a series of studies investigating the diagnostic and clinical utility of this autoantibody and its relationship to the patterns of SMA. These studies show that the sensitivity and specificity of the anti-F-actin antibody tests are lower than that of the SMA VGT positive tests. Anti-F-actin antibodies are detected in up to 25% of cases with AIH-2, PBC, PSC, viral hepatitis, alcoholic liver disease, and celiac disease, a finding which is in sharp contrast to the high specificity of the IFL VGT test. The results obtained with the IFL and molecular assays overlap considerably, but by no means completely and the discrepant cases vary among studies. The presence or absence of anti-F-actin antibodies does not appear to be prognostically useful at present. One can reasonably assume that based upon the results of these studies, at present anti-F-actin antibody kits are inadequate for autoantibody screening in patients with suspected AIH. Notwithstanding these observations, high titer anti-F-actin antibodies have good correlation with the SMA VGT immunofluorescent staining and are associated with AIH-1.

The prognostic significance of these antibodies is not clear, though some early data indicate that anti-F-actin antibodies are more prevalent in patients who have younger age at presentation, a poorer response to treatment, and faster progression to liver failure compared to patients who are seronegative for this antibody [61]. A more recent study has reported anti-F-actin antibodies by ELISA in individuals with normal liver function and no other evidence of liver disease [62]. Larger clinical studies are necessary to provide outcome data to validate the rationale for testing of these antibodies in the clinic.

Antiliver Kidney Microsomal Antibody Testing

This antibody stains the Liver and Kidney and is absorbed out by the Microsomal fraction of liver homogenate. These three characteristics of the antibody have led to labelling it as anti-LKM antibody. [63, 64]. "Microsomal" is something of a misnomer as "microsomes" are the constituents derived mostly from the endoplasmic reticulum wherein the anti-LKM autoantigens are located. These fractions are obtained by differential ultracentrifugation of liver homogenates and are mainly used for research purposes. Anti-LKM1 brightly characteristically stains the third portion of the proximal renal tubules and the cytoplasm of the hepatocytes but it spares cells of the gastric mucosa [1, 2, 12, 63, 64].

Clinical Interpretation of Anti-LKM Antibody Testing

Homberg et al. were the first to suggest that anti-LKM antibodies define a second type of AIH, which they named AIH-2 [14]. Anti-LKM antibody was soon renamed anti-LKM1 because other LKM immunofluorescent patterns were detected in cases of tienilic acid-induced hepatitis (anti-LKM2) and chronic viral hepatitis D infection (anti-LKM3) [65, 66]. A fourth type of anti-LKM antibodies has been described in patients with AIH associated with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), in which the pattern of staining is indistinguishable from that of anti-LKM1 [67]. Confusing though it might be, type 1 anti-LKM is detected in type 2 AIH!

Anti-LKM1 is a frequently misdiagnosed autoantibody, being commonly misinterpreted as AMA and this information is important for the clinical interpretation of the autoantibody test results [68–70]. The confusion between AMA and anti-LKM1 arises because of the ability of these autoantibodies to stain the renal tubules [1, 2]. The difference between the two patterns can be seen by a trained eye and is readily appreciated when the kidney tissue section contains both distal and proximal tubules [1, 2]. AMA stains strongly the mitochondria-rich distal tubules which are smaller than the proximal tubules stained by anti-LKM1 antibodies [1, 2]. AMA also stains the gastric parietal cells within the stomach, which are spared by anti-LKM1, whereas AMA stains hepatocytes much less brightly than does anti-LKM1 [1, 2]. This is why several clinical laboratories analyze samples using the three-tissue substrate instead of kidney/stomach or kidney tissue substrate alone. The combination of the three tissues allows better serological interpretation and minimizes the probability of diagnostic errors. The clinician must understand that the interpretation of staining fluorescent patterns relies heavily on the experience and the skills of the reader. Quality assurance programs around the world have noted inability of several laboratories to report correctly the presence of anti-LKM1 antibodies by IFL [2]. Physicians must always be in a

position to challenge autoantibody test results reports which are inconsistent with their clinical findings. For example, AMA is extremely rare in pediatric patients and children with PBC 15 yrs, when AMA is reported in a child with clinical and histological characteristics of AIH, the serological report is almost certainly incorrect [68, 70–72].

Anti-LKM1 seropositivity strongly supports the diagnosis of AIH-2, particularly in the absence of HCV because these antibodies in isolation or combination with anti-LC1 (discussed later) are present virtually in all newly diagnosed AIH-2 patients [3, 15]. In practical terms (by definition), the likelihood of a diagnosis of AIH-2 in a patient with anti-LKM1 antibody positivity and negative viral hepatitis C markers is approximately 100% [3, 4, 15]. Anti-LKM1 antibodies are reported in a proportion of adult (0–6%) or pediatric (0–11%) cases with chronic hepatitis C infection [1, 2, 51, 73, 74]. Thus, a clinician cannot simply rely on an anti-LKM1 antibody positive test to make a definite diagnosis of AIH-2. Experts in busy liver outpatient clinics may see chronic HCV infected patients with anti-LKM1 positive results unrelated to AIH-2. Some of those experience transaminase flares and adverse reactions during antiviral treatment and need to be monitored at regular intervals [75]. A recent study in a large cohort of anti-LKM1 antibody positive suggests that antiviral treatment is as beneficial in these patients as in anti-LKM1 negative HCV patients, and that the rare liver enzyme flares are sufficiently controlled by corticosteroids, allowing continuation of antiviral therapy [75].

Compared with pediatric AIH-1 patients, those with AIH-2 tend to be younger, have partial IgA deficiency, have higher levels of bilirubin and transaminases at diagnosis, and present more frequently with fulminant hepatic failure [15]. Early studies conducted on French and Italian patients support the contention that pediatric AIH-2 cases have more frequently aggressive disease compared to children with AIH-1 but this does not appear to be the case in series followed up at King's College London [14, 15, 37, 76].

While the target antigens of ANA and SMA certainly need better definition at the molecular level, that of anti-LKM1 antibody has been clearly identified in late 1980s as cytochrome P450 IID6 (CYP2D6), a member of the hepatic P450 microsomal family of enzymes [77–80]. Subsequent studies have reported that the target of anti-LKM2 antibody is CYP2C9 [80]. The autoantigens of anti-LKM3 antibody are members of the uridine diphosphate glucuronosyl transferases (UGT) family of enzymes and those of the APECED-associated AIH anti-LKM antibody are the CYP1A2, CYP2A6, and CYP2D6 enzymes [80, 81]. The identification of CYP2D6 as the target antigens of anti-LKM1 has enabled the development of enzyme immunoassays based on the use of the recombinant antigen which have proven useful in assisting the detection of anti-LKM1 antibodies [1, 2]. Usually, the titers of IFL-detected anti-LKM1 antibodies correlate with anti-CYP2D6 antibody concentrations by ELISA, but at times weak positive or borderline anti-LKM1 test results are true positive by anti-CYP2D6 antibody ELISA assays and such complementary testing can solve diagnostic uncertainties. The inability of these ELISAs to detect anti-LKM1 antibodies in patients with chronic hepatitis C virus infection compared

to IFL and radioimmunoprecipitation is possibly because of the advantage of the latter assays to identify conformational epitopes undetectable by ELISA [82, 83] . Short CYP2D6 peptides used as antigenic preparations perform less well than those using full-length protein and their diagnostic use is limited [82, 84]. The laboratories must ensure that anti-LKM1 or anti-CYP2D6 positive tests are "true positives." In case of a positive test, the physicians must be informed of the outcome of the test and the diagnostic importance of the finding. The experience of a West Coast American Liver Transplantation Center has shown that lack of timely anti-LKM1 detection can lead to labeling AIH-2 as cryptogenic cirrhosis [31, 69]. Misdiagnosis or late diagnosis ultimately leads to liver transplantation, or even death, for a condition exquisitely responsive to immunosuppressive treatment and for an antibody that is easily detectable by commercially available ELISAs [2, 15, 30, 69].

Antiliver Cytosol-1 Antibody Testing

Anti-LC1 antibodies have been known for more than 20 years [85]. It is the second serological marker of AIH-2. When present in isolation it stains the cytoplasm and spared the cellular layer around the central veins of juxtavenous hepatocytes [1, 12, 85]. In two third of the cases, however, anti-LC1 is obscured by the simultaneous presence of anti-LKM1 and cannot be detected by IFL [1, 12, 85]. Alternative methods for its detection include double dimension immunodiffusion, counter immunoelectrophoresis, and immunoblotting [86, 87]. The target antigen of anti-LC1 has been identified as formiminotransferase cyclodeaminase [88, 89]

Clinical Interpretation of Anti-LC1 Antibody Testing

Anti-LC1 antibody can be the sole marker in one third of cases with AIH-2. Like anti-LKM1, anti-LC1 antibodies have been reported in a proportion of chronic hepatitis C virus infected patients, most of whom had anti-LKM1 antibody positive tests [86]. An Italian study conducted in a relatively small number of anti-LKM1- and/or anti-LC1-positive AIH-2 patients has indicated that reactivity to LKM1 was unaffected during treatment, whereas reactivity to LC1 disappeared or significantly decreased (>50%) during remission and flared up during relapse, but this observation requires confirmation [90].

Nowadays, molecular assays for the detection of anti-FTCD antibodies are commercially available and can be used as alternative or complementary test to assist the diagnosis of anti-LC1. It has become evident that there is a wide variability in clinical practice regarding the utility of autoantibodies in the diagnosis and care of patients with AIH-2. A small number of clinical laboratories have implemented this test in their routine diagnostic testing.

Antineutrophil Cytoplasmic Antibody Testing

ANCA is detected by IFL using as substrate human ethanol-fixed neutrophils. Two patterns of staining can be recognized: a cytoplasmic (c-ANCA) and a perinuclear (p-ANCA). The classical p-ANCA staining is the result of an artifact caused by the ethanol fixation of the neutrophils [91]. The fixation procedure leads to the migration of some positively charged cytoplasmic antigens to the negatively charged nuclear envelope, giving the characteristic p-ANCA staining. The classical p-ANCAs are serological markers of microscopic polyangiitis and their major target autoantigen is myeloperoxidase. c-ANCAs are found in patients with Wegener's granulomatosis and are directed mainly against proteinase 3. A third type of ANCA, called atypical p-ANCA is seen in patients with AIH-1, ulcerative colitis, and PSC [92]. Unlike classical c-ANCA and p-ANCA which recognize components of the neutrophilic cytoplasm, atypical p-ANCAs with antigen(s) localized within the nuclear envelope of neutrophils [93]. An experienced reader can differentiate between typical and atypical p-ANCA using both ethanol and paraformaldehyde-fixed neutrophils [92, 94]. In our experience, atypical p-ANCA antibody positive tests must be checked by F-ANA and with specific assays for myeloperoxidase and proteinase 3.

Clinical Interpretation of ANCA Antibody Testing

Atypical p-ANCA have been reported in 50–92% in patients with AIH. A significant number of laboratories do not report the presence of the atypical pattern. Most clinicians are unaware of the lack of clinical utility of atypical ANCA. The presence of atypical p-ANCA should not be measured for screening purposes in patients with suspected AIH. Atypical p-ANCA antibody positive samples recognize a 50-kDa nuclear pore complex antigen which has been identified as the tubulin beta chain 5 (TBB5) [95, 96].

Antisoluble Liver Antigen Antibody Testing

Anti-SLA (also known as antisoluble liver antigen-liver pancreas) antibody has emerged as one of the most specific markers of AIH [17, 18, 96, 97]. This antibody cannot be detected by IFL and its testing has relied on molecular-based assays [17, 18, 97–100]. It can be the only antibody present in a small group of patients with AIH, but most frequently is present in typical cases of AIH-1 [13, 17, 98, 101, 102]. The target antigen of anti-SLA antibodies is a selenocysteine synthase critical for the metabolism of selenocysteine called *Sep* (*O*-phosphoserine) tRNA:*Sec*

(selenocysteine) tRNA synthase or simply SepSecS, an acronym which has been approved by the Gene Nomenclature Committee of the Human Genome Organization (HUGO) [101, 103]. It is anticipated that it will take a long time for clinical immunology laboratories to become familiar with the formal name of the antigen. Commercial ELISA, immunoblotting, and dot or line immunoassays, which are increasingly replacing the inhibition ELISAs originally used for anti-SLA antibody detection, are currently used in routine practice [40, 99, 100, 102, 104, 105].

Clinical Interpretation of Anti-SLA Antibody Testing

Depending on the geographic origin of the patients with AIH or the conventional assays used for their detection, anti-SLA antibodies are present in 5–22% of patients with AIH-1 [32, 102, 105]. Provided that a high sensitivity method is used for its detection, anti-SLA antibody is also present in up to 50% of patients with AIH-2 and also in patients with the pediatric form of autoimmune sclerosing cholangitis [19]. More recently, anti-SLA antibody has been described in 22% of patients with acute liver failure using a very sensitive, radiological assay but it is not clear whether the presence of these antibodies in patients with this condition reflects an underlying component responsible for the induction of acute liver failure [19, 106]. In a similar fashion, a study based on a radioisotopic immunoprecipitation method has reported anti-SLA antibodies in chronic hepatitis C virus infected patients [107]. However, a series of studies using *in house* or commercially available molecular-based assays were unable to detect anti-SLA antibodies in this group of patients [19, 104, 106]. Recently, a multicenter study from France has reported that among 81 cases with true-positive anti-SLA antibody positive tests, 3 (4%) had evidence of chronic HCV infection unrelated to AIH [108]. The significance of these findings is far from clear, and the need to screen for this marker in this group of patients is uncertain at best.

Some experts have proposed that testing of anti-SLA antibodies maybe useful to identify a subset of individuals initially thought to have cryptogenic cirrhosis [18, 30, 32]. Interestingly, the simplified criteria proposed by the IAIHG have included anti-SLA in the routine autoantibody tests used for the diagnostic testing of AIH [3]. Whether this will work in practice remains to be seen. Most clinical laboratories are unlikely to implement this test for diagnostic use. Anti-SLA antibodies denote patients with a more severe course of AIH and a propensity for relapse during maintenance therapy or after corticosteroid withdrawal compared to those without anti-SLA antibodies [19, 30, 102]. There is no unanimity of opinion, but the presence of anti-SLA antibody may alter therapeutic strategy for a subset of patients. If further prospective studies confirm a consistent relationship between anti-SLA antibody positivity and clinical indexes, it will be possible to use this antibody as a rational approach to monitor disease's activity and outcome.

Antiasialoglycoprotein Receptor Antibodies

Antibodies to liver membrane antigens received special attention in the 1980s and early 1990s since their concentration correlated with the histological severity of AIH. Attempts to identify antigens specifically expressed on the hepatocyte surface which could serve as targets in autoimmune liver diseases have led to the identification of the asialoglycoprotein receptor (ASGPR). ASGPR is a type II transmembrane glycoprotein. Anti-ASGPR antibodies cannot be detected by IFL. The detection of these antibodies relies on molecularly based assays. A limited number of studies investigated the diagnostic and clinical utility of this marker. Early studies have reported the presence of anti-ASGPR antibodies in ~90% of patients with AIH but are also in up to 14% of patients with PBC, chronic hepatitis B and C, and alcoholic hepatitis. Thus, anti-ASGPR antibodies can co-occur with AMA, SMA, ANCA, and even anti-LKM1 antibodies. Persistence of anti-ASGPR was indicative of unresponsiveness to immunosuppressive treatment and reappearance was highly suggestive of relapse especially after corticosteroid withdrawal.

Because of the difficulty to establish a reliable assay, a commercial kit was not available and for many anti-ASGPR antibody testing was limited to academic research laboratories.

A recent study has assessed the performance of a new commercial kit which detects anti-ASGPR antibodies by ELISA [109]. Anti-ASGPR antibody positive tests were reported in 70% of patients with AIH at diagnosis and in only 1/262 (0.4%) of the pathological and normal controls demonstrating a specificity of 99.4% [109]. According to the authors, anti-ASGPR antibody levels significantly correlated with transaminase and IgG levels. The number of patients and controls is relatively small to draw meaningful conclusions. Moreover, the correlations between disease's activity indices and the levels of autoantibodies are modest. Thus, though interesting these data should be viewed as provisional and will need to be validated externally in larger cohorts.

Conclusion

In conclusion, a considerable number of autoantibody tests are available to assist the diagnosis of AIH. Not one of these tests is, however, perfect, and most of them have significant constraints. Moreover, the interpretation of the autoantibody test results is not an easy task. The strength of most of these tests is their high negative predictive value, that is, they have the ability to exclude disease; for example, a negative SMA, ANA, anti-LKM1, or anti-SLA test makes AIH much less likely. Conversely, in a minority of autoantibody tests and under certain circumstances there is a high probability of confirming the presence of the disease, for example, anti-LKM1 is a good marker of AIH-2 in patients not-infected with viral hepatitis C. However, the predictive values are rarely sufficient per se to be definitive regarding the diagnosis. Nevertheless, when used wisely, they can provide answers to differential diagnostic dilemmas.

Chapter Summary

1. AIH is characterized by positive autoantibody tests: Type 1 is marked by a positive test result for antinuclear and/or antismooth muscle antibody and type 2 for antiliver kidney microsomal type 1 and/or antiliver cytosol type 1.
2. AIH is an unlikely diagnosis in patients with chronic hepatitis and true autoantibody negative tests; the results of autoantibody testing form part of the criteria for routine diagnosis of AIH. Most tests, however, lack prognostic value and repeated testing is discouraged.
3. Detection of autoantibodies is mainly performed by indirect immunofluorescence or ELISA; the first detects a broad array of autoantibody specificities of relevance to AIH while the second allows the identification of reactivities to specific autoantigens and assists the diagnosis of the disease in suspicious cases with equivocal, undetermined, or perplexing immunofluorescent patterns.

Useful Tips for Practitioners

1. Avoid unnecessary repeating of tests without proper justification worthwhile if seen very early in course of disease.
2. Be careful with the diagnostic value and clinical interpretation of "borderline positive" autoantibody test results.
3. Combination of testing by indirect immunofluorescence and molecularly based assays is unnecessary in the great majority of the cases. It can prove of help in cases with clinical suspicion of AIH and unspecified fluorescent patterns of borderline results.

Common Pitfalls in Practice

1. Clinicians should not rely on autoantibody test results to make a diagnosis: their role is to confirm the diagnosis.
2. In the correct context, moderate or low positive titers may still be of considerable diagnostic significance.
3. Physicians should engage with the clinical immunology labs if results are not in agreement with the clinical or histological findings.

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Chapter 5
Mimics of Autoimmune Hepatitis: Drug Induced and Immune Mediated Liver Disease

Arndt Vogel and Michael P. Manns

Keywords Hepatotropic viruses • Drug toxicity • Autoimmune reactions • Autoimmune diseases • Primary biliary cirrhosis

Introduction

The liver is the target of numerous acute and chronic inflammatory processes. Major causes are hepatotropic viruses, toxicity of drugs and their metabolites, autoimmune processes, or genetic defects. Serological markers of autoimmunity can be detected in serveral of these disorders including alcoholic [1] and non-alcoholic fatty liver disease [2, 3], acute [4] and chronic [5–12] viral hepatitis, and drug-induced hepatitis [13, 14]. It is however important to distinguish between autoimmune reactions and autoimmune diseases. The pathogenetic role of most, if not all, autoantibodies is still unclear and it is not known whether autoantibodies that are observed in primarily non-autoimmune liver disease contribute to tissue damage. In most liver diseases associated with serological markers of AIH, autoantibodies against phase I and phase II drug metabolizing enzymes may also be detected (Fig. 5.1). Autoimmune reactions against members of the families of cytochromes P450 (P450) and UDP – glucuronosyltransferases (UGT) will also be discussed.

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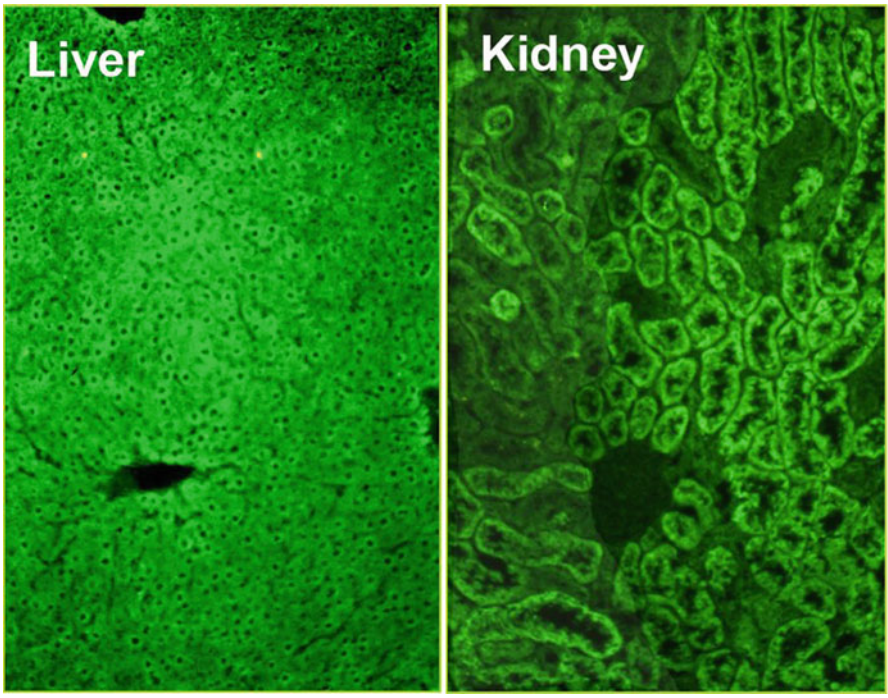

Fig. 5.1 Detection of LKM-1 antibodies by immunoflourescence

Cytochrome P450 Family and Immune-Mediated Liver Injury

Cytochrome P450 (CYP) is a very large and diverse superfamily of drug-metabolizing enzymes, which are predominately expressed in the liver, but which can also be found in several other organs such as testes, ovaries, adrenals, respiratory tract, lungs, kidney, brain, the small intestine, and in the adipose tissue of the breast. The initial nomenclature of CYPs relied on evolutionary relationships as depicted in phylogenetic trees using number–letter–number combinations. The first number designates a family that shared 40% or greater amino acid sequences. The subsequent letter denotes a particular subfamily within each family that contains CYPs that are at least 55% identical. The last number in the name identifies the specific CYP enzyme. Recently, a web page has been constructed that contains a continuously updated list of allelic variants of CYP genes (http://www.imm.ki.se/CYPalleles).

CYPs use a plethora of both exogenous and endogenous compounds as substrates in enzymatic reactions. They are also involved in the biosynthesis of steroids, fatty acids, and bile acids. Beside CYP3A and CYP2C, which represent 30% and 20% of all CYPs, other important isoenzymes are CYP 1A2 (13%), CYP2E1 (7%), CYP2A6 (4%), CYP 2D6 (2%), and CYP2B6 (0.4%) [15]. CYPs have been identified

as self-antigens in different forms of autoimmune reactions, including drug-related, viral, or autoimmune hepatitis. Though many studies investigated CYPs in autoimmune disease, the role of CYPs as self-antigens remains incompletely understood. In some cases, the capacity of CYPs to activate substrates to highly reactive metabolites that can bind to cellular marcomolecules including the CYP catalyzing the reaction may be an initiating step that triggers the immune response to the newly formed neoantigen.

At least ten human CYPs are recognized by autoantibodies associated with either acute or chronic liver diseases. This text summarizes the current knowledge about the role of CYPs in different forms of hepatitis and underlines the importance of CYPs as potential self-antigens in disease pathogenesis. Possible mechanisms that induce loss of self-tolerance leading to autoimmune attack will be illustrated.

CYPs in Autoimmune Hepatitis

Autoantibodies against microsomal proteins form a heterogeneous group and are associated with several immune-mediated diseases including AIH, drug-induced hepatitis, the autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), and chronic hepatitis C and D infection [16]. Antibodies to LKM first discovered in 1973 by indirect immunofluorescence are regarded as serological markers of AIH type 2 (Fig. 5.2). They are reactive with the proximal renal tubule and hepatocellular cytoplasma [17, 18]. Subclassification is achieved by ELISA and Western blot, preferably with recombinant antigens. The 50 kD antigen of LKM-1 was identified as CYP2D6 by two independent approaches. The first approach involved screening of human liver complementary DNA libraries and the identification of CYP2D6 by sequence analysis [19, 20]. The second approach used the specific inhibition of the enzymatic activity of the target protein for identification, showing that LKM-1 autoantibodies inhibit the hydroxylation of bufuralol, a substrate of CYP2D6 in isolated liver microsomes. Extensive mapping studies identified several epitopes recognized by sera of patients with AIH type 2 and HCV infection [21–25]. LKM-1 antibodies in AIH mainly recognize short linear epitopes of CYP2D6 ($CYP2D6_{196–218}$, $CYP2D6_{254–271}$, and $CYP2D6_{321–351}$) [21–23] (Fig. 5.3). Minor epitopes are $CYP2D6_{373–389}$ and $CYP2D6_{410–429}$. Some of these epitopes exclusively react with sera from patients with AIH (such as $CYP2D6_{254–271}$) or with sera from patients with HCV (such as $CYP2D6_{200–214}$), whereas other are recognized by LKM1 in both diseases (such as $CYP2D6_{193–212}$).

Whether anti-CYP2D6 antibodies are only of diagnostic value or also play a role in the pathogenesis of AIH remains unclear. Of note, 5–10% of the population are CYP2D6 deficient and to date, AIH type 2 has never been described in CYP2D6-deficient individuals suggesting that the expression of CYP2D6 might be a condition sine qua non for developing AIH type 2 [26].

Human CYP enzymes are primarily membrane-associated proteins located either in the inner membrane of mitochondria or in the endoplasmic reticulum of cells.

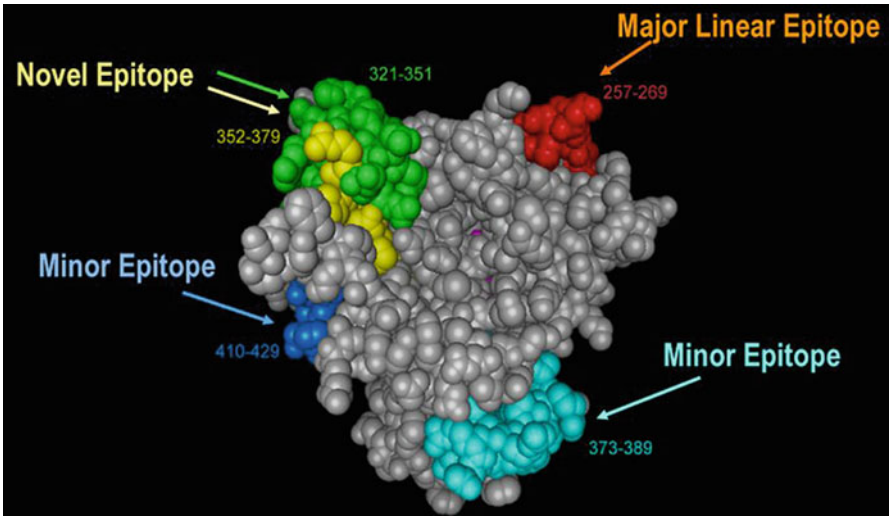

Fig. 5.2 Three-dimensional structure of CYP2D6 with known epitopes detected by AIH patients. Manns MP, Vogel A. Hepatology. 2006;43:S132–44. Used with permission

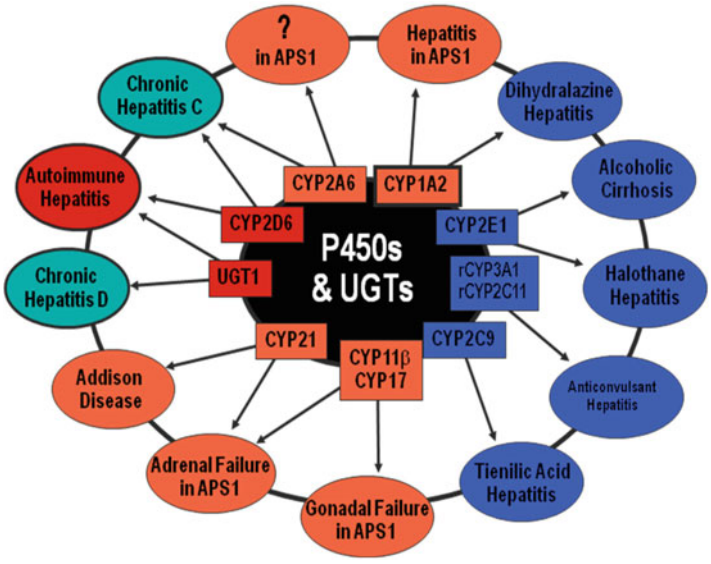

Fig. 5.3 Cytchrome P 450 Enzymes and UDP-Glucuronosyltransferases as Autoantigens and their Disease Associations. Manns MP, Obermayer-Straub P. Hepatology. 1997 Oct;26(4): 1054–66. Used with permission

One potential prerequisite for an immune-pathogenic relevance is that the antigenic regions of CYP2D6 are exposed on the external hepatocellular membrane, where antibodies that induce an immune reaction can detect them. Several studies indicate that CYP2D6 may access the cell membrane via a vesicular transport route from the endoplasmic reticulum [27]. Moreover, expression of CYP2D6 on the surface of hepatocytes can be detected by indirect immunofluorescence and confocal laser microscopy of isolated rat hepatocytes probed with LKM-1 positive sera from patients with chronic hepatitis C or AIH type 2 [28].

Some studies revealed that antibodies directed against CYPs are able to inhibit the activity of the enzyme in vitro suggesting that these antibodies might have an additional biological significance [24, 29]. However, this observation has not been confirmed in in vivo experiments. Two amino acids (Asp-301 and Glu-216), which are relevant for substrates and inhibitors, are located in proximity to the main epitopes on CYP2D6. Thus, it is possible that antibody-binding to those epitopes results in an interference with the enzyme's function [30]. Another option is an antibody-mediated conformational change of CYP2D6, which impairs its function.

CYPs in the Autoimmune Polyendocrine Syndrome Type 1 (APECED)

Autoimmunity against organ-specific CYP enzymes is also characteristic for APECED syndrome. APECED is a rare autosomal recessive disorder, which is characterized by an immune-mediated destruction of endocrine tissues, chronic candidiasis, and additional ectodermal disorders [31]. In the majority of cases candidiasis is the first clinical manifestation to appear before the age of 5 years. This is followed by hypoparathyroidism before the age of 10 years, and later by the onset of Addison's disease before the age of 15 years. Immunosuppressive therapy has been successfully used to treat various complications of APECED. In contrast to many other autoimmune diseases, APECED is associated with mutations of a single gene, designated autoimmune regulator (*AIRE*) [32]. *AIRE* up-regulates the transcription of certain organ-specific self-antigens in medullary thymic epithelial cells, and has a role in the negative selection of organ-specific thymocytes. So far, more than 50 different mutations of the *AIRE* gene have been identified and are distributed throughout the entire non-coding and coding region [33, 34]. The variety of autoimmune diseases reported in patients with APECED suggests that AIRE might contribute to the etiology of other autoimmune disorders. Recent studies however indicate that common mutations in the AIRE gene do not play a major role in autoimmune liver diseases, and are therefore a unique feature of APECED [35, 36].

Chronic hepatitis affects only 10–18% of the patients. Similar to AIH-2, autoantibodies to CYP1A2 and CYP2A6 have been described as markers of liver disease in patients with APECED, but antibodies to tryptophan hydroxylase are the best predictors for hepatitis in APECED [37–40]. Other organ-specific antibodies

detected in these patients are directed against CYPc21 (21-hydroxylase), CYPscc (side chain cleavage enzyme), and CYPc17 (17-hydroxylase) [41–43]. While antibodies directed against CYPc17 and CYPscc correlate with hypergonadotropic hypogonadism, the combination of the antibodies against CYPc21, CYPc17, and CYPscc is associated with adrenal insufficiency. However, not only CYPs but also many other antigens such as tryptophan hydroxylase and tyrosin may also be self targets in APECED.

CYPs in Hepatitis C Virus Infection

Hepatic infection with the hepatitis C virus is known to induce several hepatic and extrahepatic autoimmune manifestations. Extrahepatic manifestations include mixed cryoglobulinemia, membrano-proliferative glomerulonephritis, polyarthritis, porphyria cutanea tarda, and Sjögren syndrome. Similar to AIH patients, sera of hepatitis C virus (HCV)-infected patients if looked for are frequently positive for ANA, SMA, and LKM. LKM-1 antibodies are found in 5–10% of patients with chronic HCV infection. Additionally, anti-CYP2A6-antibodies are reported in LKM-1-positive patients with HCV [44]. In contrast to HCV infection, autoantibodies are rather infrequent in patients suffering from chronic hepatitis B. In light of pronounced differences in the capacity to induce autoimmune reactions, it seems unlikely that nonspecific reactions of the immune system, e.g., reactions against necrotic liver tissue, cause the phenomenon of virus-induced autoimmunity. It is more reasonable to assume that specific mechanisms are at work. Such processes may be cross-reactions of viral antigens with native hepatic antigens due to sequence similarity or as a consequence of mimicking tertiary structures.

LKM-1 antibodies in AIH-2 and HCV-infection do not react identically. While LKM-1 antibodies in AIH primarily recognize small linear epitopes of CYP2D6, HCV-associated LKM-1 antibodies are more heterogeneous and detect mainly conformational epitopes of CYP2D6. However, some target-epitopes of LKM-1 antibodies in AIH-2 and HCV-infection overlap. For example, the major B cell epitope $CYP2D6_{193–212}$ was recognized by sera from 93% of patients with AIH-2 and 50% of patients with LKM-1 positive-HCV-infection [24].

The clinical relevance of these autoantibodies in patients with Hepatitis C is so far not completely understood. LKM in low titer is usually regarded as an epiphenomenon and only high antibody titers are considered to be a sign for a relevant autoimmune reaction. Nevertheless, it is reported that LKM-1-positive patients with a chronic hepatitis C may develop hepatic flares under the antiviral therapy with interferon. This phenomenon has been further investigated in smaller studies with 6 or 7 LKM-1-positive HCV-infected patients, in whom exacerbation was observed in 3/6 as compared to 1/7 without LKM-1 Ab [45, 46]. Recently, Ferri et al. tested a larger group of 26 LKM-1-positive patients and found that the exacerbation rate was negligible and reversible after immunosuppressive therapy [47].

CYPs in Drug-Induced Hepatitis

Drug-induced hepatitis is albeit rare but sometimes may cause a life-threatening hepatitis if exposure to that drug is prolonged. Common to most patients is the metabolism of the drug by CYPs. A reactive metabolite is formed during the hydroxylation process, which may either bind to the active center of the CYP or which may leave the active center and covalently modify other hepatic proteins. Up to this point processes seem to be similar in all patients treated. However, in few susceptible patients drug-induced adducts are formed during these processes which are then recognized by the immune system as non-self. If this immune reaction is not suppressed, immune attacks directed against all cells, which harbor these modified proteins may follow. This immune reaction may also be directed against native and modified hepatic proteins. Since the induction of an immune reaction is the critical event in drug-induced hepatitis, the severity and onset of disease are essentially independent of drug dosage. Nevertheless, time is necessary for the induction of the specific immune response, therefore a significant latency period is observed, which may vary from a few weeks to several months. After withdrawal of the drug, the targets of the immune response is no longer available and the hepatitis usually declines. The disease recurs upon re-challenge with the drug, this time after a shorter latency period and the "hepatitis" tends to be more severe upon re-exposure. Females tend to be more frequently affected by drug-induced hepatitis than men.

Tienilic acid, a uricosuric diuretic withdrawn from clinical use in 1980, is one example for immune-mediated drug-induced hepatitis. One-tenth to 0.7% of patients treated with tienilic acid developed a hepatitis. [48]; the reaction occurred with significant delay and liver damage was found to be dose independent. After discontinuation of the drug, liver damage resolved, but recurred after re-challenge [49]. Affected livers were infiltrated with neutrophils, eosinophils, and lymphocytes. In 60% of patients suffering from severe hepatitis after administration of tienilic acid, a specific antibody directed against unmodified liver and kidney microsomal proteins was detected, LKM-2 [50]. The molecular target of this LKM-2 autoantibody is CYP2C9, which is the major tienilic acid metabolizing enzyme. Based on the available data, a hypothetical mechanism for LKM2 antibody induction in patients with tienilic acid-induced hepatitis has been proposed. Accordingly, tienilic acid is activated by CYP2C9 during the degradation process to form a reactive sulfoxide, which binds to the enzyme and then forms a neoantigen resulting in the production of the LKM-2 autoantibody [51]. It is not known whether the LKM-2 autoantibodies are simply side-products of the underlying pathogenic process or whether they are directly involved in the pathogenesis of the disease.

Dihydralazine has been shown to induce a hepatitis in some patients, which is characterized by antibodies that very specifically react with liver membranes (LM), but do not stain kidney sections. Subsequently, CYP1A2 was identified as the molecular target of these autoantibodies [52–54]. Dihydralazine-induced hepatitis affects more frequently females than males [55]. The hepatitis usually developed with a lag period of several months, resolved after discontinuation of treatment and

was re-induced upon re-challenge with the drug. A mechanism was proposed for the development of LM autoantibodies based on evidence that CYP1A2 act as the dihydralazine-activating enzyme. Dihydralazine is mainly metabolized via two pathways: One pathway is dependent of the *N*-acetyltransferase, resulting in an acetyl conjugate, the other one is an oxidative pathway, which is probably catalyzed by CYP1A2 and which contributes to the metabolic activation of dihydralazine. The latter leads to the formation of neoantigens, namely adducts with CYP1A2, which may induce an immune response and LM-antibody production. In 50% of the Caucasian population *N*-acetyltransferase activity is absent, resulting in a slow acetylator phenotype. Slow acetylators only can use the pathway mediated by CYP1A2 for detoxification of dihydralazine. Their risk for adduct formation is higher and in accordance slow acetylators are strongly overrepresented in the patient population affected by dihydralazine hepatitis [55].

Halothane hepatitis is a rare but sometimes fatal complication of halothane anesthesia. Studies in animals and humans have provided evidence for a complex multifactorial basis for halothane-induced hepatotoxicity. Immunologic changes can be detected in a high percentage of cases of halothane hepatitis. A reductive and an oxidative pathway for halothane metabolism have been previously described. The reductive pathway is regarded as the cause of the mild form of liver injury that results from direct toxicity of halothane. In contrast, in the process of halothane oxidation by CYP2E1, the highly reactive trifluoroacetylchloride is formed, which can bind to lysine residues of proteins forming trifluoroacetyl (TFA) protein adducts [56]. These adducts may act as neoantigens and trigger an immune response. Subsequently, trifluoroacetylated CYP2E1 was detected immunochemically in livers of rats treated with halothane [57]. Furthermore, high levels of autoantibodies that recognized purified rat CYP2E1 but not purified rat CYP3A were detected by enzyme-linked immunosorbent assay in 14 of 20 (70%) sera from patients with halothane hepatitis suggesting that immune responses to cell surface CYP2E1 could be involved in the pathogenesis of halothane hepatitis.

Patients receiving anti-convulsants such as phenobarbital, phenytoin, or carbamazepine, occasionally develop potentially life-threatening, idiosyncratic reactions [58]. Characteristically, the adverse effects occur within 3 months after the initiation of the therapy, and patients develop febrile illness affecting several organs and lymph nodes. The symptoms do not appear to be dose dependent. Some of the patients treated subsequently with other anti-convulsants may also develop adverse reactions against the other drugs [59]. Anti-microsomal antibodies have been found in sera of 9 out of 24 patients with hypersensitivity reactions after phenytoin treatment. These autoantibodies were neither detected in sera from healthy individuals nor in sera from patients treated with anti-convulsants without side effects. The antigen was constitutively expressed in rat liver and was inducible by phenobarbital treatment. Immunoblotting experiments conducted with a series of purified rat cytochrome P450 showed that all sera tested from eight patients reacted with rat CYP3A1, and that sera from six patients reacted with CYP2C11. The human antigen however has not yet been identified yet [60].

CYPs in Alcohol-Induced Liver Disease

As only 10–20% of alcohol abusing patients develop liver disease, host factors may be important in alcohol-induced liver disease. There is growing evidence that inflammatory reactions play an important role in the pathogenesis of alcoholic liver disease (ALD) and autoimmune reactions are frequently observed in these patients [61]. Autoantibodies directed toward alcohol dehydrogenase, hepatic asialoglycoprotein receptor, heat shock protein 65, and phospholipids are present in 25–50% of patients with alcoholic hepatitis or cirrhosis [62, 63]. The implication that immunity contributes to chronic inflammation in ALD has further emerged from clinical and experimental evidence showing recruitment and activation of lymphocytes in the inflammatory infiltrates of ALD. Several investigations performed in different animal models and in humans revealed that patients with advanced ALD show a high prevalence of circulating IgG and T-lymphocytes to covalently modified neoantigens [64–66]. Mice exposed to alcohol generate persistent antibodies against acetaldehyde-protein adducts. The reactivity to the acetaldehyde-protein adducts is interestingly independent of the protein carrier used [66]. During ethanol oxidation by hepatic microsomes not only the reactive intermediate acetaldehyde is formed, but also other free hydroxyethyl radicals [67]. Using adducts of human serum albumin or bovine fibrinogen modified by hydroxyethyl radicals, Clot et al. showed that both hydroxyethylated proteins were recognized by the patient sera and that binding was again independent of the protein carrier used [64]. Furthermore, the authors found that two populations of autoantibodies existed, because autoantibodies directed against hydroxyethyl domains did not cross-react with autoantibodies directed against acetaldehyde-modified proteins. Incubation of liver microsomes with ethanol resulted in formation of four hydroxyethyl radical-derived liver antigens. Antibodies of most patients with alcoholic cirrhosis were bound by a 52 kd protein, which was later identified as CYP2E1. The conclusion of this study was that CYP2E1 leads to formation of hydroxyethyl-adducts with CYP2E1 and three other proteins, which are recognized by the immune system resulting in formation of antibodies [65]. The titers of anti-CYP2E1 but not those of anti-CYP3A antibodies are interestingly associated with the severity of alcohol liver damage, and the inhibition of CYP2E1-mediated ethanol metabolism by chlormethiazole prevented both liver injury and anti-CYP2E1 auto-reactivity [66]. The potential role of an autoimmune response toward CYP2E1 in the pathogenesis of alcohol liver damage in humans is further supported by the demonstration that CYP2E1 is present on the surface of both rat and human hepatocytes [27, 68, 69]. Thus, both in immune-mediated drug-induced hepatitis and in alcoholic liver disease adduct-formation seems to be a crucial mechanism for establishing immune reaction against the metabolizing enzymes. Indeed, protein fragments modified by drug metabolites have been shown to induce T-cell clones recognizing as “nonself,” short linear peptides derived from the native unmodified protein. In turn, these T lymphocytes are capable of activating B lymphocytes to produce antibodies directed against both drug-modified and non-modified proteins [70, 71].

Table 5.1 Viruses proposed as trigger for autoimmune hepatitis

Virus	
Hepatitis C virus	Autoimmune hepatitis type 2 induced by HCV and persisting viral clearance [109]
Hepatitis A virus	Identification of hepatitis A virus as a trigger for AIH type 1 insusceptible individuals [110]
Measles	Autoimmune hepatitis type 1 after measles [111]
Epstein-Barr-virus	Epstein-Barr virus as a trigger for autoimmune hepatitis insusceptible individuals [112]
Herpes simplex virus	LKM-1 autoantibodies recognize a short linear sequence in P450IID6, a cytochrome P-450 monooxygenase [21]
Herpervirus-6	Autoimmune hepatitis and adrenal insufficiency in an infant with human herpesvirus-6 infection [113]

All the described forms of inflammatory liver diseases have in common specific antibodies directed against CYP enzymes as diagnostic markers. The same CYPs may serve as an autoantigen in different diseases (Fig. 5.1). I.e., anti-CYP2D6 antibodies can be found in patients with AIH and patients with hepatitis C, anti-CYP1A2 antibodies in APECED and dihydralazine-associated hepatitis, and anti-CYP2A6 antibodies in HCV and APECED (Table 5.1). However, different sequences may serve as target epitopes or, in case of anti-CYP1A2 and dihydralazine- and APECED-mediated hepatitis, different ways of immunization may result in antibody induction.

Role of Molecular Mimicry for Induction of Autoantibodies to CYP Enzymes

Molecular mimicry is one of the favored, yet still controversial theories by which infectious agents may activate these cells against self-antigens. During the last two decades hundreds of articles have focused on epitope mimicry and have provided many arguments supporting this hypothesis but sound proof is elusive. According to the hypothesis a susceptible individual acquires an infection with an agent that has antigens immunologically similar to the host antigens which can induce an immune response when presented to T cells. As a result, the immune response generated cross-reacts with host tissues leading to tissue destruction. The immune responses can either be on the humeral (antibody) or T cell-mediated level or both. Several criteria need to be fulfilled to establish evidence that molecular mimicry is involved in the pathogenesis of autoimmune diseases. (1) There has to be an association between the infection and the autoimmune disease, which has to persist in the absence of the initiating microbe. (2) The cross-reacting epitopes should be identified and the corresponding immunological cells such as antigen presenting

cells and effector cells like T cells has to be determined during the infection and the autoimmune diseases. (3) It needs to be demonstrated that both the critical epitopes and the corresponding T cells are required and necessary to provoke the autoimmune disease.

There is evidence suggesting that molecular mimicry might also act as a trigger for induction of liver-specific autoantibodies. Sera of patients with different diseases can react to some extent with the same epitopes on CYPs as shown for $CYP2D6_{193–212}$ and HCV- and AIH-2-patients. Similarly, sequence homologies between HCV and the B cell epitope $CYP2D6_{254–271}$ as well as homologies between CYP2D6 and common viruses like herpes simplex virus type 1 (HSV1), cytomegalus virus (CMV), Ebstein-Barr-Virus (EBV), and human adenovirus have been discovered [72, 73]. Furthermore, Ma et al. found that disease-specific CD4+ T cells in patients infected with HCV detect $CYP2D6_{313–332}$, which in turn is homologous to the sequence 794–801 of HCV [74]. Kammer et al. found a striking homology between the HCV core 178–187 peptide and CYP2A6 and CYP2A7 [75]. Intriguingly, HCV-induced cytotoxic T-cells recognize these CYPs and lyse cells transfected with a plasmid coding for the whole CYP2A6 protein. But a reaction against CYP2A6 and CYP2A7 at B-cell level could not be detected. These observations led to the suggestion that molecular mimicry may play a role in the pathogenesis of AIH-2. According to the "multiple hit-theory," in genetically predisposed patients multiple contacts to viruses might induce a cross-reactive subset of T-cells and permit a loss of immunological self-tolerance. This hypothesis might be expanded to incorporate cross-reactive responses involving various self-antigens specific for autoimmune diseases, which then lead to the development of multiple autoimmune diseases in the same patient. Studies by Choudhuri et al., for example, revealed that antibodies detecting the AIH-related antigen $CYP2D6_{321–351}$ can cross-react with carboxypeptidase H, an autoantigen in type I diabetes and with CYP21, an autoantigen in Addison's disease suggesting that autoimmunity to one CYP might spread to other CYPs via molecular mimicry [76].

The role of molecular mimicry in autoimmune reactions against CYP2D6 was further analyzed in a recently developed mouse model for AIH type 2 [77]. In this model "molecular mimicry" was compared to "molecular identity" in response to infection with an adenovirus, expressing the human CYP2D6 (Ad-2D6) [78]. Either wildtype FVB/N mice, which express mouse CYP isoenzymes with a structural and sequential similarity to human CYP2D6 (molecular mimicry) or transgenic CYP2D6 mice, which express in addition the identically human CYP2D6 (molecular identity), were infected with Ad-2D6. Infection of FVB/N mice led to a rapid development of persistent AIH with typical histological features like infiltration of mononuclear cells and severe fibrosis as well as the formation of CYP2D6 antibodies and CYP2D6-specific T-cells. In contrast, liver damage in the transgenic mice progressed significantly slower was less severe and was associated with lower antibody titers. Furthermore, a significantly lower frequency (10–20×) of CYP2D6-specific T-cells was found in the transgenic mouse model. The authors concluded that the threshold of specific T-cells rather than the antibody titer is responsible for the severity of the disease.

Role of Cellular Autoimmunity to CYP Enzymes

In the histological picture of AIH the main feature is a mononuclear cell infiltrate, containing lymphocytes, plasma cells, and macrophages. Immunohistochemical studies revealed a predominance of CD4+ T-cells and a minority of CD8+ T-cells [79].

CD4+ T lymphocytes play a major role in inflammation processes of autoimmune hepatitis. Studies with lymphocytes demonstrated proliferation of T-Helper-cells specifically in response to human recombinant CYP2D6 [80]. In studies analyzing the proliferative response of mononuclear cells of patient sera after contact with $CYP2D6_{262–285}$ Löhr et al. found that eight of eight patients with AIH-2, 6 of 12 with AIH-1, and 4 out of 31 patients with chronic hepatitis C reacted with the antigen. After immunosuppressive treatment T cell response decreased [81]. Recent studies investigated T cell responses to peptides covering the whole CYP2D6 molecule. They demonstrated that multiple epitopes on CYP2D6 were detected by T-cells and that the production of cytokines was not uniform. The number of recognized epitopes as well as the quantity of cytokine production directly correlated with disease activity. The authors concluded that the T cell response to CYP2D6 is polyclonal, involves multiple effector cell types targeting different epitopes and is associated with hepatocyte damage [74].

There is growing evidence pointing to a crucial role of CD8+ autoreactive T-cells for liver cell injury. CYP2D6-specific CD8+ T-cells were shown to secret interferon-γ and to be cytotoxic after recognition of CYP2D6-epitopes. In this study, frequency, IFN- production, and cytotoxicity of CYP2D6-specific CD8 T-cells were higher at diagnosis than during treatment. Furthermore, intensity of CYP2D6-specific CD8 T-cell responses correlated with disease activity [82].

Recently, a HLA B8/DR3 dependent impairment of T regulatory cell (Tregs) number and function has been considered as a permissive factor leading to autoimmune reactions [83]. Tregs are CD4+ CD25+ cells representing 5–10% of all CD4+ T-cells in healthy individuals [84]. They control the proliferation of autoreactive T-cells by direct contact to the target cell and to a lesser extent by releasing cytokines. The number of Tregs inversely correlates with markers of the disease and LKM-antibody titers. Remarkably, the number of Tregs increases after the administration of corticosteroids [85, 86].

Uridine Diphosphate 5′-Glucuronosyltransferases and Immune-Mediated Liver Injury

Uridine diphosphate 5′-glucuronosyltransferases are a superfamily of drug metabolizing enzymes located in the inner membrane of the endoplasmatic reticulum [87]. UGTs have evolved in vertebrate species and more than 50 isoforms have been so far identified. The UGT enzymes catalyze the transfer of the glucuronic acid moiety of UDP glucuronic acid (UDPGA) to a wide range of acceptor molecules, including bilirubin, sex steroids, numerous prescribed drugs, and environmental toxins [88].

The sugar acid can be coupled to the substrate through the –OR, –SR, or –N.R′R″ forming a P-D-glucopyranosiduronic acid or glucuronide. This reaction leads almost always to inactive metabolites that are excreted into bile or urine. In their ability to glucuronize oxidized compounds, UGT enzymes complement the metabolic function of phase I enzymes such as CYPs, which are localized in the external membrane of the ER.

The mammalian UGT1 gene superfamily currently has more than 117 members that can be divided into four families, UGT1, UGT2, UGT3, and UGT8 [89]. Based on an agreed system of gene nomenclature, members of the UGT superfamily have been named based on divergent evolution, with each gene given the symbol UGT, followed by a number representing the family, a letter to denote the subfamily, and a number for the individual gene within that family or subfamily similar to the nomenclature of the CYP enzymes. Human UGT1 is located on chromosome 2q37 and spans approximately 200 kb, which is composed of 17 exons. To synthesize the final UGT1 protein, one of 13 different exon-1 on the locus is spliced to four downstream exons (exon 2–5), common to all UGT1A isoforms. The exon-1 sequence of UGTs codes for the substrate-binding domain, while the four common exons code for the cosubstrate-binding domain. The human UGT2 gene family is located on chromosome 4q13 and is divided into two subfamilies, UGT2A and UGT2B. Similar to the UGT1 family, members of the UGT2B subfamily, which comprises several independent genes, share a high degree of similarity in the C-terminal portion of the protein and the highest degree of divergence in sequences encoded by exons 1. Although the liver is the main site of glucuronidation, expression of UGTs can also be found in extrahepatic tissues, including the kidney, lung, gastrointestinal tract, prostate, spleen, skin, and brain. Some isoforms appear to have a broad distribution, whereas others may be specific to the liver or may be restricted to specific extrahepatic tissues.

UGTs in Hepatitis D Virus Infection

The hepatitis D virus, an RNA viroid dependent on hepatitis B co-infection, was discovered in 1977 [17]. It was soon recognized that in 13% of Italian patients infected with HDV had LKM autoantibodies in serum, that differed from LKM-1 autoantibodies in autoimmune hepatitis type 2, and LKM-2 autoantibodies in dihydralazine-induced hepatitis directed. These antibodies gave a distinct immunofluorescence pattern on rat liver-kidney cryostat sections with no or weak staining of the proximal renal tubules and stomach [90]. These autoantibodies were termed LKM-3. Just as in chronic hepatitis C a multitude of serum autoantibodies were subsequently identified in sera of patients suffering from chronic HDV infection. In 1994, family 1 UDP glucuronosyltransferases (UGT-1) were identified as molecular target of LKM-3 autoantibodies [91–93]. This discovery now allows for the characterization of another form of virus-associated autoimmunity. As in HCV-associated LKM-1 autoantibodies, LKM-3 autoantibodies in HDV infection are present at lower titers than in AIH. LKM-3 autoantibodies appear to be specific for HDV and are not found in the sera of HCV infection [91, 94].

These preliminary data indicate, that the immune response characterized by the molecular analysis of LKM-3 autoantibodies may differ in HDV-associated autoimmunity and AIH. Further studies are needed to evaluate the clinical and pathophysiological significance of LKM-3 autoantibodies.

UGTs in Autoimmune Hepatitis

LKM-3 antibodies were subsequently also found in patients with AIH and can be regarded as rare markers of AIH [95]. Epitope mapping identified a mayor epitope in the C-terminal region of the protein (AA 264–373). The signal was significantly stronger when the N-terminal sequence was included in the clones indicating that the epitope is conformation dependent [96]. In addition to the mayor epitope, a minor epitope was found on a family II UGT, UGT2B13. Overall, LKM-3 antibodies have been shown to react with UGT1A1, 1A6, 1A4, and the UGT2B isoform. These antibodies can occasionally be the only marker of AIH, but most AIH type II patients test positive for LKM-1 antibodies.

The molecular events leading to the formation of antibodies directed to UGTs are not known so far. In analogy to antibodies to CYPs, it can be postulated that UGTs might also lead to the formation of adducts thereby generating neoantigens that trigger an immune response.

Drug-Induced Liver Without Antibodies Directed Against CYP or UGT Enzymes

Drugs such as minocycline [97–99], diclofenac [100, 101], infliximab [102], propylthiouracil [103], atorvastatin [104], nitrofurantoin [105], methyl dopa [106], and isoniazid [107] can cause a syndrome that resembles AIH [108] with autoantibodies which however generally disappear after discontinuation of the drug and which usually do not require treatment.

Conclusion

Autoimmune reactions against drug metabolizing enzymes are involved in several liver diseases of different origin, mainly seen in autoimmune hepatitis, viral hepatitis, and hepatitis induced by xenobiotics. In genetically predisposed individuals, antigen-antibody reactions and mislead cellular immune responses apparently lead to persistent and perpetuating liver damage. The immune reaction against self might be triggered by repeated virus-infections via molecular mimicry. However, the prime mechanism for breakdown of self-tolerance remains unclear and have to be addressed in future studies.

Chapter Summary

1. Serological markers of autoimmunity can be detected in alcoholic and non-alcoholic fatty liver disease, acute and chronic viral hepatitis, and drug-induced hepatitis.
2. The liver contains the greatest abundance of phase I and phase II xenobiotic-metabolzing enzymes in the body. In most liver diseases associated with serological markers of AIH, autoantibodies against cytochrome P450 (CYP) enzymes and UDP – glucuronosyltransferases (UGT) are detected.
3. The pathogenetic role of most, if not all, autoantibodies is still unclear and it is not known whether autoantibodies that are observed in primarily non-autoimmune liver disease contribute to tissue damage.

Useful Tips for Practitioners

1. LKM-1-positive patients with a chronic hepatitis C may develop hepatic flares under antiviral therapy with interferon. However, antiviral treatment is as beneficial in these patients as in anti-LKM1-negative patients, and the rare heptic flares can be effectively treated with corticosteroids.
2. Several drugs can cause a syndrome that resembles AIH with autoantibodies which however generally disappear after discontinuation of the drug and which usually do not require treatment.
3. Drug-induced hepatitis is rare but may cause a life-threatening hepatitis if exposure to that drug is prolonged. Since the induction of an immune reaction is the critical event in drug-induced hepatitis, the severity and onset of disease are essentially independent of drug dosage.

Common Pitfalls in Practice

1. Antibodies directed against CYP enzymes may serve as diagnostic markers in several inflammatory liver disease. These antibodies are not disease specific and the same CYPs may serve as an autoantigen in different diseases, i.e., anti-CYP2D6 antibodies can be found in patients with AIH and patients with hepatitis C, anti-CYP1A2 antibodies in APECED and dihydralazine associated hepatitis, and anti-CYP2A6 antibodies in HCV and APECED.

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Chapter 6
When and How to Treat the Adult Patient

Aldo J. Montano-Loza

Keywords Autoimmune hepatitis • Corticosteroids • Prednisone • Azathioprine • Immunosuppressive treatments

Introduction

During the 1970s, three clinical controlled trials demonstrated the lifesaving properties of corticosteroid therapy in patients with autoimmune hepatitis (AIH) [1–3]; and since then treatment with prednisone alone or a lower dose in combination with azathioprine has become the standard therapy for AIH [4, 5]. However, not all patients with AIH respond to this therapeutic regimen, and of those who do, many relapse after corticosteroid withdrawal [6, 7]. In addition, the success of corticosteroid treatment must be counterbalanced against the development of side effects to the medications [8, 9]. New pharmacological agents have emerged that may promise better immunosuppression and tolerance than the conventional corticosteroid regimens [10, 11].

Indications for Treatment

The indications for treatment of adult patients with AIH are based on the risk factors for disease progression, and for practical purposes can be classified as absolute, relative or uncertain indications, and no indications for treatment. Special consideration should be made to the presence or absence of symptoms and patients with fulminant presentation of AIH.

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Table 6.1 Treatment indications for adult patients with autoimmune hepatitis

	Indications		
Findings	Absolute	Relative	None
Clinical	Incapacitating symptoms Relentless clinical progression Fulminant presentation	Mild or no symptoms	Asymptomatic with mild laboratory changes Previous intolerance of prednisone and/or azathioprine
Laboratory	AST ≥ tenfold normal AST ≥ fivefold normal and γ-globulin ≥ twofold normal	AST 3–9-fold normal AST ≥ fivefold normal and γ-globulin < twofold normal	AST < threefold normal Severe cytopenias (White blood cell count below 2.5×10^9/L or platelet count below 50×10^9/L)
Histologic	Bridging necrosis Multi-acinar necrosis	Interface hepatitis	Inactive cirrhosis Focal interface hepatitis Portal hepatitis Decompensated inactive cirrhosis with variceal bleeding or hepatic encephalopathy

AST = serum aspartate aminotransferase level
Adapted from Montano-Loza AJ, and Czaja AJ [10]

Absolute Indications

Severe laboratory abnormalities defined as serum AST levels of at least tenfold the upper limit of the normal (ULN) or more than fivefold ULN in conjunction with a serum γ(gamma)-globulin level more than twofold ULN, incapacitating symptoms (fatigue and arthralgia), and histological changes of moderate-to-severe interface hepatitis are absolute indications for corticosteroid treatment. In addition, if bridging necrosis or multi-acinar collapse is seen on liver biopsy but the other criteria are absent, immediate corticosteroid therapy should be started (Table 6.1). These patients progress to cirrhosis in 80% if untreated and mortality could be as high as 60% at 6 months [1–3].

Relative or Uncertain Indications

Patients with laboratory and histological features that are less severe and not immediately life-threatening have not been studied by controlled clinical trials. The benefit–risk ratio of corticosteroid treatment in these patients has not been thoroughly determined, and the institution of treatment is an individualized clinical decision that is generally influenced by the presence of symptoms and histological changes of mild–moderate interface hepatitis. Laboratory abnormalities of a mild-to-moderate degree are associated with cirrhosis in 49% within 15 years and a 10-year survival

of 90%, and untreated patients with interface hepatitis have a 17% probability of cirrhosis within 5 years [12–14] (Table 6.1). Therefore, diagnosis of AIH does not compel therapy, and retrospective analyses of patients with mild disease have demonstrated the possibility of long-term survival without treatment [12–14].

No Indication for Treatment

The risk of the disease must be counterbalanced by the risks of the treatment, especially in patients with mild-to-moderate disease activity who are likely to be intolerant of the medication, such as those with advanced but inactive cirrhosis, postmenopausal osteopenia or vertebral compression, emotional lability or psychosis, poorly controlled hypertension, and difficult to control diabetes mellitus [8, 9]. Patients with histological features of focal interface hepatitis, portal hepatitis or inactive cirrhosis, or advanced stages of liver decompensation in the absence of severe inflammatory activity necessitate close observation (i.e. 3–6 months), symptomatic care, or be considered for liver transplantation (Table 6.1) [12–14].

Presence of Symptoms and Decision of Treatment

AIH can be asymptomatic in as many as 34% of patients at presentation [14, 15]. Asymptomatic individuals are commonly men, and they have lower serum levels of aminotransferases and immunoglobulin G (IgG) at presentation than symptomatic patients. Histological features are similar between symptomatic and asymptomatic patients, and there is no significant difference in the occurrence of cirrhosis [15]. Frequently, asymptomatic patients have inactive cirrhosis, and their survival is not enhanced by corticosteroid treatment. Asymptomatic patients without cirrhosis may have 10-year survival probabilities greater than 80% without treatment [14]. Disease severity as reflected in the laboratory and histological features of inflammatory activity and not the presence or absence of symptoms is the principal justification for corticosteroid treatment. Asymptomatic patients commonly become symptomatic [14, 15]. The absence of symptoms at presentation should not decide treatment in patients who otherwise satisfy criteria for severe disease [1–3].

Fulminant Autoimmune Hepatitis

Several clinical descriptions of severe acute and fulminant AIH have emerged from small retrospective analyses within single institutions [16–22]. The efficacy of corticosteroids in the treatment of severe acute and fulminant AIH has not been established, but these experiences have suggested that the prompt institution of

corticosteroid therapy may be beneficial in 36–100% of such patients [17–21]. The diagnosis of AIH should be considered in all patients with acute and chronic liver disease, including those patients with allograft dysfunction who have undergone liver transplantation for autoimmune [23, 24] and non-autoimmune conditions [25, 26], and its presentation warrants an increase in immunosuppressive therapy.

Conventional Treatment Schedules

The preferred treatment schedule for adults with severe AIH is prednisone (prednisolone can be used in equivalent doses) in combination with azathioprine (50 mg/day generally used in North America and 1–2 mg/kg/day in Europe) [4, 5] (Table 6.2). Prednisone alone in higher dose is as effective as the combination regimen, but it is associated with a higher frequency of drug-related side effects (44 versus 10%) [27].

Prednisone should be tapered down slowly when at 20 mg/day as long as the ALT becomes close to normal, the reduction should be done by 5 mg every week or two until 10 mg per day are achieved; and even further reduction by 2.5 mg per week have been considered up to 5 mg daily. The maintenance regimen is then continued until resolution of the disease, treatment failure, or drug-intolerance. It is usual that patients going into complete remission can be maintained in remission on azathioprine alone (Fig. 6.1).

In patients with advanced cirrhosis impairment of the conversion of prednisone to prednisolone may be present, but this impairment is insufficient to alter treatment response or mandate the administration of prednisolone [28]

Table 6.2 Treatment schedules for adult patients with autoimmune hepatitis recommended by the AASLD

	Combination therapy			Prednisone therapy
		Azathioprine		
Weeks administered	Prednisone (mg daily)	NA (mg daily)	EU (mg/kg/day)	Prednisone (mg daily)
1	30	50	1–2	60
1	20	50	1–2	40
2	15	50	1–2	30
Maintenance until end point	10	50	1–2	20
Relative Contraindications	Cytopenias Pregnancy Active malignancy Short course (less than 6 months) Thiopurine methyltransferase deficiency			Post-menopausal state Osteoporosis Diabetes Hypertension Obesity Emotional lability

AASLD American Association for the Study of the Liver Diseases, *NA* North America, *EU* Europe. Adapted from Montano-Loza AJ, and Czaja AJ [10]

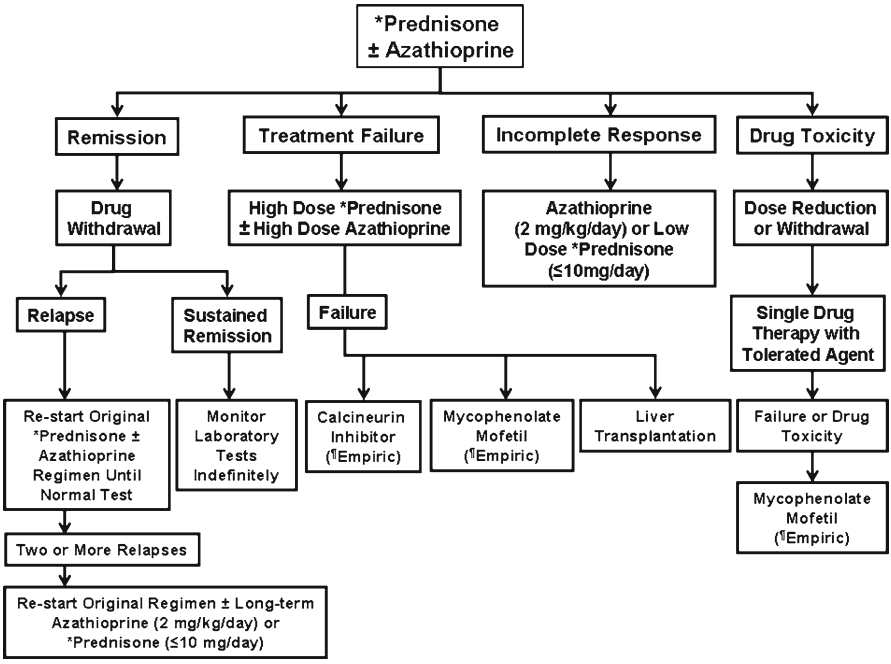

Fig. 6.1 Algorithm of therapy for patients with autoimmune hepatitis. The preferred initial regimen is prednisone alone or a lower dose of prednisone in combination with azathioprine. Outcomes after the initial therapy dictate changes in the initial treatment strategy. *Prednisolone can be used in equivalent doses. Lack of clinical trials. Adapted from Czaja AJ, and Manns MP [11]

Some centers advocate for the use of maintenance low dose corticosteroid and/or azathioprine monotherapy treatment in patients with AIH to avoid the high frequency of relapse after drug withdrawal but such schedules in adults may not be as successful as fixed daily dose regimens in inducing histological resolution [27].

Treatment End Points

Therapy should continue until remission, treatment failure, incomplete response, or drug toxicity. There is no prescribed minimum or maximum duration of treatment. The length of therapy should be based on a fixed minimum duration that is usually associated with a complete response or on a variable duration that is individualized to the desired result and tolerance. The average duration of treatment required for disappearance of symptoms, normalization of laboratory indices, and histological resolution is 22 months [29]. Histological improvement lags behind clinical and laboratory resolution by 3–8 months, and therapy should be continued for at least 3 months beyond this point of improvement [30]. In Europe, treatment is maintained for at least 2 years before considering drug withdrawal [31].

The ideal end point of therapy in patients with AIH is after resolution of all clinical, laboratory, and histological manifestations of disease activity [32–34]. Normalization of serum aspartate aminotransferase (AST), γ(gamma)-globulin, and IgG levels in conjunction with histological resolution reduces the relative risk of relapse after drug withdrawal by 3–11-fold [34]. The frequency of sustained remission after initial treatment is still low (approximately 25%), but 87% of patients who achieve an inactive state have normal laboratory indices prior to the termination of therapy. The normalization of tests and tissue does not protect against relapse, and 60% of patients who relapse do so despite prior disappearance on treatment of inflammatory features [34]. The frequency at which corticosteroid treatment can induce resolution of the disease is unclear, and the pursuit of an idealized end point may be futile in some patients or may be associated with serious drug-related side effects. Under such circumstances, management strategies must be individualized to patient tolerance.

Liver biopsy evaluation before considering ending treatment is one approach to confirm complete resolution of the disease. Up to 50% of patients with AIH and normal serum aminotransferases and γ-globulin levels during therapy have interface hepatitis, and these patients usually relapse after cessation of treatment; therefore, their recognition by liver biopsy should indicate prolongation of immunosuppressive treatment. Thus, a liver biopsy is often suggested prior to termination of treatment in patients with AIH [4, 5].

Termination of immunosuppressive treatment should be considered after at least 2 years, when serum aminotransferases and γ(gamma)-globulin levels have been constantly normal. Termination of therapy after induction of remission should follow by gradual and monitored dose reduction over a 6-week period. The activity of the disease during and after drug withdrawal is assessed by the appearance of symptoms (fatigue and arthralgias) and laboratory indices (serum AST/ALT and γ[gamma]-globulin levels). Laboratory tests should be done as often sometimes as every 3 weeks during drug withdrawal and for 3 months after termination of therapy. After that, laboratory test should be repeated every 3 months and then every 6 months for 1 year, and finally every year for life [4, 5].

Nine percent of patients have worsening serum aminotransferases or bilirubin levels, unchanged or worse histological activity indices, and develop ascites or hepatic encephalopathy despite compliance with the treatment regimen [35, 36]. These individuals have treatment failure, and they are candidates for either high dose corticosteroid therapy after their original diagnosis has been reconfirmed and different or superimposed diseases have been excluded. They commonly become candidates for liver transplantation [37].

Thirteen percent of patients with AIH improve but not to a point of complete resolution [38]. These individuals have an incomplete response, and they may represent those patients who require indefinite treatment and who remain at risk for disease progression. Serious side effects associated with corticosteroids or azathioprine that require premature termination of treatment are present in 13% of patients with AIH [8, 29].

Treatment Outcomes

Approximately, 80% of treated patients satisfy remission criteria within 3 years [1–3], and the life expectancies of treated patients exceed 85% at 10 years [39] and 74% at 20 years even in those with cirrhosis at baseline [40]. These survivals are comparable to those of an age- and sex-matched normal population from the same geographical region. Patients with cirrhosis respond as well to treatment as patients without cirrhosis, and they should be treated similarly with the same expectation of success [39]. Twenty-one percent of individuals who enter remission sustain this result long term after drug withdrawal (median interval of follow-up, 76 months), and an effort should be made to discontinue initial therapy in all patients with inactive disease [41].

Corticosteroid therapy may also reduce or prevent hepatic fibrosis [42, 43]. Fifty-six percent of patients have lower hepatic fibrosis scores on repeat liver biopsy performed after 55 ± 9 months of follow-up, and 33% have stable hepatic fibrosis during 62 ± 14 months of observation. Improvement in hepatic fibrosis occurs in association with reduction in liver inflammation secondary to corticosteroid therapy [44]. Case series studies have also suggested that cirrhosis may regress on treatment [42, 43].

Treatment of Suboptimal Responses

Treatment failure is defined as deterioration during therapy, characterized by a progressive increase in serum aminotransferases or bilirubin at presentation. Consultation with an expert center is important. Management is individualized after compliance is confirmed. This suboptimal response can be managed by administering high dose prednisone alone (60 mg daily) or prednisone (30 mg daily) in conjunction with azathioprine (150 mg daily) (Table 6.3) [45]. Doses of prednisone and azathioprine should be reduced by 10 and 50 mg respectively, for each month of laboratory improvement until conventional maintenance levels of drug are achieved. Seventy-five percent of patients treated with this regimen achieve clinical and laboratory remission, but only 20% have histological resolution. These patients remain at risk for progressive liver disease and drug toxicity.

Drug toxicity requires premature dose reduction or discontinuation of the offending drug and continued use of the other tolerated medication in adjusted dose (Table 6.3). Corticosteroid-related side effects are the most common causes for drug withdrawal, and they include intolerable cosmetic changes or obesity (47%), osteoporosis with vertebral compression (27%), brittle diabetes (20%), and peptic ulcer disease (6%) [29]. Azathioprine can be administered as a corticosteroid-sparing agent with doses increased to 2 mg/kg daily. The emergence of a cholestatic hepatitis, pancreatitis, rash, progressive cytopenia, or gastrointestinal symptoms indicates azathioprine toxicity and the need for its withdrawal.

Table 6.3 Conventional and empiric treatments of suboptimal responses in autoimmune hepatitis

	Conventional treatments		Possible empiric treatments	
Clinical event	First choice	Second choice	Third choice	Fourth choice
Treatment failure	Prednisone (30 mg daily) and azathioprine (150 mg daily)	Prednisone (30 mg daily) and Mercaptopurine (1.5 mg/kg daily)	Cyclosporine (5–6 mg/kg daily) or Prednisone (30 mg daily) and Mycophenolate mofetil (2 g daily)	Tacrolimus (4 mg twice daily)
Drug toxicity	Azathioprine (2 mg/kg daily) if prednisone intolerance	Prednisone (20 mg daily) if azathioprine intolerance	Budesonide (3 mg twice daily)	UDA (13–15 mg/kg daily)
Incomplete response	Prednisone maintenance ≤10 mg daily if serum AST < threefold normal	Azathioprine maintenance (2 mg/kg daily) if serum AST < threefold normal	Budesonide maintenance (3 mg twice daily)	UDA maintenance (13–15 mg/kg daily)
Relapse	Azathioprine maintenance (2 mg/kg daily) if serum AST < threefold normal	Prednisone maintenance reduced to ≤10 mg daily if serum AST < threefold normal	Mycophenolate mofetil maintenance (1 g twice daily)	Cyclosporine maintenance (5–6 mg/kg daily)

UDA ursodeoxycholic acid
Adapted from Montano-Loza AJ, and Czaja AJ [10]

However, this complication is rare. Routine genotyping and phenotyping for thiopurine methyltransferase deficiency prior to the institution of azathioprine therapy does not correlate with the occurrence, nature, or severity of the hematologic or somatic complications of azathioprine treatment, and the value of routine testing for this enzyme is uncertain [46–48].

An *incomplete response* is arbitrarily declared after 3 years of conventional therapy without complete remission [5, 33]. Patients improve but not to a degree to satisfy remission criteria and they are at risk for drug-related side effects associated with standard doses of prednisone. Low dose prednisone or long-term maintenance therapy with azathioprine (2 mg/kg daily) is a treatment option (Table 6.3).

The emergence of new immune-modulating drugs in the liver transplant arena has led to their empiric use in the treatment of suboptimal responses to conventional therapies in AIH. These empiric applications are indicated in Table 6.3, but none has been evaluated in randomized control trials and are thus not incorporated into established treatment algorithms.

Relapse After Drug Withdrawal

Relapse occurs in 20–86% of patients depending on the laboratory and histological findings prior to drug withdrawal [6, 7, 34]. Generalized weakness, arthralgias, and increase in the serum AST level characterize this occurrence. Examination of liver tissue typically reveals moderate–severe interface hepatitis in patients in whom the serum AST level increases above threefold normal [30]. Re-treatment with the original regimen typically induces another remission, but relapse recurs in 79% within 6 months after drug withdrawal [7]. With each relapse and re-treatment, the frequency of drug-related side effects increases, as does the occurrence of cirrhosis, death from hepatic failure, or requirement for liver transplantation [41]. Thus these patients are candidates for alternative therapies with either low dose prednisone or long-term azathioprine maintenance (Table 6.3).

The low dose prednisone regimen requires first the induction of clinical and laboratory remission on standard therapy and then reduction in the dose of prednisone by 2.5 mg each month of clinical and laboratory stability [48]. The lowest dose that prevents symptoms and keeps serum AST levels within the normal range is maintained. More than 80% of patients can be managed on prednisone, 10 mg daily or less (median dose, 7.5 mg daily). Side effects associated with earlier conventional treatments improve or disappear in 85%; and in most cases new side effects do not develop and survival is unaffected.

Maintenance therapy with azathioprine also requires the initial induction of clinical and laboratory remission by conventional treatments [49, 50]. The corticosteroid component is then withdrawn, and the dose of azathioprine may be increased to up to 2 mg per kg daily and maintained indefinitely. Eighty-seven percent of adult patients managed in this fashion remain in remission during a median observation period longer than 60 months. Follow-up liver biopsy assessments reveal inactive or minimal

histological disease in 94% of patients; corticosteroid-related side effects improve or disappear in the majority of cases; and the drug is generally well-tolerated. The most common side effects are steroid withdrawal arthralgias (63%), lymphopenia (57%), and myelosuppression (7%). Malignancies involving diverse cell types occur in 8% of patients, but their association with the treatment strategy is unclear [51].

Relapse does not prohibit permanent discontinuation of medication later in the course of the disease [41]. Twenty-eight percent of patients who relapse and are re-treated develop inactive disease and can be withdrawn from medication. The probability of a sustained remission after initial or subsequent therapy is 47% during 10 years of follow-up. As with other suboptimal responses in AIH, pharmacological agents of theoretical but unconfirmed efficacy have been used empirically for the treatment of relapse. These novel but unendorsed treatments are indicated in Table 6.3.

Different Treatment Schedules

The outcomes associated with conventional medications may be improved by altering doses and routes of delivery. Under these circumstances, success is measured as a favorable balance between the response of the disease and the tolerance of the medication. Alternate day corticosteroid regimens may not induce histological remission of the disease in adults, but they may have fewer side effects than conventional schedules. Consequently, they may be an appropriate strategy for individuals with obesity, osteoporosis, and difficult to control diabetes [27]. Pulse therapy regimens based on oral medication have not been well tolerated in adults [51]. Therefore, it is appropriate and important to individualize therapies according to patient response and tolerance. Recommended schedules are not inflexible, and they must be adapted to suit the individual need.

Screening of Patients Receiving Treatment

Patients with AIH receiving prednisone or azathioprine should be aware of possible complications of these medications, and they should be introduced to appropriate adjunctive treatment programs to reduce the risk of drug-related complications. Such therapies should include regular weight bearing exercise program, vitamin D and calcium supplementation. The administration of bisphosphonates may be appropriate for individual patients. Patients on long-term (>6 months) prednisone should be monitored for bone disease at baseline and periodically thereafter according to local protocol with bone densitometry of the lumbar spine and hip.

Also, patients on prednisone should undergo periodic eye examinations during treatment looking for cataracts and glaucoma. Patients receiving azathioprine in any dose should be monitored at 3–6-month intervals for leucopenia and thrombocytopenia.

Lastly, patients with AIH should be protected against hepatitis B virus (HBV) and hepatitis A virus (HAV), and vaccination should be done as early as possible even before immunosuppression is started because of lower response rates [5].

Liver Transplantation for Autoimmune Hepatitis

Liver transplantation is an effective treatment for the decompensated patient with AIH. Patient and graft survival after liver transplantation ranges from 83 to 92%, and the actuarial 10-year survival after transplantation is around 75% [23, 37, 52]. Recurrence of AIH is recognized in at least 17% of patients after 5 ± 1 years, especially in individuals receiving inadequate immune suppression [22, 23, 37, 53]. Adjustments in the immunosuppressive regimen are usually able to suppress recurrent disease, and infrequently cirrhosis or graft failure occurs [22]. Patients transplanted for AIH may also have a greater frequency of acute and chronic rejection than patients transplanted for non-autoimmune conditions, and some but not all studies have suggested they may be more difficult to wean from corticosteroid therapy [53, 54]. These potential consequences have tempered efforts to rapidly withdraw corticosteroids after the procedure [54, 55]. De novo AIH can develop in 1–3% of adult recipients who undergo transplantation for non-autoimmune liver disease, and it can result in graft loss if not treated with corticosteroids [25, 26, 56]. Recent studies indicated that sirolimus (rapamycin) can be effective in controlling this process [57]. The pathogenic mechanisms resulting in de novo AIH are unknown, but they may reflect reduced thymic clearance of autoreactive cells or impaired apoptosis of activated lymphocytes by the calcineurin inhibitors used in the post-transplantation period [58–60].

The early response to corticosteroid treatment is predictive of the need for liver transplantation [61, 62]. Histological features of multilobular collapse and lack of improvement in laboratory indices within 2 weeks of corticosteroid treatment characterizes patients who die of liver failure within 6 months of presentation. A hyperbilirubinemia that does not improve or that worsens during this interval is highly predictive of early mortality and the need for liver transplantation [35]. Patients with high Model for End-stage Liver Disease (MELD) score who do not respond rapidly to corticosteroid therapy, especially those in whom there is an emerging cholestasis, should also be considered for liver transplantation [63].

New Drug Therapies for Autoimmune Hepatitis

Drugs already exist that can interfere selectively with each co-stimulatory signal of lymphocytes activation, and agents that block transendothelial migration of T cells into target tissues are also in development [64–66]. The specificity of action of these new drugs in various combinations may have complementary effects that will provide greater efficacy than current non-selective immunosuppressive regimens (Table 6.4).

Table 6.4 Promising drug therapies for autoimmune hepatitis

Drug	Dose	Actions	Experience
Cyclosporine	5–6 mg per kg daily	Calcineurin inhibitor; impairs transcription of IL 2; prevents T lymphocyte proliferation; increases hepatic TGF-β(beta)	Empiric first-line therapy in adults; empiric salvage therapy
Tacrolimus	4 mg twice daily	Calcineurin inhibitor; impairs transcription of IL 2; limits expression of IL 2 receptors; increases hepatic TGF-β(beta)	Beneficial in three small clinical experiences
Mycophenolate mofetil	1 g twice daily	Purine inhibitor; independent of thiopurine methyltransferase; impairs DNA synthesis; reduces T lymphocyte proliferation	Beneficial as steroid-sparing agent in five small clinical experiences
Budesonide	3 mg thrice daily	Corticosteroid actions; high first pass clearance by liver; metabolites devoid of glucocorticoid activity	Effective as frontline therapy of treatment-naïve mild disease; not in cirrhotics
6-thioguanine nucleotides	0.3 mg/kg daily	Active metabolites of azathioprine	Effective in three patients intolerant of azathioprine
Rituximab	375 mg/m^2 IV weekly ×4 weeks	Chimeric monoclonal anti-CD20 antibody that induces B lymphocyte depletion	Improvement in one patient with concomitant ITP, and six patients refractory or intolerant of standard therapy
Ursodeoxycholic acid	13–15 mg per kg daily	Reduces HLA class I expression; inhibits IL 2, IL 4 and interferon-γ(gamma) production; inhibits apoptosis; eliminates hydrophobic bile acids	Effective in eight Japanese patients with mild disease; not in severe disease

IL interleukin, *TGF-β(beta)* transforming growth factor-beta, *DNA* deoxyribonucleic acid, *IV* intravenous, *ITP* idiopathic thrombocytopenic purpura
Adapted from Montano-Loza AJ, and Czaja AJ [10]

Cyclosporine A binds to cyclophilin and inhibits the phosphatase activity of calcineurin. Consequently, it impairs transcription of interleukin (IL)-2 and the downstream activation events dependent on this cytokine. In doses of 5–6 mg/kg daily, it has been used successfully as "salvage" therapy in patients who have failed or have been intolerant of conventional corticosteroid therapy, and it has also been used as first-line therapy [67–72]. However, cyclosporine A has not been compared to conventional treatments in control-randomized trials, and its use, especially as a first-line drug, remains controversial. Side effects of cyclosporine A include renal insufficiency, hypertension, and malignancy.

Tacrolimus inhibits expression of the IL-2 receptor and as a result impairs cell cycle progression and expansion of cytotoxic T lymphocytes. At doses of 4 mg twice daily, it has improved serum aminotransferase and bilirubin levels at acceptable risk in a small, open-labeled treatment trial in which the drug was given for 3 months [73]. In another study, 11 patients who were treated an average of 25 months with doses of tacrolimus titrated to serum concentrations between 1.7 and 10.7 ng/mL (mean, 3 ng/mL) experienced significant improvements in serum aminotransferase levels and histological activity indices [74]. Tacrolimus is an empiric therapy for the refractory patient, and it may allow corticosteroid withdrawal [75]. Clinical control and randomized trials are needed to fully justify this expensive and potentially toxic treatment option.

Mycophenolate mofetil (MMF) is an ester prodrug of mycophenolic acid, and it inhibits inosine monophosphate dehydrogenase. Conversion of inosine monophosphate to xanthosine monophosphate is impaired, and subsequently DNA synthesis is reduced as a result of depleted stores of guanine nucleotides and lymphocyte proliferation is inhibited. Four small studies, using a typical dose of 1 g twice daily, have demonstrated the potential value of MMF as a substitute for azathioprine in patients intolerant of the conventional drug regimens [76–79]. These reports have been countered by another small study in which five of eight patients receiving MMF had laboratory improvement but not resolution, histological improvement did not occur in four patients sampled during treatment, and successive liver tissue specimens in two patients showed progressive fibrosis. Furthermore, corticosteroids could not be withdrawn in any patient [80]. Another study with 15 patients with AIH demonstrated that administration of MMF, either as monotherapy or in combination with prednisone, results in biochemical and histologic improvement in patients who were previously prednisone and/or azathioprine intolerant or resistant without the development of significant complications [81]. Recently, the largest cohort treated with MMF, including 39 patients with AIH showed a low frequency of response to treatment as only 14 patients (39%) achieved remission, defined as AST less than twice the upper limit of normal. In a subset analysis, the authors further demonstrated that most patients with prior non-response to azathioprine did not response to MMF either [82].

MMF may be useful in corticosteroid-responsive patients who require a non-steroidal maintenance regimen, whereas it may be less effective as a salvage therapy for corticosteroid-dependent or unresponsive patients. A potential advantage over

azathioprine is the independence of MMF from the thiopurine methyltransferase pathway; therefore it may be an alternative for patients who could not tolerate azathioprine previously.

Budesonide (3 mg thrice daily) is a second generation glucocorticoid with high first pass clearance by the liver and metabolites that are devoid of glucocorticoid activity. Preliminary studies in patients with mild disease have suggested that it is useful in selected patients as a frontline treatment [83–85], and these small reports justified the performance of a controlled clinical trial. Recently, a German study compared combined budesonide and azathioprine to prednisolone and azathioprine treatment in 208 patients with AIH. The primary end point was to achieve complete remission without the typical steroids side effects, defined as acne, hirsutism, diabetes, striae, glaucoma and facial swelling. In this study the outcome used was a combination of biochemical remission without the typical steroids side effects (47% versus 18%, $P<0.001$). Furthermore, the frequency of biochemical remission was superior in the budesonide group (60% versus 39%, $P=0.001$); however, for the long-term normalization of bilirubin and IgG, budesonide was not superior to prednisolone. There is controversy with respect to the prednisolone effect, as the remission rates seems poor when compared to the 90% remission rates achieved in previous studies [86]. Moreover, budesonide cannot be used in cirrhotics and has not been effective as a salvage therapy for patients who are refractory or on corticosteroid-dependent treatment [87].

6-mercaptopurine (6-MP) is the active metabolite of azathioprine and a purine antagonist, but the drugs are not interchangeable equivalents. 6-MP, administered initially as 50 mg daily and increased to 1.5 mg per kg daily, has salvaged patients who have failed therapy with azathioprine, and it should be considered as empiric treatment in such patients [88]. Its advantage over azathioprine may reflect differences in intestinal absorption and metabolism. 6-MP generates thioguanine nucleotides that can accumulate in hematopoietic tissue and cause myelosuppression, and its clearance involves the thiopurine methyltransferase pathway.

6-thioguanine nucleotides are the active metabolites of azathioprine, and they have been effective in three patients intolerant of the parent drug [89]. Their use in AIH represents a logical addition of the use of purine antagonists for this disease, and the strategy takes advantage of the downstream metabolic products of the parent drug to minimize intolerance and perhaps improve efficacy. The discordance between blood levels, laboratory response, and treatment tolerance underscores the lack of effective dosing and monitoring schedules for the 6-thioguanine nucleotides and cautions against its premature clinical application.

Rituximab is a chimeric monoclonal anti-CD20 antibody which can induce depletion of B lymphocytes by targeting their CD20 cell surface receptor. This interaction may affect complement activation, antibody-dependent cytotoxicity, and the induction of apoptosis. Its rationale in AIH is based on a presumption that B-cell expansion and hyperactivity can be suppressed by blocking the CD20 cell surface receptor of these cells. The unregulated proliferation of activated plasma cells can result

in the production of immunoglobulins that adhere to normal membrane constituents of the hepatocytes. These aggregates can then become targets of natural killer cells with Fc receptors, and the antigen–antibody complex on the hepatocytes surface can induce an antibody-dependent cytotoxicity. These presumptions have been supported by the successful treatment of one patient with AIH and idiopathic thrombocytopenic purpura with the drug [90]. Moreover, a recent study described six patients with AIH intolerant of or refractory to prednisone and azathioprine who received rituximab 1,000 mg IV at days 1 and 15. In all cases rituximab was well-tolerated and there were no serious adverse events. All patients entered biochemical remission and IgG and γ-globulin levels significantly improved [91]. These results support the ongoing investigation of rituximab for AIH patients who are refractory to or intolerant of standard therapy.

Ursodeoxycholic acid (13–15 mg/kg daily) has putative choleretic, cytoprotective and immunomodulatory actions that could benefit patients with AIH. Its ability to reduce expression of class I human leukocyte antigens, reduce immunoglobulin production, inhibit IL-2, IL-4 and interferon-γ production, impair nitric oxide synthetase, and reduce production of reactive oxygen species might attenuate the autoimmune response. Ursodeoxycholic acid has been used as primary therapy for mild AIH, and treatment with 600 mg daily for 2 years has resulted in clinical, laboratory, and histological improvement in eight Japanese patients [92]. Similar improvements have not been achieved in North American patients with severe disease who experienced relapse or treatment failure after corticosteroid therapy [93]. The role of ursodeoxycholic acid as primary or adjunctive treatment for AIH is limited, and its value may be in the treatment of individuals with the clinical phenotype of those in Japan who have mild or uncomplicated disease.

None of the new drugs that have been used in AIH have been formally incorporated into management algorithms, but each has been administered empirically with reported success and safety. These agents increase the treatment options for the difficult patient, who is refractory to conventional regimens or intolerant of first-line medications, but they have not been established as effective treatments and their application is associated with an uncertain benefit–risk ratio.

Conclusion

Progress toward a more rational therapy of AIH is being made because of a better understanding of pathogenic mechanisms and the emergence of potent site-specific immunosuppressive agents. Improved prognostic instruments and recognition of modifying genetic and regional factors will allow modifications of therapy to suit individual clinical situations. The drugs and science are available to improve treatment of AIH; nevertheless, reliable animal models of the human disease and a multi-center, collaborative network of clinical investigators are the remaining requisites for success.

Chapter Summary

1. Autoimmune hepatitis may behave aggressively and can result in cirrhosis, mortality caused by liver failure, or the need for liver transplantation.
2. Prednisone alone or at a reduced dose combined with azathioprine remains the first-line treatment for autoimmune hepatitis.
3. Not all patients with autoimmune hepatitis respond to corticosteroid therapy, and those who do can relapse after withdrawal of this therapy.

Useful Tips for Practitioners

1. Therapy to the point when liver test results and histological findings are normal reduces, but does not eliminate, the occurrence of relapse.
2. Termination of immunosuppressive treatment can be considered after at least 2 years of treatment, when serum aminotransferases and immunoglobulin levels have been persistently normal; an individualized approach is however needed.
3. Treatment failure warrants reassessment with regard to the accuracy of the original diagnosis and the exclusion of variant forms of hepatitis or concomitant alternative diseases.

Common Pitfalls in Practice

1. The diagnosis of autoimmune hepatitis does not compel therapy, and retrospective analyses of patients with mild disease have demonstrated the possibility of long-term survival without treatment.
2. Treatment of patients presenting with inactive cirrhosis with immunosuppressants is generally to be avoided.
3. Immunosuppressive treatment must ideally be continued until resolution of symptoms, laboratory indices of liver inflammation, and histological features of disease activity.

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Chapter 7
When and How to Treat the Pediatric Patient

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Keywords Children • Autoimmune hepatitis • Autoimmune sclerosing cholangitis • De novo autoimmune hepatitis post-liver transplant • Corticosteroids • Azathioprine • Calcineurin inhibitors • Mycophenolate mofetil • Ursodeoxycholic acid • Rituximab • Anti-IL2 receptor monoclonal antibody • Regulatory T cells

Introduction

Autoimmune hepatitis (AIH) is a progressive inflammatory liver disorder affecting mainly females, characterized serologically by high levels of transaminases and immunoglobulin G (IgG), and presence of autoantibodies, and histologically by interface hepatitis, in the absence of a known etiology [1]. AIH responds satisfactorily to immunosuppressive treatment. If left untreated, it progresses rapidly to cirrhosis and liver failure.

The clinical phenotype of AIH in children differs from that of adults [2, 3]. The juvenile form of AIH is subdivided into two types according to the autoantibody profile: type 1, positive for anti-nuclear (ANA) and/or anti-smooth muscle (SMA)

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antibody, and/or type 2, positive for anti-liver kidney microsomal antibody type 1 (anti-LKM-1) or anti-liver cytosol type 1 (anti-LC1), autoantibodies rarely described in adult patients [4]. Both AIH types are associated with other autoimmune disorders (about 20%) and a family history of autoimmune disease (40%) [5]. Type 2 AIH can be part of the autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) syndrome, an autosomal recessive genetic disorder in which the liver disease is reportedly present in some 20% of patients [6].

The epidemiology of childhood AIH is unknown, but type 1 AIH accounts for two-thirds of the cases and presents usually during adolescence, while type 2 AIH presents at a younger age and also during infancy. In both types there is a female preponderance (75%) and IgG levels are usually raised at presentation, though 15% of children with AIH type 1 and 25% of those with AIH type 2 have normal levels [5], especially those with an acute presentation. IgA deficiency is common in AIH type 2 [5].

AIH in children is usually more advanced at presentation than in adults [7]. Severity of disease is similar in the two subtypes, though anti-LKM-1-positive children have a higher tendency to present as fulminant liver failure than those with type 1 AIH [5]. Most children with either subtype of AIH have an acute onset, indistinguishable from severe acute infectious hepatitis, and over 40% already have cirrhosis at diagnosis, the milder forms of the disease described in adults being rarely reported in childhood [1, 8]. The aggressive course of juvenile AIH requires a low threshold of suspicion for this condition in children with unexplained abnormal liver function tests, so that treatment can be started early to avoid progression of disease and worse long-term outcome [9].

In pediatrics, sclerosing cholangitis is often associated with florid autoimmune features, including elevated titers of autoantibodies, in particular ANA and SMA, elevated IgG, and interface hepatitis [10]. Since these features are shared in common with AIH and are often not accompanied by elevated alkaline phosphatase or gamma glutamyl transpeptidase levels at disease onset, the diagnosis of sclerosing cholangitis relies on cholangiographic studies. In the absence of cholangiographic studies at presentation many of these children are diagnosed and treated as AIH, though the diagnosis of sclerosing cholangitis often becomes apparent during follow-up. This condition, referred to as autoimmune sclerosing cholangitis (ASC), is as prevalent as AIH type 1 in childhood, but in contrast to AIH it affects equally boys and girls [10]. ASC responds satisfactorily to immunosuppression, at least in regard to the parenchymal inflammation, if treatment is started early. Current International Autoimmune Hepatitis Group (IAIHG) diagnostic criteria [2, 11] do not allow distinction between AIH and ASC.

Treatment

The goal of treatment is to obtain early complete remission to prevent disease progression, and maintain it long term on the lowest dose of medication possible.

Definition of Remission and Relapse

Remission is defined as complete clinical recovery, normal transaminase and IgG levels, negative or very low titer autoantibodies (tested by immunofluorescence on rodent liver stomach and kidney [12]) and histological resolution of inflammation. The histological response lags behind the biochemical response [13–15] and clinical/biochemical remission does not necessarily reflect histological resolution. After a mean duration of 4 years of treatment, improvement of the intensity of portal inflammation is observed in up to 95% of cases and is accompanied by an improvement of the fibrosis scores [14]. Histological regression of cirrhotic changes after immune suppressive treatment with steroids and azathioprine has been recently reported in a child with AIH type 1 [16].

Relapse is characterized by increase of serum transaminase levels after remission has been achieved. Relapse during treatment is common, occurring in about 40% of patients requiring a temporary increase in the steroid dose. An important role in relapse is played by nonadherence, which is common, particularly in adolescents [17]. In more aggressive cases, the risk of relapse is higher if steroids are administered on an alternate-day schedule, which is often instituted in the belief that it may have a less negative effect on the child's growth. Small daily doses are more effective in maintaining disease control and minimize the need for high-dose steroid pulses during relapses (with consequent more severe side effects). Importantly, these small daily doses have been shown not to affect final height [18].

When to Treat

AIH should be suspected and sought in all children with evidence of liver disease after exclusion of infectious and metabolic etiologies. Autoimmune hepatitis is exquisitely responsive to immunosuppression and treatment should be initiated promptly to avoid progression of disease. The aim of treatment is to reduce or eliminate liver inflammation, to induce remission, improve symptoms, and prolong survival [19, 20]. The rapidity and degree of the response depends on disease severity at presentation. Although cirrhosis is reported to be present in 44–80% of children at the time of diagnosis [5, 8, 21], mortality is low and most children remain clinically stable, with a good quality of life on long-term treatment.

How to Treat

With the exception of a fulminant presentation with encephalopathy, AIH responds satisfactorily to immunosuppressive treatment whatever the degree of liver impairment, with a reported remission rate around 80%.

There have been no randomized, controlled, treatment trials in children with autoimmune hepatitis, but several reports have documented the efficacy of regimens similar to those used in adults [9].

Standard Treatment

Successful treatment is obtained in most cases of AIH with inexpensive, well-tested drugs. The mode of administration over time is key to success. Treatment of juvenile AIH is initiated with prednisolone (or prednisone) 2 mg/kg/day (maximum 40–60 mg/day). This dose should be gradually decreased over a period of 4–8 weeks, guided by the decline of transaminase levels, to a maintenance dose of 2.5–5 mg/day [5, 22, 23]. The target should be an 80% decrease of the transaminase levels by the first 2 months of treatment, and not their complete normalization, which may take several months [5, 24]. During the first 6–8 weeks of treatment, liver biochemical tests should be checked weekly to allow frequent dose adjustments. The attempt to attain normal transaminase levels more rapidly would require a prolonged use of high-dose steroids with attendant severe side effects. The timing for the addition of azathioprine as a steroid-sparing agent varies according to the protocols used in different centers. In our center [5], azathioprine is added if the transaminase levels stop decreasing on steroid treatment alone, or in the presence of steroid side effects, at a starting dose of 0.5 mg/kg/day, which in the absence of signs of toxicity is increased up to a maximum of 2.0–2.5 mg/kg/day until biochemical control is achieved. In other centers azathioprine is added at a dose of 0.5–2 mg/kg/day in all cases after a few weeks of steroid treatment, when the serum aminotransferase levels begin to decrease. Whatever the protocol, 85% of the patients eventually require the addition of azathioprine to steroids. Some centers use a combination of steroids and azathioprine from the beginning, but caution is recommended because azathioprine can be hepatotoxic, and should be used in severely jaundiced patients only when jaundice subsides.

Measurement of thiopurine methyltransferase activity level before initiating azathioprine therapy has been advocated to predict azathioprine metabolism and toxicity. Measurement of the azathioprine metabolites 6-thioguanine and 6-methylmercaptopurine has been reported to help in identifying drug toxicity and nonadherence and in achieving a level of 6-thioguanine considered therapeutic for inflammatory bowel disease [25], though an ideal therapeutic level for AIH has not been determined. Prospective studies are needed.

In adults, azathioprine alone has been shown to maintain remission in AIH type 1. A similar experience has been recently reported in pediatrics, successful withdrawal of steroids and maintenance of remission with azathioprine alone having been described in 18/37 children with AIH type 1 and 4/11 children with AIH type 2 in one center [26] and in 5/7 children with AIH type 1 in another [27].

Alternative Treatments

Calcineurin inhibitors. The use of cyclosporine A to induce remission without high-dose steroid side effects has been advocated both in type 1 [28, 29] and 2 AIH [30]. In treatment naïve children, mostly with AIH type 1, Alvarez' group reports attainment of remission using cyclosporine A alone for 6 months, followed by the addition of prednisone and azathioprine [28, 29]. One month later, cyclosporine is discontinued.

Cyclosporine is used at the dose of 4 mg/kg/day in three divided doses, increased every 2–3 days to achieve a whole-blood trough level of 250±50 ng/ml for 3 months. If there is clinical and biochemical response in the first 3 months, cyclosporine is reduced to achieve trough levels of 200±50 ng/ml for the following 3 months, before discontinuing it. Normalization of transaminases was obtained in 94% of subjects treated, with 72% responding within the first 6 months of treatment. Transition to azathioprine and low-dose prednisone after 6 months was possible in all patients and resulted in sustained remission in the majority for more than 2 years. Cyclosporine side effects included hypertrichosis (55%), gingival hyperplasia (39%), elevation of creatinine (8/84 children), and hypertension (3/84 children). Whether this mode of induction has any advantage over the standard treatment has yet to be evaluated in controlled studies.

Tacrolimus, a second calcineurin inhibitor, is a more potent immunosuppressive agent than cyclosporine, with fewer cosmetic consequences, but significantly higher toxicity. There is limited evidence supporting its role in the treatment of AIH apart from anecdotal reports in adults.

There are theoretical disadvantages in the use of calcineurin inhibitors for the treatment of AIH, since these drugs have been shown to favor autoimmunity in experimental models both by depressing regulatory T-cell function and favoring thymus egress of self-reactive T cells [31–35]. Calcineurin inhibitors may in fact be involved in the development of autoimmune reactions after transplantation, including de novo AIH after liver grafting [35].

Both cyclosporine A and tacrolimus are used as rescue treatment for difficult-to-treat cases, but since no large series in this subgroup of patients has been published, they should be used cautiously.

Treatment of refractory cases. Mycophenolate mofetil (MMF) is the prodrug of mycophenolic acid. Its effect on purine synthesis leads to decreased T and B lymphocyte proliferation. In patients in whom standard immunosuppression is unable to induce stable remission, or who are intolerant to azathioprine, mycophenolate mofetil at a dose of 20 mg/kg twice daily, together with prednisolone, has been used with success [36]. If there is a persistent absence of response or if there is intolerance to mycophenolate mofetil (headache, diarrhea, nausea, dizziness, hair loss, and neutropenia), the use of calcineurin inhibitors may be considered. Tacrolimus may be useful in combination with prednisolone as second-line therapy.

Other treatments. No data are available on the effectiveness of budesonide or ursodeoxycholic acid (UDCA) in childhood AIH. Data on budesonide treatment in childhood AIH have been presented at an EASL meeting but await peer review.

Treatment of Autoimmune Sclerosing Cholangitis

ASC responds to the same immunosuppressive treatment described above for AIH. However, while steroids and azathioprine are beneficial in abating the parenchymal inflammatory lesions, they appear to be less effective in controlling the bile duct

disease [10]. UDCA is usually added to steroids and azathioprine for the treatment of ASC, but whether it is helpful in arresting the progression of the bile duct disease remains to be established. In adults with primary sclerosing cholangitis high-dose UDCA has been reported as more beneficial than standard doses [37], but a randomized double-blind controlled study by the Mayo Clinic group shows that very high-dose UDCA (30 mg/kg/day) has a negative long-term effect [38]. It is prudent, therefore, to use doses not exceeding 15 mg/kg/day. Both AIH and, more commonly, ASC can be associated with inflammatory bowel disease which should be investigated even in the absence of symptoms and appropriately treated.

Duration of Treatment and Prognosis

The optimal duration of immunosuppressive treatment for AIH is unknown. Treatment withdrawal is successful only if there is histological resolution of inflammation. Hence, cessation of treatment should be considered if a liver biopsy shows minimal or no inflammatory changes after 1–2 years of normal liver function and biochemical tests, normal IgG levels and negative, or low titer autoantibodies. However, it is advisable not to attempt treatment withdrawal within 3 years of diagnosis or during or immediately before puberty, when relapses are more common. It has been reported that 20% of patients with AIH type 1 can successfully and permanently stop treatment, while this is rarely achieved in AIH type 2 [5]. Long-term treatment is required for the majority of patients and parents and patients should be counseled accordingly. In the pediatric setting, an important role in monitoring the response to treatment is the measurement of autoantibody titers and IgG levels, the fluctuation of which correlates with disease activity [39]. In particular, for patients with high IgG levels, their decrease is a reliable, objective and inexpensive measure of disease control.

The prognosis of those children with AIH who respond to immunosuppressive treatment is generally good, with most patients surviving long-term with excellent quality of life on low dose medication. Development of end-stage liver disease requiring liver transplantation despite treatment, however, has been reported 8–14 years after diagnosis in 8.5% of children with AIH [5].

A question frequently asked by parents and teenage girls is the effect of treatment on pregnancy and its safety for the fetus. A few published reports demonstrate that treatment with steroids and azathioprine is safe for the mother and the baby and not associated with an increased risk of fetal defects or mortality [40–42].

Liver Transplantation

Liver transplantation is indicated in patients who present with fulminant hepatic failure (with encephalopathy) and those who progress to end-stage liver disease despite treatment (10–20%). The latter is more likely when established cirrhosis is

present at diagnosis, or if there is a long history before the start of treatment. After transplantation, recurrent AIH develops in some 20% of cases [43]. Diagnosis of recurrent AIH is based on biochemical abnormalities, presence of autoantibodies, interface hepatitis on liver histology, and/or steroid dependence. Recurrence may occur even years after transplantation, and consequently maintenance of steroid-based immunosuppression at a higher dose than that used for patients transplanted for conditions other than AIH is generally recommended. Additionally, a form of graft dysfunction called de novo AIH, associated with positive autoantibodies, high IgG, histological features of interface hepatitis has been described in 6–10% of children transplanted for nonautoimmune disorders [35, 44]. This condition does not respond satisfactorily to anti-rejection regimens, but only to the standard treatment for AIH [35, 44], or, in resistant cases, to rapamycin [45].

Future Treatment Options

New immunosuppressive agents effective in the 10–15% of children who fail to respond to conventional corticosteroid treatment, and which may promote permanent resolution of the disease in all patients are the ultimate goal for childhood AIH.

The armamentarium available to manipulate the immune system in the field of organ transplantation and in other autoimmune diseases may ultimately provide important information for the treatment of autoimmune hepatitis and prompt multicenter, collaborative studies in large numbers of children.

Immunosuppressant medications that may theoretically be useful in the treatment of AIH include selective monoclonal antibodies directed against the IL-2 receptor, a high number of activated lymphocytes bearing this receptor being characteristic of the active phase of the disease [46]. However, regulatory T cells also express IL-2 receptor and a fine balance will need to be found between curbing effector function while avoiding interfering with regulatory mechanisms. In view of the elevated level of IgG and high titers of autoantibodies, rituximab is a possible mode of treatment for particularly aggressive cases. Rapamycin, reportedly successful in the control of post-transplant de novo AIH [45], could also have a role in difficult-to-treat AIH.

All the above drugs, however, in common with conventional immunosuppressive drugs, do not only suppress the autoimmune process causing liver damage, but also weaken the physiological immune responses, with consequent systemic side effects.

Recent studies showing that a decrease in number and function of regulatory T cells (Tregs) characterizes childhood AIH, particularly when the disease is active [47–49], and that defective liver antigen-specific Tregs can be cultured, re-educated and expanded in vitro [50, 51], lay the foundation for treatment based on adoptive transfer of re-educated antigen-specific Tregs. This would be able to treat, possibly cure, liver-directed autoimmunity without impairing the overall function of the immune system.

Chapter Summary

1. In childhood, autoimmune hepatitis has a particularly aggressive course and should be diagnosed and treated as early as possible to avoid rapid progression to cirrhosis and liver failure.
2. The goal of treatment is to obtain early complete remission (complete clinical recovery, normal transaminase and IgG levels, negative or very low titer autoantibodies and histological resolution of inflammation) and maintain it long term on the lowest dose of medication possible.
3. The vast majority of children with autoimmune hepatitis respond to steroids, usually with the addition of azathioprine as a steroid sparing treatment. Alternative treatments should only be offered to difficult-to-treat patients. Medium and long-term prognosis of treated autoimmune hepatitis is excellent.

Useful Tips for Practitioners

1. Autoimmune hepatitis should be considered in the differential diagnosis of all children with abnormal liver function tests.
2. An acute hepatitis with a fluctuating course, negative for hepatitis A, B or C infection, is highly suggestive of autoimmune hepatitis.
3. Relapse of autoimmune hepatitis on treatment, in particular in teenagers, is usually due to poor adherence to treatment.
4. Progression of disease despite effective treatment suggests presence of bile duct disease (autoimmune sclerosing cholangitis).

Common Pitfalls in Practice

1. To delay investigating children with a cryptogenic acute hepatitis in the belief that it is a benign condition.
2. To continue high-dose steroid treatment in autoimmune hepatitis until the transaminase levels return to normal: this will cause severe steroid side effects. The aim should be to achieve at least an 80% reduction of baseline transaminase levels within 6–8 weeks, and decrease the steroid dose weekly while the transaminase levels improve. Azathioprine should be added if the transaminase values plateau.
3. To give alternate days steroid treatment to children with autoimmune hepatitis in the belief that this will have less negative effect on growth. In children with severe disease, an alternate day steroid schedule is associated with a high relapse rate, requiring increased steroid doses, with attendant more severe side effects and growth impairment.

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Chapter 8
Strategies for Treatment Nonresponders: Second-Line Therapies and Novel Approaches

Elizabeth Mileti and Marion Peters

Keywords Alternative medications • Side effects • Mycophenylate mofetil • 6-Mercaptopurine • Cyclophosphamide • Budesonide • Deflazacort • Tacrolimus • Cyclosporine • Ursodeoxycholic acid • Inflizamab

Introduction

Conventional treatment of autoimmune hepatitis (AIH) with prednisone alone or in combination with azathioprine has been well studied over the last 40 years and found to be effective in approximately 80% of patients. The other 20% of patients are not successfully treated for various reasons including nonresponse, partial response, or noncompliance because they are unable to tolerate the side effects of these medications. Side effects of corticosteroids can be quite severe and include diabetes, hypertension, Cushingoid facies, behavioral changes, osteopenia, and growth retardation. These side effects are especially undesirable in children and the elderly. Azathioprine was initially studied as medication to maintain remission so that steroids could be weaned and side effects minimized. While azathioprine does allow for some steroid-sparing effects, it has its own adverse events which may limit tolerance to the medication.

Over recent decades, newer immunosuppressive medications have become available mainly for use in transplantation. Figure 8.1 provides the dates they were first reported for use in AIH. These medications have been evaluated for use in patients intolerant to first-line therapy for AIH. As more studies and experience with

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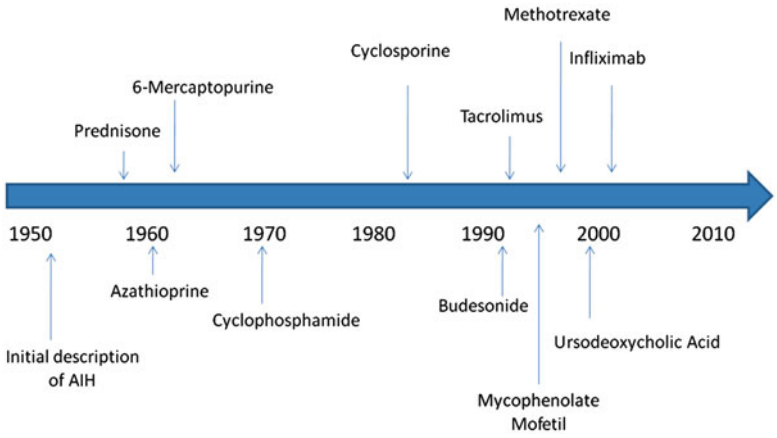

Fig. 8.1 Timeline of immunosuppressive medications usage for autoimmune hepatitis

Table 8.1 Quality of studies reported using alternative therapies for autoimmune hepatitis

	Primary therapy		Rescue therapy	
Drug	Studies (*n*)*	RCT* (*n*)	Studies (*n*)	RCT* (*n*)
MMF	0	0	Case series (36)	0
Budesonide	Case series (13)	Y* (203)	Case series (11)	0
Tacrolimus	Open-label prospective trial (21)	0	Case series (11)	0
Cyclosporine	Open-label prospective trial in children (84)	0	Case series (10)	0
UDCA	Retrospective study (65)	0	Case reports	ns* (147)

*(*n*) = number of patients in largest study reported
RTC = randomized controlled trial
Y = RCT with significant benefit and fewer side effects than prednisone
ns = RCT not significant

these medications continue to grow, the therapeutic approach to AIH is evolving. These alternative therapies include: mycophenolate mofetil, budesonide, cyclosporine, tacrolimus, cyclophosphamide, methotrexate, 6-mercaptopurine, not an immunosuppressive acid, and infliximab. These mostly immunosuppressive medications have generally been studied as second-line therapy after failure or intolerance of azathioprine and prednisone. There are few studies using these medications as alternative first-line approach or for induction of remission prior to transition to prednisone and azathioprine. There are only two randomized controlled studies of new medications for AIH which are described below. This review will outline the data supporting use of these alternative therapies for AIH. Table 8.1 shows the quality of studies reported for each drug and whether the drug was studied as primary or rescue therapy. In parentheses is the number of subjects in the largest study reported. Table 8.2 shows the side effects of these alternative therapies.

Table 8.2 Side effects of alternative therapies for autoimmune hepatitis

Medication	Side effects >10%	1–10%	<1%
Mycophenolate mofetil	Asthenia, backache, cytopenias Electrolyte abnormalities, GI symptoms Headache, insomnia, tremor Hypercholesterolemia, hyperglycemia Hypertension, nephrotoxicity	Severe neutropenia Gastrointestinal hemorrhage	Lymphoma Malignant epithelial skin neoplasm Progressive multifocal leukoencephalopathy Pulmonary fibrosis
Cyclophosphamide	Alopecia, amenorrhea, GI symptoms Hemorrhagic cystitis Interstitial pneumonia, leukopenia	Infectious disease	Azoopermia Cardiomyopathy Stevens-Johnson syndrome Toxic epidermal necrolysis
6-Mercaptopurine	Diarrhea hyperpigmentation of skin, rash	Hepatotoxicity	Hyperuricemia Myelosuppression Ulceration of intestine
Methotrexate	GI symptoms, mucositis, cytopenias Nephrotoxicity	Alopecia, photosensitivity, rash, pneumonitis, liver fibrosis, cystitis	Acute neurologic syndrome Alveolitis Erythema multiforme
Budesonide	Headache	GI symptoms arthralgia	Cataract, glaucoma, hypocortisolism
Tacrolimus	Headache, insomnia, tremor, parasthesias, alopecia, erythema, pruritus, GI symptoms, cytopenias, hypertension, diabetes, hyperlipidemia, nephrotoxicity, electrolyte abnormalities	Seizures	Lymphoproliferative disorders, Leukoencephalopathy
Cyclosporin	Electrolyte abnormalities, gingival hyperplasia, headache, tremor, High triglycerides, hirsutism, hypertension, nephrotoxicity	Seizures	Lymphoproliferative disorders
Ursodeoxycholic acid	Backache, dizziness, GI symptoms	Rash	
Infliximab	Abdominal pain, headache, nausea	Fatigue, leukopenia, neutropenic disorder	Acute coronary syndrome, drug induced lupus erythematosus, erythema multiforme, hepatosplenic T-cell lymphoma, hepatotoxicity, immune hypersensitivity reaction, Stevens-Johnson syndrome, toxic epidermal necrolysis

Antimetabolites

Mycophenolate Mofetil

Mycophenolate mofetil (MMF) is a popular second-line immunosuppressive medication for the treatment of autoimmune hepatitis. MMF is a pro-drug that is converted to mycophenolic acid (MPA), a noncompetitive inhibitor of inosine monophosphate dehydrogenase. MPA blocks purine synthesis, thereby stopping DNA replication in T and B lymphocytes which are unable to use the salvage pathway. Initial small case series of patients who failed standard therapy reported success rates of 63–75% [1, 2]. One of the first studies to use mycophenolate mofetil, published in 2000, had seven patients who were either intolerant or failed treatment with prednisone and azathioprine. This study used 20 mg/day of prednisone and 1,000 mg MMF twice a day. Five of these seven patients (71%) had normalized transaminases after 3 months of therapy with subsequent decrease in prednisone dose from 20 mg/day to 2 mg/day. Hepatic activity index scores were measured in this study with median scores decreasing from 11 to 3 after 7 months of therapy. Side effects included leucopenia in one patient, which resolved after dose reduction of MMF [3]. A similar study reported biochemical remission in all five patients on 1,000 mg MMF twice a day with continued steroid dose. All patients were able to decrease their steroid dose while on MMF [4]. A larger Canadian study reported normalization of ALT within 4.2 months in 13 of their 16 patients with MMF. Prednisone was able to be reduced in those who achieved biochemical remission [5]. One case series reported 34% dropout rates, due to side effects of the medication [6]. The most recent study in children by Aw et al. evaluated mycophenolate mofetil as rescue treatment for AIH. Twenty-six children with median age 9.9 years were treated with MMF. Indications for use of MMF included failure to induce or maintain remission in 20 subjects and intolerance of side effects in the other six children. Sixty-nine percent of children in this study achieved biochemical remission in this study [7].

In contrast, Czaja et al. reported only 25% achieved biochemical improvement with MMF and 38% actually had worsening transaminases [8]. Similarly disappointing results were seen in the largest case series performed thus far with MMF. Hennes et al. studied 36 individuals who failed standard therapy: 27 were intolerant to the side effects of steroids and azathioprine, and nine were nonresponders to standard treatment. Overall, only 14 out of 36 subjects responded to MMF (39%) but the results varied based on the reason for switching to MMF. Those subjects who were intolerant to azathioprine had a better response to MMF (43% remission) compared with subjects who did not respond to azathioprine (25% remission). Nausea, vomiting abdominal pain, and diarrhea were the most common side effects seen in 31% of patients and four patients stopped therapy due to side effects [9]. No randomized controlled studies have been performed in treatment naïve or as rescue therapy in AIH. While MMF has gained popularity, there are no data to show that it is more efficacious than azathioprine.

6-Mercaptopurine

6-Mercaptopurine was one of the initial medications used to treat AIH in the early 1960s. Azathioprine is a derivative of 6-Mercaptopurine and both often used interchangeably. Despite being a derivative of azathioprine, 6-Mercaptopurine has a different side effect profile and patients who are intolerant of azathioprine can sometime tolerate and respond to 6-Mercaptopurine and prednisone and be able to be weaned off prednisone [10].

Cyclophosphamide

Cyclophosphamide has been used for AIH since the 1970s. Several case reports have been published with the use of cyclophosphamide for patients intolerant or unresponsive to standard therapy with normalization of liver enzymes on a regimen of cyclophosphamide and steroids [11, 12]. However, it is not often used because of incidence of side effects, especially hematologic effects.

In addition to the above anti-metabolites, there are a few case reports highlighting the use of methotrexate in autoimmune hepatitis. Much like other anti-metabolites, methotrexate has shown promising results in limited case reports [13, 14].

Steroid Alternatives

Budesonide

Budesonide is a synthetic steroid with 90% first-pass metabolism in the liver. It also has a 15-fold higher affinity for the glucocorticoid receptor making it an attractive alternative to prednisone with potentially fewer side effects. Initial case series of 13 patients reported normalization of aminotransferases and the drug was well tolerated with fewer side effects [15]. A follow-up study of ten patients who were treatment-dependent were not stable on budesonide: remission was only seen in three patients and significant side effects were noted [16].

The largest multicenter study of budesonide as an alternative to prednisone has been performed in 30 centers in Europe and Israel and was recently published [17]. This was a 6-month, prospective, double-blind, randomized, active-controlled, multi-center, phase IIb trial (RTC) of 203 (all but five naïve) patients with AIH without evidence of cirrhosis. Treatment was randomized to 40 mg prednisone plus 1–2 mg/kg/day azathioprine or budesonide (3 mg tid) plus azathioprine with differing regimens for dose reduction subsequently. After 6 months, all patients

received budesonide and azathioprine for a further 6 months. Both prednisone and budesonide were decreased when biochemical remission was noted. A complete response was defined as biochemical remission (normal AST and ALT) as well as lack of steroid side effects at 6 and 12 months after therapy was started, i.e., a combined response. By intention to treat, a complete combined response was noted in 47.0% of 19/103 patients on budesonide compared to 18.4% on prednisone-based therapy ($p < 0.001$). Biochemical remission at 6 months was seen in 60% of patients on budesonide and 38.8% of patients on prednisone ($p = 0.001$). Steroid-related side effects were higher in the prednisone group (53.4%) compared to budesonide (28%) ($p < 0.001$) [17]. These side effects included weight gain, headache, mood changes, muscle weakness, moon facies, hypertension, and insomnia. Steroid side effects also decreased from 44.8 to 26.4% at 12 months in those 87 patients who were initially given prednisone and then received budesonide after 6 months ($p < 0.002$). Thus, budesonide is clearly beneficial in achieving biochemical remission as well as decreasing unwanted steroid side effects as shown in this RTC. The caveat is that not all clinicians treat AIH with such a high starting dose and the side effect profile might not match their own clinical experience.

Deflazacort

There is only one study and case report of deflazacort, an oxazolinic derivative of prednisolone with fewer effects on bone and glucose metabolism. Fifteen patients stable on therapy were switched to deflazacort with or without azathioprine. No patient flared and 94% remained in sustained remission [18]. No further studies have been reported.

Calcineurin Inhibitors

Tacrolimus

Tacrolimus is a macrolide that binds to an intracellular protein, FK506 binding protein. This bound complex competitively binds to and inhibits the phosphatase activity of calcineurin. Inhibition of calcineurin inhibits IL-2 transcription and subsequent T-lymphocyte activation. The initial study of tacrolimus was first-line sole treatment in untreated AIH patients and was published in 1995. Twenty-one patients with biochemical and histological evidence of autoimmune hepatitis were started on tacrolimus. Mean duration of disease was 4 years and approximately half of the patients had cirrhosis prior to therapy. Subjects were treated with 3 mg of tacrolimus twice a day and levels were followed. Results showed a reduction in ALT level by 80% and a reduction in AST level by 70% after 3 months of therapy with

normalization of levels by 1 year. Serum creatinine increased during this study from 0.9 to 1.3 mg/dL. The mean tacrolimus dose was 7.2 ± 0.8 mg/day. Four patients required liver transplantation [19].

Tacrolimus as rescue therapy has been reported in small case series with variable results. Aqel et al. reported 11 subjects refractory to standard therapy, who were subsequently treated with tacrolimus: ten subjects achieved clinical remission with normalization of transaminases. In one individual who did not achieve remission, rapamycin was added and remission was subsequently achieved [20]. In contrast, a Canadian study in 2005 of three subjects did not report such promising results with tacrolimus. Three patients received tacrolimus as monotherapy while an additional two patients received it in conjunction with MMF. None of the three subjects on tacrolimus alone achieved remission [5]. Finally, a study by Larsen et al. used low-dose tacrolimus in nine patients refractory to steroids and azathioprine or MMF. ALT normalized in all subjects and liver biopsies showed improvement in inflammatory activity as determined Ishak score. These patients, however, were unable to be weaned off steroids completely [21]. No randomized controlled trials have been performed to date.

Cyclosporine

Cyclosporine is another calcineurin inhibitor. Cyclosporine binds to different intracellular proteins than does tacrolimus, called cyclophilins. The cyclophilin/cyclopsporine complex can bind to calcineurin, thereby inhibiting its phosphatase activity. Like tacrolimus, the complex inhibits the action of calcineurin, required for transcription of cytokines, IL-2 in particular, which drive the T-cell proliferative response. Much like the other second-line medications, many of the studies related to cyclosporine have been small case series. The larger studies on cyclosporine for AIH have been performed in children and used to induce remission. The first published use of cyclosporine for the treatment of autoimmune hepatitis was by Mistilis et al. in 1985 in a case report of a 51-year-old man who was unresponsive to corticosteroids and was intolerant of azathioprine. He was placed on cyclosporine with normalization of his ALT [22]. Several other case reports followed showing similar results. A case series of six patients with type I autoimmune chronic active hepatitis unable to achieve remission with standard therapy and were intolerant to the side effects of the medications also showed promising results. Five out of the six patients had improved ALT levels, which were normal or near normal and remained in remission for up to 1 year. Post-treatment liver biopsies were performed in three subjects and all showed histological improvement [23].

The largest open-label trial of cyclosporine for autoimmune hepatitis was performed in medication naïve children as induction therapy. The study was initially reported in 1994 with 32 subjects and updated in 2006 with 84 subjects [24, 25]. This was a multi-centered clinical trial that enrolled 84 children prospectively. Subjects were started on cyclosporine at a dose of 4 mg/kg/day divided into three

daily doses. The dosing was adjusted to achieve blood concentration levels of 250 ± 50 ng/ml. Subjects remained on this for 3 months. Once transaminases had improved to three times the upper limit of normal, the dose was decreased to achieve blood concentrations of 200 ± 50 ng/ml for another 3 months. Prednisone and azathioprine were then started 6 months after diagnosis. Dosing for prednisone and azathioprine were significantly lower than usual therapy dose. Prednisone was started at 0.3 mg/kg/day in patients less than 20 kg and 0.5 mg/kg/day if greater than 20 kg. Azathioprine was started at 1.5 mg/kg/day divided into two daily doses. Cyclosporine was gradually decreased over 15 days as the other two medications were started. Prednisone was then reduced slowly 1 month later, and azathioprine was kept at the same dosage. Normalization of ALT was seen in 94% of subjects (79/84) by 1 year and no relapses were seen during treatment. Side effects of cyclosporine were transient but included hypertrichosis and gingival hyperplasia [25].

There have been open-label prospective studies and case series reporting use of cyclosporine both as first-line treatment and as a rescue therapy for AIH. One prospective open-label study had 19 patients aged 15–46, ten previously on steroids and azathioprine at the start of the study. Subjects were started on cyclosporine at doses of 2–5 mg/kg/day divided twice a day and followed up for 26 weeks of treatment. Cyclosporine levels were monitored and kept between 100 and 300 ng/ml. If ALT was not decreased by at least 50% at 4 weeks of treatment, prednisone was added at 10 mg/day. After 26 weeks of treatment, subjects were placed back on prednisone 10 mg/day plus azathioprine at 1–2 mg/kg/day as a maintenance therapy. Fifteen of the 19 patients completed the 26-week course of cyclosporine. The four subjects that stopped cyclosporine prematurely did so because of side effects or nonresponse to treatment. Of the 15 subjects remaining, all had improvement in transaminases to normal or near normal levels [26]. Similar results were noted in other small case series [27, 28]. No randomized controlled trials of cyclosporine in AIH have been performed.

Other Medications

There are two other medications that have been used in patients with autoimmune hepatitis who have failed treatment, are nonresponsive or only have a partial response to azathioprine and prednisone. There are limited data on these agents.

Ursodeoxycholic Acid

Ursodeoxycholic acid (UDCA) is a 7α(alpha)-hydroxyl epimer of chenodeoxycholic acid that has been used in the treatment of cholestatic liver disease. It has been studied

in primary biliary cirrhosis and is thought to may have immunomodulatory properties. Several papers have been published looking at the role of UDCA in AIH, including several case reports, a larger Japanese study looking at UCDA in initial therapy with and without steroids and a randomized placebo-controlled trial using UCDA as adjunctive therapy after failure of conventional treatment [29].

The larger Japanese study described their experience over 18 years of 147 patients with AIH were treated with UDCA monotherapy ($N=25$), UDCA plus prednisolone ($N=40$), prednisolone monotherapy ($N=68$), or other therapies ($N=14$). The characteristics of the patients in each group prior to medication were dissimilar, however, the degree of histologic changes at baseline did not differ. UDCA monotherapy was used in patients who were elderly or had co-morbidities such as osteoporosis and diabetes. Of the 25 patients treated with UDCA alone, ten subsequently required prednisone because they did not achieve a biochemical response. Of the remaining 15, 11 achieved remission. Higher responses were noted in those receiving UDCA plus prednisolone group or prednisolone alone: both regimens had similar remission rates in terms of ALT normalization (94–95%) and similar relapse rates (57–58%). While biochemical remission was achieved with UDCA alone, it was slower than in the groups with corticosteroid use. Additionally, UDCA did not decrease relapse rates when combined with prednisolone [29]. No postdiagnosis biopsies were obtained to assess histologic improvement.

In 1999, Czaja and colleagues evaluated UDCA as an adjunctive treatment in problematic type I AIH patients. A randomized placebo-controlled trial was performed with 37 patients who had multiple relapses, partial response, or treatment failure on conventional therapy. These patients were randomized to receive UDCA or placebo for 6 months in conjunction with their corticosteroid treatment. The trial unfortunately did not enroll enough patients to detect statistical differences in efficacy between the two groups. The study reported a reduction in AST and alkaline phosphatase levels in the UDCA group compared to placebo, but this was not statistically significant. During the 6 months that patients were on UDCA or placebo, UDCA did not allow for reduction in corticosteroid use nor did UDCA improve histological activity [30]. Thus while there are cases with improvement of biochemical markers of AIH with UDCA, there is not enough evidence to support routine use of UDCA in the treatment of AIH.

Infliximab

A recent case report was published in 2009 of use of Infliximab, a monoclonal antibody against TNF-alpha, for treatment of a particularly difficult case of autoimmune hepatitis who had failed prednisone, azathioprine, mycophenolate mofetil, and cyclophosphamide. Infliximab was used only to induce remission and azathioprine and prednisone were used as maintenance medications. Even with the evidence of cirrhosis on biopsy, the patient responded biochemically to Infliximab. During her

treatment course, she had two relapses and Infliximab was successful at inducing remission both times. However, infectious complications occurred during infliximab treatment [31]. This agent remains experimental.

Conclusion

The current available evidence for second-line treatments of autoimmune hepatitis is based mostly on small retrospective case series. In patients who are not responding to therapy, it is imperative to ensure that these subjects are being compliant with their medications. In those who cannot tolerate the side effects or are true nonresponders, treatment with alternative medications should be considered. However, it is critical that both provider and patient understand the side effects of the medication, which can be quite severe as noted in Table 8.2. Education about these medications, their side effects, and the different treatment options available should be discussed with patients prior to initiating alternative therapy. While results of these studies are promising, with many agents inducing biochemical remission and allowing for weaning of steroids, they are difficult to interpret due to the lack of a common protocol for selection or management of these patients. Randomized control trials (RCT) are needed to compare these medications to the conventional therapy of prednisone and azathioprine. Only two randomized controlled trials have been performed: UDCA failed to show benefit perhaps related to study size; but budesonide appears to have benefit in patients without cirrhosis with similar efficacy to standard therapy and likely fewer steroid-induced side effects. Only RCT can allow the development of a paradigm to determine which patients will benefit from which alternative therapies.

Chapter Summary

1. Second-line therapies for the autoimmune hepatitis should be reserved only for those individuals who are true nonresponders or are unable to tolerate the side effects of conventional therapy.
2. Current evidence surrounding efficacy of second-line treatments results from mainly retrospective case series. The majority of empiric experience has been with mycophenolate mofetil and cyclosporine. Only budesonide has shown benefit in a randomized control trial.
3. It is critical when considering alternative medications for patients with treatment failure that both providers and patients understand the potential side effects, which can be quite severe.

Useful Tips for Practitioners

1. Steroid and azathioprine dosing should be optimized to induce remission prior to using second-line therapies for AIH.
2. Budesonide is a beneficial alternative and first-line therapy in patients without cirrhosis who are unable to tolerate the side effects of corticosteroids.
3. Biochemical monitoring varies depending upon the side effect profile of the second-line therapy being used.

Common Pitfalls in Practice

1. Lack of adequate initial therapy may be misinterpreted as treatment failure.
2. Rapid weaning of steroids may result in loss of response which is not due to treatment failure.
3. Budesonide should only be used in patients without cirrhosis.

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Chapter 9
Liver Transplantation for Autoimmune Hepatitis

James Neuberger

Keywords Autoimmune hepatitis • Indications • Liver transplantation • Outcomes • Recurrent disease

Abbreviations

AIH	Autoimmune hepatitis
ANA	Anti-nuclear antibodies
ASM	Anti-smooth muscle antibodies
AST	Aspartate aminotransferase
CNI	Calcineurin inhibitor
GSTT1	Glutathione S-transferase T1
HCC	Liver cell cancer(hepatocellular carcinoma)
HCV	Hepatitis C virus
HLA	Human leucocyte antigen
IgG	Immunoglobulin G
INR	International normalised ratio
LKM	Liver-kidney microsomal antibody
MELD	Model for end-stage liver disease
MMF	Mycophenolate mofetil
OLT	Orthotopic liver transplant
PBC	Primary biliary cirrhosis
PEI	Percutaneous ethanol injection

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PT	Prothrombin time
PTLD	Post-transplant lymphoproliferative disease
rAIH	Recurrent AIH
RFA	Radiofrequency ablation
TACE	Trans-arterial chemo-embolisation
TIPS	Transjugular intra-hepatic shunt
TMPT	Thiopurine methyl transferase
UKELD	United Kingdom model for end-stage liver disease

Introduction

Although immunosuppressive therapy is usually very effective in preventing progression of autoimmune hepatitis (AIH), a small minority of patients may become transplant candidates. Some will have presented too late for treatment to prevent the onset of life-reducing complications (such as hepatocellular cancer), others will develop intractable symptoms such as hepatic encephalopathy and others may have failed to respond fully to treatment. A small proportion will have failed to comply with treatment and progress to end-stage disease. In such cases, liver replacement remains the only therapeutic option to increase the quantity or quality of life, or both.

Indications for Liver Transplantation

AIH account for just over 5% of liver transplants [1]. Of the various subtypes of AIH, those with type 2 (liver kidney microsomal antibody positive) and those with cholestatic hepatitis may have a more aggressive pattern and so come to transplant sooner than those with type 1.

Chronic Liver Disease

There is a significant imbalance between the number of people who would benefit from a liver transplant and the number of grafts available. The increasing use of organs from non-heart-beating donors (donation after circulatory death – DCD) and live donors has failed to bridge the gap. Thus, there is a need for rationing: this remains a controversial area where the often conflicting demands of justice, equity, utility, and benefit have to be balanced. Selection (the process that determines who is placed on the transplant waiting list) is usually based on need and benefit, whereas allocation (deciding which recipient will be given a donated organ) is based on different criteria and considerations such as benefit and utility are important. With mortality

of those on the waiting list between 10 and 15%, the role of transplantation for those with a relatively good liver prognosis but a poor quality of life because of the complications is more controversial. In some administrations, such as in North America, those with relatively good graft function but a very poor quality of life because of, for example, intractable encephalopathy, will be less likely to get a deceased-donor graft than those with end-stage disease.

For those with chronic liver disease, the MELD (Model for end-stage liver disease) score has been validated and is utilised extensively [2]. Although MELD was originally developed to predict short-term survival following a transjugular intra-hepatic shunt (TIPS) [3], it has been shown in many studies to be an effective predictive model for patients with a wide variety of chronic liver diseases. The MELD score is derived from the serum creatinine, bilirubin, and International Normalised Ratio (INR).

There are some well-founded criticisms of the use of the MELD score: with time, it has been necessary to amend the coefficients of some of the variables; some of these variables can be affected by nonhepatic conditions (as, for example, the serum bilirubin can be elevated by hemolysis rather than liver disease) or serum creatinine can be "artificially" increased by over-use of diuretics. Furthermore, the creatinine value differs between the two genders (as the normal range is lower in females than males, females may be disadvantaged by the use of the model) and different laboratories may give different values for some analytes.

In not all cases will the MELD score predict prognosis (as in the development of cancer, or the presence of ascites and hyponatremia). Thus, there are some indications for which additional points can be given: these include hepatopulmonary syndrome and porto-pulmonary hypertension. Liver cancer is discussed separately. Finally, it must be emphasised that these prognostic models, which have been well validated in many centres and countries, give useful information for a population and extrapolation to the individual must be done with caution.

In the UK [4], a similar approach was taken to develop a model to predict short-term survival in those with advanced liver disease; the model developed (UKELD – UK model for end-stage liver disease) included most of the variables included in the MELD model – serum sodium, creatinine, prothrombin time and bilirubin – but with different coefficients and, in a validation set, was better associated with outcome than MELD, suggesting that different countries may need to adapt MELD better to suit the local population.

The introduction of the MELD system has been shown, in the United States, to be very effective in reducing the mortality of patients on the patient list without having a significant deleterious impact on post-transplant outcomes however, resource utilisation may be increased.

A MELD score of 16 or above is indicative of the need for transplant listing (for those without liver cell cancer) since at this level and above, the survival advantages of liver replacement exceeds the risk of dying without a transplant.

In those systems where selection and allocation are not based solely on the risk of death without transplant, other indications for liver transplantation include an

impaired quality of life due to intractable encephalopathy, those rare cases of refractory ascites where the MELD is less than 15 and shunting is either ineffective, technically not possible or otherwise contraindicated [4]. Other factors that suggest the need for transplantation include developing hepatopulmonary syndrome, portopulmonary hypertension, progressive muscle wasting, and progressive unresponsive hepatic osteodystrophy.

There needs to be caution with children, where the course of AIH may be different to that seen in adults [5]: in particular, in some an initial syndrome, identical to classical AIH, may evolve into classical primary sclerosing cholangitis. Most patients transplanted for primary sclerosing cholangitis will have a Roux-en-y hepatic anastomosis because of the need to avoid doing a bile duct anastomosis with diseased tissue and the potential for development of cholangiocarcinoma in the native biliary tree, either before or after transplantation; hence it is important to determine the state of the biliary tree prior to transplant.

Hepatocellular Carcinoma

Patients with HCC pose additional challenges: the recipient has two life-threatening diseases (the parenchymal liver disease and the cancer). The MELD score will reflect prognosis from the former but not the latter. An allocation system based on prognosis (as death on the list or progression of the cancer so a transplant becomes futile) must therefore reflect these changes and balance the needs of these patients with those who have parenchymal disease.

In recent years, the range of treatment options for those with liver cell cancer (HCC) has increased, with successful pharmacological therapy (such as sorafenib [6]), loco-regional such as radiofrequency ablation (RFA) or percutaneous ethanol injection (PEI), trans-arterial chemo-embolisation (TACE) and resection being shown to be effective [7]. There remains no clear consensus as to the optimal treatment for the individual with HCC.

One concern for those with HCC is the need to identify those in whom recurrence of the cancer will preclude a successful long-term outcome. The impact of surgery and most immunosuppressive agents will enhance tumour growth. Thus, if there is tumour outside the liver, then transplantation may be futile. Clearly, detectable tumour extension beyond the liver will preclude transplantation. Several studies have suggested that tumour size and number may be a good surrogate for predicting the likelihood of tumour recurrence. However, it is likely that such a crude measure will be replaced in time by more sensitive predictive markers: the absolute level and rate of rise of α-fetoprotein, the rate of growth of tumour, and the molecular signature of the HCC may all prove to be superior but none yet has been robustly validated.

The introduction of the Milan criteria, where the likelihood of recurrence was determined by the size and number of lesions, allowed greater use of transplantation for this indication [8]. However, it became clear that some patients with HCC that fell outside the Milan criteria would have an excellent prognosis after transplant

so these criteria were too restrictive and those with HCC were not treated equitably. Extension of the Milan criteria has been advocated by some and early outcomes are not dissimilar from those within the Milan criteria [9]. Revision (the Metro-ticket approach) has further refined selection of those who might benefit from transplantation and justify the use of a scarce resource [10]. The extent to which the newer immunosuppressive agents, such as sirolimus [11], may inhibit the recurrence rate is uncertain and is likely further to modify the role of transplantation in this group.

A reasonable approach is, for those with good liver function is, to consider first RFA or even resection and offer transplantation where this approach is not possible or likely to be ineffective. Some centres use resection as a first-line treatment where possible, and reserve salvage transplantation where recurrence or decompensation occurs.

In the North American model, at present those with a documented HCC, greater than 2 cm in diameter, will be given additional MELD points to ensure that the revised MELD score reflects survival to the stage where transplantation is futile from the cancer rather than the underlying liver disease. This has resulted in many more patients being grafted for HCC: whether this will adversely affect long-term outcomes is not clear but early data show little effect.

Criteria for living donor liver transplantation: The shortage of livers has resulted in the development of new surgical techniques, including splitting livers, greater use of extended criteria donor livers living donation. For most centres, the indications for transplantation from living donors are similar to those from deceased and non-heart-beating donors.

Fulminant AIH

In those with fulminant liver failure due to AIH, the Kings College Hospital Criteria are widely utilised to identify those patients in whom the chance of survival with full medical support although these criteria were not developed for those with fulminant AIH, therefore may not be applicable in this situation [12]. As with patients with fulminant hepatic failure from other aetiologies, adverse serological happy, the development of cerebral oedema and cardiovascular instability should alert the clinician to the possible need for transplantation; contraindications include active sepsis, severe cardio-respiratory instability, and significant extra-hepatic disease.

The criteria used in the UK for consideration of liver transplantation in this situation include either prothrombin time >100 s *or* INR >6.5, and any grade of encephalopathy or any grade of encephalopathy, *and* any three from the following:

> Age >40 years, jaundice to encephalopathy time >7 days, serum bilirubin >300 μmol/l, or prothrombin time >50 s or INR >3.5.

Whether specific medical therapy with corticosteroids affects the natural history and reduces the need for liver transplantation remains controversial. A recent study,

where corticosteroid therapy using prednisolone at a dose of 1 mg/kg/day in 12 patients with type 1 fulminant liver failure, showed that steroids did not obviate the need for liver replacement [13] but did highlight the risk of septic complications associated with high-dose steroids, with five patients developing sepsis, which, in one case was fatal. The response to steroids varied from 36 to 100%, although the severity of the disease was not clearly defined [14–20]. Villamil [20] identified adverse prognostic criteria as: prothrombin time (PT) less than 20%, encephalopathy on admission, massive or sub-massive necrosis on histology, type II AIH, or 20% increase in PT at day 3 post-steroid therapy.

Contraindications to Liver Transplantation

There are relatively few contraindications for liver transplantation. These can be classified as those factors that make the procedure not technically possible or too high risk factors that make the survival after transplantation limited.

Technical factors that make transplantation futile include extensive vascular thrombosis, advanced pulmonary or cardiovascular disease that mean the patient would not survive the procedure. Previous upper abdominal surgery will add to the risks of the procedure but may not preclude it. The presence of active cancer and active bacterial and mycobacterial infection also contraindicate transplantation. HIV infection may not be a contraindication for transplantation but many units consider active AIDS an absolute contraindication. Severe hyponatremia (<120 mmol/L) is associated with the risk of central pontine myelinolysis and so should be corrected prior to surgery. Renal impairment is not a contraindication to transplantation but is associated with increased post-transplant poor survival.

A previous history of malignancy may contraindicate surgery: colorectal cancer, melanoma, thyroid carcinoma, oral squamous carcinoma, breast cancer, and vulvovaginal cancers are associated with more than 10% probability of tumour recurrence and so may contraindicate surgery but every case needs individual consideration.

The presence of hepatopulmonary syndrome and porto-pulmonary hypertension may be contraindications for the procedure but, as indicated above and if not too advanced, may be indications. Porto-pulmonary hypertension is defined as a mean pulmonary artery pressure of more than 15 mm Hg (normal pulmonary artery occlusion pressure [<15 mm Hg]) and pulmonary vascular resistance of more than 250 dyn.s.cm^{-5}. However, a mean pulmonary artery pressure greater than 40 mm Hg classifies those at high risk as does a pre-transplant partial pressure of oxygen of less than 50 mm Hg and a macroaggregated albumin lung scan showing more than 20% brain uptake in those with hepatopulmonary syndrome.

Post-transplant, any factor that would prevent the recipient having a reasonable expectation of life or quality of life acceptable to the patient would also contraindicate the procedure. Alcohol abuse, illicit drug use, noncompliance with medications, and active smoking may be contraindications but will depend on the individual. The key question is whether the recipient will comply with the medication and need

for life-long follow-up and will not indulge in behaviour that will damage the graft. An agreed support package of care may be required before transplantation can be considered.

Age itself is not an absolute contraindication: older patients do fare less well after transplantation than younger ones (as with any surgical procedure), but there is no absolute bar. Clearly, those aged over 60 years will require closer evaluation to look for cardiovascular and pulmonary disease and malignancy.

Outcome of Patients Following Liver Transplantation for AIH

Liver transplantation for AIH is associated with 5-year patient survival between 80 and 90% [21, 22]. The quality of life after transplantation is usually excellent, although only half are able to return to full-time employment.

The optimal immunosuppression remains uncertain. As discussed below, patients grafted for AIH are at greater risk of developing acute cellular and possibly ductopenic rejection. We found severe acute rejection occurred in 61% of those grafted for AIH compared with 42% for those grafted for alcohol-related liver disease [23]. Reasons for this increased susceptibility to rejection are not clear although it should be noted that those grafted for other indications with a presumed autoimmune aetiology (such as primary biliary cirrhosis and primary sclerosing cholangitis) have a rejection rate similar to that seen in AIH.

The mainstay of immunosuppression includes a calcineurin inhibitor (usually tacrolimus), either alone of with an anti-metabolite (mycophenolate or azathioprine). Whether corticosteroids should be used with other agents remains controversial. Our own practice is to use a calcineurin inhibitor in combination with low-dose steroids (such as prednisolone 5–7.5 mg/day given with bone protection therapy) maintained long-term.

Recurrent AIH (rAIH)

Since the first report in 1984 [24], recurrent AIH is well recognised with [1, 22, 25–34] recurrence rates of 20–30%. (Table 9.1) However, serological and histologic features of AIH can also occur in those grafted for other conditions; this is termed "de novo AIH" or, more accurately, graft dysfunction mimicking autoimmune hepatitis [35]. A more appropriate term, therefore, may be alloimmune hepatitis.

Because of a lack of consensus about diagnostic criteria and difficulties in interpreting the nonspecific histological findings, the diagnosis can be challenging. As protocol biopsies are not routinely done, the incidence of recurrent AIH may be under-reported.

Table 9.1 Published series of recurrent autoimmune hepatitis

Study	No. of patients transplanted for AIH	No. with recurrence	Autoantibodies				Therapy at recurrence	Outcome
			ANA	ASMA	LKM	IgG		
Wright, 1992 [65]	43	11	6	8	–	Elevated	C	
Bimbaum, 1997 [48]	6	5	4	4	–	Elevated	C (4), T(4)	2nd OLT (2), PTLD (1)
Prados, 1998 [39]	27	9	17	11	7	n/a	C (26), T(1)	Resolution
Ratzui, 1999 [31]	15	3	15	21	5	2.6	C, + prednisolone/ azathioprine	2nd OLT (1), cirrhosis and death (1), no change (1)
Narumi, 1999 [66]	40	5	–	–	–	n/a	T or C. Prednisolone (1)	No graft loss
Milkiewicz, 1999 [30]	47	13	–	–	–	16.5	–	2nd OLT (3)
Reich, 2000 [49]	32	6	23	24		3	T	2nd OLT (3)
Ayata, 2000 [25]	12	5	6	6	1	Elevated	T (3), T & C (2), + azathioprine/ prednisolone	Cirrhosis (2), Chronic rejection (2)
Gonzalez-Koch, 2001 [29]	41	7	–	–	–	–	T (4), C (3), + azathio-prine/prednisolone	Lymphoma (2), 2nd OLT (1)
Molmenti, 2002 [22]	55	11	–	–	–	–	C (82% or T (18%), + prednisolone	Lymphoma (1), no graft loss
Yusaff, 2002 [67]	12	2	–	–	–	–		
Heffron, 2002 [42]	52	9	–	–	–	–	Prednisolone (6), no further data	

Duclos-Vallee, 2003 [28]	17		7	9	14	4	23.2	C, Prednisolone, Azathioprine	2nd OLT (2)
Vogel, 2004 [33]	28		9	24	14	16	6	T	
Renz, 2002 [32]	37		12	–	–	–	–	C	Cirrhosis (1)
Khalaf, 2007 [41]	16		3	–	–	–	–	Steroids	Graft failure (1)
Rowe, 2008 [1]	103		28%	–	–	–	–	Steroids (last 5 years of study)	6.2% graft loss
Campsen, 2008 [27]	66		23	–	–	–	–	C (26%), T (64%), prednisolone (50%), azathioprine/MMF (28%)	No graft loss
Montanoi-Loza [43]	46		11						

Key: *T* Tacrolimus, *C* Cyclosporine, *ANA* anti-nuclear antibodies, *ASM* anti-smooth muscle antibody, *LKM* liver kidney microsomal antibody, *IgG* immunoglobulin G, *OLT* orthotopic liver transplant, *PTLD* post-transplant lymphoproliferative disease, *AIH* autoimmune hepatitis

Table 9.2 Criteria for the diagnosis of recurrent autoimmune hepatitis

Liver transplant for AIH
Autoantibodies in significant titre (>1:40)
Sustained rise in serum aminotransferase activity (> twice normal)
Elevated serum immunoglobulins (especially IgG)
Compatible liver histology, i.e. chronic inflammatory cell infiltrate consisting of • Plasma cells • Interface hepatitis (piece meal necrosis) • Bridging necrosis and fibrosis
Corticosteroid dependency
Exclusion of other causes of graft dysfunction (e.g. rejection, HCV infection)

HCV Hepatitis C virus, *AIH* autoimmune hepatitis

Diagnosis

Criteria for the diagnosis of rAIH have been proposed (Table 9.2) [36]. Diagnostic criteria for AIH in the native liver should not be directly applied to the liver allograft recipient: the liver recipient is usually taking immunosuppressive agents, there is usually a different HLA (human leukocyte antigen) and other antigenic environment and there are many other causes of potential graft damage which, of course, may co-exist with rAIH.

Organ nonspecific autoantibodies can be present in low titre post-transplant, and histological features of graft inflammation do not always correlate with the presence of antibodies [37]. Histological abnormalities can precede changes in biochemical and immunological tests [28]. Raised transaminases do not correlate with chronic hepatitis in children following transplantation [38]; biochemical improvement does not always correlate with histological remission [39]. Assessing response to treatment of recurrent disease is best served by liver biopsy, since liver tests do not correlate with liver histology and significant histological inflammation can be present with normal biochemistry.

The use of routine protocol liver biopsies is controversial, and the risks of biopsy must be balanced with the potential benefits. While liver biopsy is associated with a small risk (and is very rarely fatal), over-immunosuppression is associated with an increased risk of sepsis, renal failure, and some malignancies. Interpretation of graft histology can be challenging due to the need to exclude rejection, although there are some typical features in both as detailed in Table 9.3 [37]. One of the earliest findings is that of lobular lymphoplasmacytic hepatitis with acidophil bodies [25]. Although it would seem likely that early diagnosis and modification of immunosuppression is desirable, this does not always prevent graft loss.

Factors Associated with Recurrence

Published series have resulted in conflicting conclusions as to the risk factors for rAIH: Factors associated with recurrence include the type of immunosuppression,

Table 9.3 Histological differences between recurrent AIH and rejection

	Recurrent AIH	Rejection
Portal and periportal changes		
Portal inflammation	Mononuclear cells (plasma cells ++)	Mixed infiltrate (lymphocytes, macrophages, blast cells, neutrophils, eosinophils)
Interface hepatitis	Variable (often prominent)	Mild
Bile duct inflammation	Mild (lymphocytes)	Prominent (mixed infiltrate)
Bile duct loss	Minimal/none	Variable (may progress to chronic rejection)
Venous endothelial inflammation	None/mild	Yes
Fibrosis	Yes	No
Parenchymal changes		
Parenchymal inflammation	Variable	Generally mild
Composition	Mononuclear (mainly plasma cells)	Mixed (mainly lymphocytes)
Pattern	Spotty or confluent	Confluent
Distribution	Random or zonal	Zonal (acinar zone 3)
Associated features	Lobular disarray	Hepatic vein endothelial inflammation
Cholestasis	Rare	Common

HLA status of donor and/or recipient, severity and type of AIH in the recipient, and the length of follow-up. Weaning of corticosteroids may be associated with a high rate of recurrence [40]. One recent study reported that attempts at complete steroid withdrawal 1 year following live donor liver transplantation were unsuccessful [41]. Others have attempted alternative immunosuppression using mycophenolate [40]. However, data are conflicting [22, 29, 41, 42] and further prospective studies are needed.

Greater necro-inflammatory activity in the recipient liver prior to transplantation is associated with rAIH [25, 43]. These patients may also be less responsive to immunosuppression post-transplantation, although the fact that patients with a fulminant course can have very good outcomes may be evidence against this argument. Indeed, one small study suggested that recurrence is less likely in patients transplanted for fulminant AIH [41].

The length of follow-up is important in assessing the probability of rAIH. The rate of recurrence has been quoted at 8% at 1 year and up to 68% after 5 years [39]. Studies following up patients over a longer period have revealed that the risk of recurrent disease persists even over 10 years post-transplantation [28]. This late recurrence may be related to reduction in immunosuppression over time. Whether the use of current immunosuppression has an impact on long-term risk remains to be seen, although the evidence so far is that the choice of calcineurin inhibitor does not appear to be risk factor for recurrence [34].

There is conflicting evidence on the role of HLA phenotype on the risk of recurrence. Some studies have noted an association between HLA-DR3+ve recipient/HLA-DR3−ve graft and recurrent disease [41, 44]. Others have demonstrated a link between

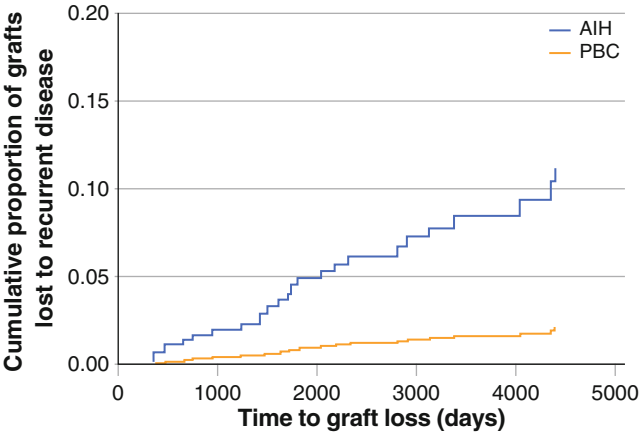

Fig. 9.1 Proportion of patients transplanted for AIH and PBC in Birmingham UK who develop recurrent disease in the graft after OLT

recipient HLA-DR3 positivity and recurrent disease, although HLA phenotype mismatch between donor and recipient was not confirmed [29]. There are other studies which have failed to demonstrate a link with the HLA-DR3 phenotype [22, 45].

Management of Recurrent Disease and Outcomes

Successful management relies on early detection, and, as mentioned above, there may be a role for protocol biopsies. In the majority of cases, increased immunosuppression is successful in controlling progression, although early reports where over half of patients failed to respond to treatment [39]. Therapy usually involved increased doses of steroids and maintaining patients on steroids [1, 25, 28–30, 32, 41], although in some cases azathioprine was introduced [22, 39] and patients were switched from cyclosporine to tacrolimus [22].

Tacrolimus has been used successfully as a salvage therapy for lack of response to steroids, azathioprine, and cyclosporine [46]. Others have used other agents such as cyclosphosphamide [25, 47]. The roles of rituximab, mycophenolate, and sirolimus have not been well studied in this context [40, 47], although anecdotal reports of benefit have appeared. Additional immunosuppression has to be balanced against the risk of adverse events, which includes fatal post-transplant lymphoproliferative disorder [22, 29].

The outcome in patients with recurrent disease in terms of graft and patient survival does not appear to be significantly worse than patients without disease recurrence, with 5-year survival is around 80% [22, 29, 33, 48, 49]. Comparison of outcomes of recurrent disease following transplantation for AIH with other indications [1] showed that when compared with recurrent primary biliary cirrhosis, there was a 4.1 times increased risk of graft loss (>90 days post-transplantation) (Fig. 9.1).

Recurrent disease can have an aggressive course unresponsive to immunosuppression, resulting in the need for re-grafting or death [48, 49] and patients who develop significant fibrosis may deteriorate despite immunosuppression [32]. This emphasises the need for early detection and treatment of these patients, and perhaps supports the argument for protocol biopsies.

De Novo AIH

The development of the clinical, serological, and histological features of autoimmune hepatitis in patients transplanted for other aetiologies was initially described in a paediatric population in 1998 [35]. There have been numerous reports since then with a predominance of paediatric patients, although adult patients also appear to be at risk [50, 51]. The condition usually presents between 2 and 10 years after transplant. The typical clinical, serological, and histological features of AIH are seen with elevated immunoglobulins, autoantibodies, and histological features of portal inflammation and interface hepatitis. Some have reported cases presenting predominantly with central peri-venulitis prior to the development of typical portal inflammation [52, 53]. There has also been a report of concurrent recurrent primary biliary cirrhosis (PBC) and de novo AIH [54].

The exact pathogenesis is not clear, and further work is necessary in this regard. It has been suggested that calcineurin inhibitors may interfere with the maturation of T cells and the function of regulatory T cells as has been demonstrated in animal studies [55]. The predominance of de novo AIH in children may be due to calcineurin inhibitors causing thymic dysfunction [56]. It is interesting that the calcineurin inhibitors, in particular tacrolimus, have been implicated in earlier and more aggressive recurrence of PBC, another disorder with a presumed autoimmune basis [57] although this observation has not been supported by others [58].

De novo AIH may represent a form of late cellular rejection, since antibodies are directed against the graft and not self, i.e. alloimmune response. Some support for this theory comes from studies where an association between de novo AIH and previous episodes of acute cellular rejection was noted. Further evidence comes from studies where patients negative for glutathione-S-transferase T1 (GSST1) antibodies were transplanted grafts positive for GSST1 subsequently developed antibodies to GSST1 [50, 59]. This observation requires further validation.

De novo AIH generally responds to modification of immunosuppression, although there are studies reporting poor outcome in certain groups of patients. Gupta and colleagues described a series with an atypical histological feature of ductal proliferation [60]. Most of the patients developed progressive fibrosis. These patients may perhaps have a form of chronic rejection. In another series of patients who had live donor liver transplantation, cirrhosis was noted in half the cases with remission of interface hepatitis in only one patient [61]. It is noteworthy that azathioprine was not used. The combination of azathioprine and steroids appears to be the key to successful therapy. Azathioprine has also successfully treated patients

who did not respond to high-dose steroids or changes in the dose of cyclosporin or tacrolimus therapy [62]. Similar findings were noted by Andries and colleagues [63], where one patient also responded to treatment with mycophenolate mofetil therapy after relapse following withdrawal of azathioprine. The lack of effect of calcineurin inhibitor was also demonstrated in a study where cyclosporine was withdrawn, and patients subsequently responded to azathioprine and steroid therapy [64]. The importance of maintenance therapy with steroid therapy was shown in a study comparing treatment with and without corticosteroids [52]. Patients on steroids did well, and all patients treated only with cyclosporine and azathioprine developed cirrhosis of the graft. Steroids were also effective in treating patients who relapsed.

Conclusion

Autoimmune hepatitis affects a diverse group of patients, and medical management is largely successful in controlling progression and prolonging survival. Alternative agents such as MMF have yet to gain universal acceptance. The indications for liver transplantation do not differ greatly from other aetiologies of chronic liver disease. The utility of MELD >16 as an indication for selection to the waiting list has been widely adopted. A further refinement with the introduction of UKELD is likely to lead to improved patient selection. Special consideration is necessary for patients with other indications such as HCC, refractory ascites not amenable to nonsurgical therapies, and intractable hepatic encephalopathy. These conditions may merit listing despite not meeting MELD criteria. Fulminant hepatic failure associated with AIH can have a poor outcome despite aggressive immunosuppression, and an early decision for transplant listing is paramount.

rAIH occurs in one-third of patients. There are no agreed diagnostic criteria, and differentiation with chronic rejection can be particularly challenging. Proposed risk factors for recurrence include level of immunosuppression HLA status, length of time following transplant, and severity of disease in the recipient explant. The lack of correlation between histology and biochemical abnormalities has led to some experts advocating routine protocol biopsies to aid early diagnosis and assessment of treatment response. Treatment involves increasing immunosuppression, and is largely successful. The outcome of rAIH is favourable, with most studies demonstrating no difference when compared with patients without rAIH. Optimisation of immunosuppression appears to have resulted in a trend toward a decline in graft loss over the years. While early detection and treatment are important, caution is necessary to minimise over-immunosuppression, which has been implicated in the development of fatal malignancies. De-novo AIH is a recent entity, where there are features of AIH in the graft of patients transplanted for other causes. CNIs may be involved in the pathogenesis, and optimal immunosuppression with steroids and anti-metabolites appears to be effective.

Chapter Summary

1. Liver transplantation is indicated in patients with AIH with end-stage disease (as evidenced by a MELD >16), liver cell cancer, intractable symptoms (such as encephalopathy), or variant syndromes such as hepatopulmonary syndrome.
2. Results after transplantation are usually excellent with 5-year survival over 70%.
3. Recurrent disease, which may develop in the presence of normal liver tests, may be present in 20–30% and may progress to end-stage graft failure, despite increased immunosuppression.
4. Long-term steroids should be considered as part of immunosuppressive regime as this may prevent recurrence and reduce the risk of graft loss.

Useful Tips for Practitioners

1. Referral for transplantation should be considered when the MELD score approaches 15 or HCC is detected.
2. Time on the transplant list should be used to optimise the patient's health, with special attention to nutrition, vaccination, and maintaining bone health.
3. Post-transplant, patients should remain under follow-up, with surveillance for recurrent disease; autoantibodies and immunoglobulins should be routinely measured as these may be abnormal while liver tests are normal.
4. Addition of long-term steroids to the immunosuppressive regime to reduce the risk and possibly the impact of recurrence.

Common Pitfalls in Practice

1. It is still assumed, wrongly, that liver cell cancer does not occur in patients with AIH: those with cirrhosis should be in a surveillance programme and, if HCC is detected, transplantation considered.
2. In fulminant AIH, a trial of high-dose steroids should be used with caution as steroids are often ineffective in this situation, increase the risk of sepsis, and delay consideration of transplantation too long.
3. Mis-timing of referral to a transplant unit: referring too early does not harm the patient but referral too late may prejudice the outcome.
4. Close follow-up is required to detect and treat recurrent disease: normal tests do not preclude recurrence of disease in the graft.

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Chapter 10
Autoimmune Hepatitis and Pregnancy

Rachel H. Westbrook and Michael A. Heneghan

Keywords Contraception • Fertility • Fetal health • Maternal health • Pregnancy

Introduction

Autoimmune hepatitis (AIH) is a condition that classically affects women of childbearing age [1, 2]. In such patients, the desire to have a family frequently raises questions around fertility and contraception in addition to the risks and outcomes of pregnancy. This chapter summarises the current literature regarding women's health in AIH. Information regarding the complications, outcomes and best practice management in AIH and pregnancy is sparse and evidence is delivered predominantly from reported case series. We discuss in detail the current evidence regarding best practice during pregnancy with an emphasis placed both on maternal and fetal health.

Background

AIH was first described in 1950 by Waldenström, when he reported a chronic form of hepatitis which had a propensity to affect young women [3]. The classical phenotype described was that of a young women with jaundice and extrahepatic manifestations including arthralgia, endocrine abnormalities and amennorhoea [3].

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While reports regarding pregnancy in patients with AIH exist from as early as the 1970s, these outcomes were largely unfavourable with a high incidence of obstetric complications including early fetal loss, prematurity, low birth weight and a high rate of caesarean section [4, 5]. Maternal complications included pre-eclampsia, flares in disease activity, hepatic decompensation and death [4, 5]. These unfavourable reports were published prior to the discovery of the hepatitis C virus. Thus, the sensitivity and specificity of the diagnosis of AIH in these studies is likely to be poor. Furthermore, these studies also lacked pertinent information regarding the severity of the underlying liver disease, therapy during pregnancy and extent of disease control achieved prior to conception. Recent case series of AIH in pregnancy report much more favourable outcomes (Table 10.1) [6–9].

Impact of Pregnancy on Disease Activity

Pregnancy impacts upon the activity of AIH. Flares in AIH activity during pregnancy and in the post-partum period have been described, in addition to index presentations of disease. Remission of AIH in association with pregnancy has also been described [6–12]. Interestingly, of those patients that have a flare associated with pregnancy the majority occur in the post-partum period with relatively few flares occurring during the pregnancy itself [6–9]. Understanding why some patients have an exacerbation during pregnancy whilst others maintain remission only to relapse post-partum remains unclear.

The phenomenon of an improvement in disease activity during pregnancy, followed by a flare in the post-partum period is not limited to AIH but has been reported in other autoimmune conditions. In patients with rheumatoid arthritis, pregnancy is associated with an improvement of symptoms in 75%, but relapses occur in up to 90% during the post-partum period [13]. The mechanism for this phenomenon in autoimmune conditions is incompletely understood. It is likely, in-part related to the fact that pregnancy induces the temporary development of immunological tolerance in order to allow the mother to tolerate the antigens expressed from the father by the foetus. Regulatory T-cells are required for the maternal immune system to tolerate the fetal allograft, and there is an increase in their circulating number during pregnancy [14, 15]. The up-regulation of T-cells during pregnancy is thought to be hormonally driven. Changes in the production of cortisol, estrogen and progesterone during pregnancy modulate cellular and humoral immune functions including the cytokine profile [16]. High concentrations of estrogens are thought to inhibit immune activities whilst progesterone promotes T helper 2 cells and in itself has anti-inflammatory properties [8, 16].

Evidence published regarding the aetiopathogenisis of AIH has demonstrated that it is an impairment in regulatory T-cells that is key to the loss of immune tolerance in AIH and thus the emergence of uncontrolled effector autoimmune responses [17, 18]. Taking into account the above factors, it becomes clearer why patients with AIH, an indeed other autoimmune conditions, may induce remission during

Table 10.1 Potential complications encountered in pregnancy and AIH

	Total number of conceptions	Incidence of post-partum flares	Flare during gestational period	Maternal deaths associated with pregnancy	Hepatic decompensation	Live birth rate	Prematurity
Heneghan et al. [6]	35	4/35 (11%)	4/35 (11%)	2	2	30 (86%)	2/30 (7%)
Schramm et al. [7]	42	22/42 (52%)	9/42 (21%)	1	1	35 (83%)	7/35 (20%)
Terrabuio et al. [9]	51	23/51 (45%)	4/51 (8%)	0	0	36 (71%)	6/36 (17%)
Buchel et al. [8]	14	12/14 (86%)	1/14 (7%)	0	0	13 (93%)	1/14 (8%)

pregnancy and then flare in the post-partum period, such that when pregnancy ends, tolerance breaks down and flares in disease activity occur. Although this hypothesis has never been scientifically proven, it is attractive on many levels.

Exacerbation of AIH in Pregnancy

An exacerbation of AIH associated with pregnancy, as manifested by a rise in the aminotransferase activity, immunoglobulin level and/or the recurrence of symptoms is common. The four largest case series in the literature provide the most useful insight into incidence of disease flares associated with pregnancy along with information regarding their severity and impact on maternal health (Table 10.2) [6–9]. Discrepancies exist between studies regarding what constituted a "flare" in disease activity. Both Heneghan et al. and Schramm at al. define a flare as a twofold increase in serum aspartate aminotransferase (AST) activity above the upper limit of normal or a lesser increase in AST in conjunction with an increase in serum globulin level. Terrabuio et al. describe a flare in AST activity of less than twice the upper limit of normal and a relapse as an elevation of AST above twice the upper limit of normal. Finally, Buchel et al. did not provide a standard definition but provided a descriptive report on each patient.

Flares in AIH during pregnancy have been reported to occur in 7–21% of patients [6–9]. In patients that do experience a flare, AIH activity was easily controlled in the majority of cases with augmentation of baseline immunosuppression, usually in the form of prednisolone. Reported augmentation dosages to treat a flare of AIH during pregnancy vary between 10 and 30 mg of prednisolone. The clinical significance of disease flares appeared minor, with the majority of patients responding to medication

Table 10.2 Maternal and fetal complication associated with pregnancy and AIH

	Non-cirrhotic	Cirrhotic
Maternal complications	Disease flares Decompensation Transplantation if sub-acute liver failure develops Death	Disease flares Sepsis Decompensation Variceal bleeding Encephalopathy Splenic artery aneurysm formation and rupture Bleeding during labour Transplantation Death
Fetal complications	Increased rate of spontaneous pregnancy loss Prematurity Low birth weight Congenital malformations	Increased rate of spontaneous pregnancy loss Prematurity Low birth weight Congenital malformations

changes. However, in a small subset of patients, a flare in AIH during pregnancy led to hepatic decompensation with the most serious consequence being death of the patient and/or foetus. Combining data from the largest four series published in the literature provides a total of 142 pregnancies in women with AIH [6–9]. Overall, a total of three maternal deaths were reported (2%), one of which was directly liver related to uncontrollable variceal haemorrhage in the post-partum period. The aetiology of the other two deaths were non-liver related and included a fatal pulmonary embolus and multi-organ failure following a septic abortion. Hepatic decompensation following a flare occurred in three additional patients, one of whom subsequently required transplantation.

Post-partum flares are common in AIH with reports of flares occurring in between 11 and 86% of patients [6–9]. Due to the relatively high post-partum flare rates, some centres advocate for the routine use of steroid augmentation after delivery. This would typically involve the commencement or augmentation of prednisolone to 20 mg soon after delivery and tapering after 3 months [8]. Other centres undertake regular monitoring of the liver function tests during the post-partum period and only augment immunosupression in those that have a true flare, thus not subjecting all patients to increased corticosteroids [6]. Terrabuio et al. routinely treated all patients with steroid augmentation post-partum. Despite this the post-partum flare rate was still 45% [9]. Interestingly, this is not significantly different to rates reported by Heneghan et al. (18%) and Schramm et al. (55%), who did not routinely augment immunosuppression post-partum. Moreover, in the study by Terrabuio et al. azathioprine dosing was reduced or discontinued in pregnancy and this in itself is likely to have contributed to the high post-partum flare rate [9]. Thus, no convincing evidence exists to support the routine augmentation of immunosuppression medication in the post-partum period. Patients should be monitored closely and have prednisolone augmentation if a flare in disease activity occurs (Fig. 10.1).

Index Presentation of AIH in Pregnancy

AIH can present as a new diagnosis in pregnancy or in the post-partum period [6, 7, 11, 19, 20]. This is important to recognise as AIH must form part of the differential diagnosis in any pregnant women who presents with jaundice or abnormal liver enzymes. The differential diagnosis includes viral infections (Hepatitis A, B, C and E viruses, Herpes simplex virus, Cytomegalovirus and Epstein Barr Virus), thrombotic complications such as Budd-Chiari Syndrome, drug reactions and liver diseases specific to the pregnant state including cholestasis of pregnancy, hypertension-related disorders and acute fatty liver of pregnancy [21, 22]. In the literature, adverse outcomes have been reported with AIH arising de novo in pregnancy. This is in part due to the delay in diagnosis and subsequent delay in commencement of potentially life-saving treatment. Schramm et al. describe one woman whose first

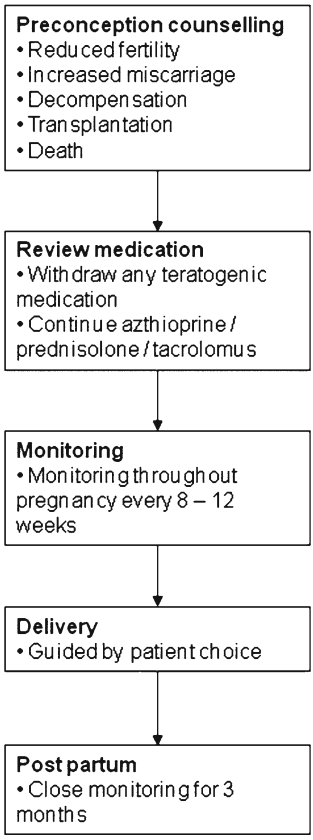

Fig. 10.1 Algorithm for management of pregnancy in women with AIH without cirrhosis

presentation was in the second trimester of her second pregnancy, but remained undiagnosed until she presented with fulminant hepatic failure in the second trimester of her third pregnancy. Emergency liver transplantation and hysterectomy was performed at 18 weeks gestation. The patient survived and remains well 6 years after transplantation [7]. Heneghan et al. described two women with index presentations of AIH during pregnancy [6]; the first presented with hepatic decompensation on a background of established cirrhosis at 24 weeks gestation. She was admitted to intensive care, and an emergency caesarean section was performed; however, the child has serious physical and mental developmental difficulties since birth. The second patient presented with pre-eclampsia at 16 weeks gestation and was noted to have abnormal hepatic biochemistry. The diagnosis was made based on the serological tests, autoantibodies and liver biochemistry. She was commenced on immunosuppression and subsequently delivered a healthy infant at 37 weeks gestation.

Cirrhosis and Pregnancy in Patients AIH

A subset of patients with AIH are cirrhotic at the time of conception. Pregnancy in patients with AIH and underlying cirrhosis, although rare, does occur and carries with it additional maternal risks and adverse fetal outcomes. It is recognised that women with cirrhosis who become pregnant are at risk of worsening liver synthetic function and hepatic decompensation including the development of ascites and encephalopathy [21, 23]. They also are at increased risk of developing varices as portal pressure rises in the second trimester of pregnancy due to a combination of the gravid uterus compressing the inferior vena cava and an increase in blood volume and flow [24, 25]. In previous small series, variceal haemorrhage has been reported to occur in 18–32% of pregnant women with cirrhosis and in up to 50% in those with pre-existing portal hypertension [25, 26]. Moreover, decompensation has been reported to affect up to 24% of pregnant women with cirrhosis and often follows an episode of variceal haemorrhage [24].

The optimal management of portal hypertension during pregnancy remains challenging with the absolute need for variceal screening, primary prophylaxis against bleeding and the management of a variceal haemorrhage during pregnancy is largely undefined. Management is based on best guess experience extrapolated from the non-pregnant literature. Currently, it is recommended by experts that once pregnant, women with cirrhosis should have a screening endoscopy in the second trimester. Previous studies have reported the prevalence of varices in the second trimester to be in excess of 50% [27]. In a patient with "at risk" for bleeding oesophageal varices, endoscopic band ligation of varices, although not proven, is appropriate. Case reports describing this strategy in pregnancy have been published [28], although no randomised trials have been carried out to prove efficacy. The efficacy of non-selective β(beta) blockers, such as propranolol, during pregnancy on portal pressure and variceal bleeding rates has never been investigated. Their use as a prophylactic agent against variceal bleeding in pregnancy has been extended from trials proving efficacy in the non-pregnant literature [29, 30]. If a patient is established on β(beta) blockers prior to conception, continuation throughout pregnancy is appropriate. Case reports exist in the literature on the commencement of β(beta) blockers following the discovery of varices in the second trimester with no adverse outcomes reported [31]. The decision to commence an individual patient on β(beta) blockers needs to be made on a case-by-case basis. Their safety profile is discussed below.

If varices are confirmed on screening endoscopy, delivery by caesarean section is often recommended. Although not proven, this strategy is thought to avoid the theoretical increased bleeding risk associated with an increase in portal pressure in the context of the valsalva manoeuvre during labour. The optimal timing of when to schedule caesarean section remains challenging and the risks between electively delivering a premature baby and the avoidance of a spontaneous labour must be balanced. In practice, patient management should be individualised and take into account local obstetric and hepatology expertise (Fig. 10.2).

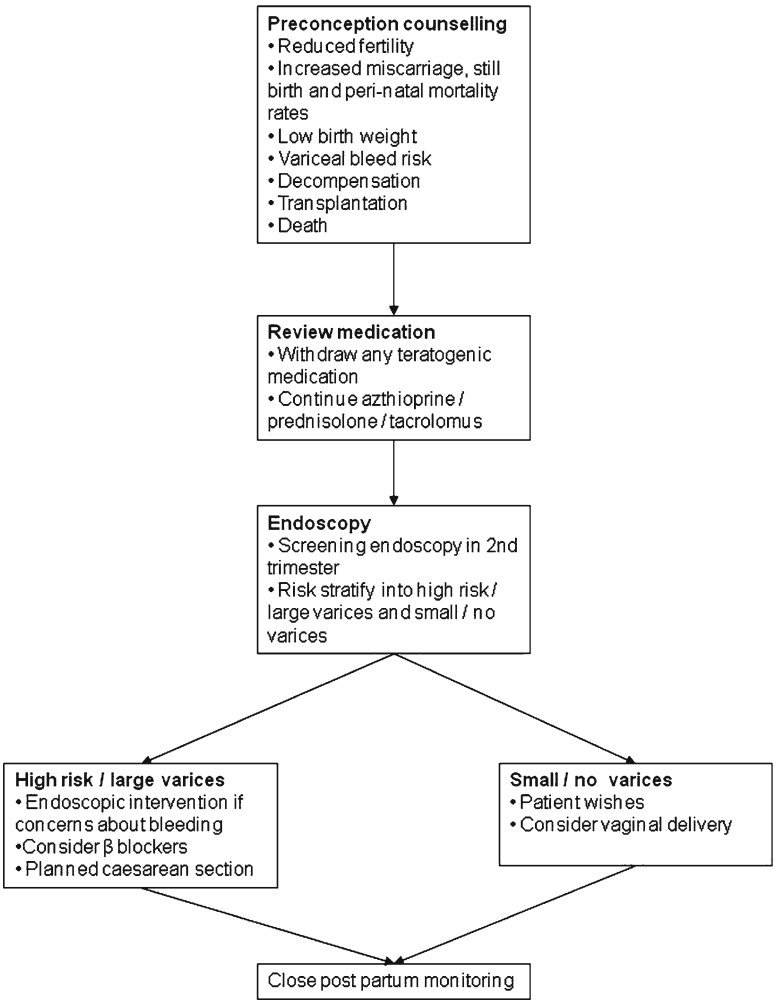

Fig. 10.2 Algorithm for management of pregnancy in women with AIH and underlying cirrhosis

Overall, maternal mortality for pregnant women with cirrhosis was reported to be as high as 10.5% in the early 1980s [32]; however, with advances in the management of liver disease and variceal haemorrhage, mortality is likely to have improved [33]. Despite this, maternal and fetal morbidity and mortality is thought to remain significantly higher than the general population and women with cirrhosis need comprehensive pre-conception counselling if pregnancy is being considered. Pre-conception counselling is challenging since the underlying severity of cirrhosis can vary widely (Table 10.2).

Fetal Outcomes

Fetal Loss

The delivery of a healthy infant can be expected in the majority of patients with AIH that become pregnant, with live birth rates in case series reported to be between 71 and 86% [6–9]. These rates are comparable to live birth rates in patients with other autoimmune conditions, but lower than rates reported for the general population [34]. The cause for increased fetal loss in patients with AIH in unknown and most cases are labelled as spontaneous abortions. In the series by Terrabuio et al., no cause for pregnancy loss could be identified in 87% of pregnancy losses [9]. Furthermore, no association between the activity of the underlying AIH, the maternal immunosuppression or the presence of cirrhosis has been shown to be associated with an increased risk of pregnancy loss in patients with AIH. However, despite these spontaneous losses of pregnancy, other isolated pregnancy losses have been reported secondary to congenital abnormalities, maternal death or transplantation [6, 7].

Affect of Maternal Antibody Profile on Fetal Loss

The effect of maternal antibody profile on outcomes of pregnancy in AIH has been investigated [7]. Investigators reported 11 fetal losses in 42 pregnancies (26%), in whom seven had no identifiable medical cause [7]. Interestingly, they demonstrated that maternal antibodies to soluble liver antigen/liver-pancreas (SLA/LP) ($p=0.003$) and Ro/SSA ($p=0.01$) were associated with an increased risk of fetal loss when compared to women without antibodies. It was postulated that a reason for this loss is the association between anti-Ro/SSA and adverse pregnancy outcomes in other autoimmune conditions, with the losses related to congenital heart conduction abnormalities. However, since pregnancy losses in this cohort all occurred early in the gestational period, heart conduction defects are unlikely in isolation, to explain these fetal losses.

Prematurity, Birth Weight and Outcomes After Delivery

Prematurity, defined as delivery of the foetus at less than 36 weeks gestation is associated with AIH and occurs in approximately 14% of live births [6–9]. Combining 114 live births from the largest reported case series, there were three neonatal deaths following delivery. These deaths occurred in babies that were born at 24, 26 and 32 weeks gestation and all deaths were directly related to prematurity. A further child has severe physical and mental handicap after an emergency caesarean delivery at 28 weeks following an index presentation of AIH during pregnancy.

Fetal birth weights were normal in babies born at term, although low birth weight defined as a weight of less than 2,500 g can be expected in the majority of premature births [6–9].

Congenital abnormalities have been reported sporadically in infants delivered by patients with AIH. Cases vary from urethral stenosis to Edward's syndrome, congenital heart block and anencephaly [6, 7]. The occurrence of a congenital anomaly appears sporadic and no evidence exists to suggest that the occurrence is related to either immunosupression or the underlying AIH. Finally, longer-term follow-up of infants born to mothers with AIH is reassuring with reports that children have normal physical and mental development [6].

Drug Safety

Data regarding the safety of medications commonly used in patients with AIH during pregnancy is scarce. Most of our information is obtained from its use in patients with other disease entities such as inflammatory bowl disease or from post-transplant patients [35]. The common medications used in AIH are discussed with respect to their safety in pregnancy.

Azathioprine

In animal models, azathioprine has been associated with skeletal abnormalities, cleft palate, hydrops fetalis and hemopoetic abnormalities of the foetus [36, 37]. In humans, lymphopenia, hypogammaglobulinaemia and thymic hypoplasia have all been reported in children born to mothers on azathioprine. However, these latter changes seem to all be reversible after birth with no long-term effects on the child. Furthermore, azathioprine has been linked to pre-term deliveries [38], and in light of the above reports, physicians historically recommended patients with AIH to discontinue azathioprine if they were trying to conceive. Azathioprine is classified as a Food and Drug administration (FDA) category as class D, which states that positive evidence of risk to the fetus exists, but it is accepted that as in many medical conditions the potential benefits of its use throughout pregnancy may outweigh the risk (Table 10.3).

Recently, experience with azathioprine in pregnancy has increased dramatically with information derived from other patient populations especially those with inflammatory bowel disease, rheumatoid arthritis or patients following solid organ transplantation [35]. Following favourable reports in other pregnant populations, the number of reported cases in AIH and pregnancy is increasing. In 2001, our group addressed the risk–benefit balance of azathioprine in AIH for the first time [6]. A total of 35 pregnancies were reported, 18 of whom were taking azathioprine in isolation or in conjunction with prednisolone. Three patients had azathioprine withdrawn

Table 10.3 United States of America Food and Drug Administration categories of the safety of drugs in pregnancy

Categories of drug safety in pregnancy
A: Controlled studies show no risk Adequate, well-controlled studies in pregnant women have failed to demonstrate risk to the fetus
B: No evidence of risk in humans Either animal findings show risk (but human findings do not) or, if no adequate human studies have been done, animal findings are negative
C: Risk cannot be ruled out Human studies are lacking and animal studies are either positive for fetal risk or lacking as well. However, potential benefits may justify the potential risk
D: Positive evidence of risk Investigational or post-marketing data show risk to fetus. Nevertheless, potential benefits may outweigh the risk
X: Contraindicated in pregnancy Studies in animals or humans, or investigational or post-marketing reports have shown fetal risk which clearly outweighs any possible benefit to the patient

prior to or on discovery of conception. In this series, little was found to suggest that azathioprine or its metabolites were toxic in pregnancy. Indeed, the two congenital abnormalities reported in this series occurred in women not on azathioprine. It was concluded that for women with AIH that became pregnant on azathioprine, no evidence existed to support reduction or withdrawal of medication. This is especially pertinent in patients where azathioprine is critical for the maintenance of remission. Moreover, in a recent case series from Terrabuio et al., azathioprine was routinely stopped if the pregnancy was planned and withdrawn after conception if the pregnancy was unplanned. Prematurity rates were no different in this study compared to ones where azathioprine was continued and interestingly the incidence of gestational flares were similar to those reported by Heneghan et al. Indeed, rates of postpartum flares were much higher (11 versus 45%) [6, 9].

In light of the above studies, it is generally recommended that azathioprine therapy should be continued during pregnancy, at the same dose used to control disease activity. At present, no evidence exists to suggest that discontinuing the azathioprine for the gestational period is beneficial for the mother or fetal outcomes.

Corticosteroids

Prednisolone can cross the placenta, thus potentially exposing the fetus to adverse effects of the drug [39]. The major fetal risks regarding steroids are cleft palate (particularly with high dose exposure in the first trimester), premature rupture of the membranes and intrauterine growth restriction (IUGR) [40, 41]. Reports of low birth weight and IUGR associated with steroid usage during pregnancy are confounded by the severity of underlying disease necessitating corticosteroid therapy.

In addition, fetal adrenal hypoplasia and suppression of the fetal pituitary have also been described, although both are uncommon due to the rapid maternal metabolism and placental breakdown of corticosteroids. Prednisolone is considered a class B drug in terms of its risk for pregnancy by the FDA. In the AIH literature, no adverse fetal outcomes have been thought to be directly related to prednisolone.

An alternative to prednisolone is the synthetic corticosteroid budesonide. Budesonide has a high first-pass metabolism and in comparison to prednisolone, it has been associated with fewer systemic side effects. In rats, budesonide is associated with fetal loss, decreased birth weight and skeletal abnormalities. Currently there are no adequate studies in pregnant women and it is considered by the FDA as a class C drug.

Overall, it is generally accepted amongst experts that control of the hepatic inflammation is crucial and that risks associated with steroids are outweighed by the benefit of good disease control. Thus, any maintenance prednisolone should be continued throughout pregnancy and flares occurring during pregnancy should be routinely treated with steroid augmentation.

Tacrolimus

In the last decade, tacrolimus has been used routinely in the post-transplant setting and consequently data regarding its safety in pregnancy is emerging. The neonatal malformation rate in reported series is approximately 4% [35, 42, 43]. Tacrolimus is considered a class C drug in terms of its risk in pregnancy by the FDA. No reports of its use or safety with regards to pregnancy, in women with AIH exist to date.

Mycophenolate Mofetil

Mycophenolate mofetil (MMF), an inhibitor of purine biosynthesis, has been shown to cause abnormal development of ova in animal models and therefore is potentially teratogenic [44]. A recent review of data of 119 human pregnancies with maternal exposure to MMF found outcome data for 65 and demonstrated a live birth rate of only 34% with miscarriage occurring in 31% and elective abortion in 20% [45]. The rate of congenital abnormalities, at 15%, was higher than that seen in the general population [46]. The most frequent congenital abnormalities reported included external ear and other facial malformations such as cleft lip and palate. Thus, when women of child-bearing age are commenced on MMF they should be counselled regarding its safety in pregnancy. If they wish to become pregnant, the drug must be discontinued with at least a 6-month wash out period before conception. MMF is considered by the FDA a class D drug in terms of the risks associated with its use in pregnancy.

β(Beta) Blockers

The safety of profile of the non-selective β(beta) blocker propranolol during pregnancy has been established from cohorts with indications other than portal hypertension, including maternal cardiac disease and hyperthyroidism. A number of fetal adverse events have been reported including neonatal hypoglycaemia bradycardia and respiratory depression in addition to unproven concerns regarding intrauterine growth retardation and reduced placental weight. Doses greater than 160 mg/day are associated with the greatest risk. Propranolol is considered by the FDA a class C drug in terms of the risks associated in pregnancy.

Breast Feeding

Most physicians advise against breastfeeding due to concerns over the safety of neonatal exposure to immunosuppressants. Corticosteroids, azathioprine and tacrolimus are all known to be excreted in breast milk. Corticosteroids, however, are excreted in extremely low concentrations and are felt to be safe during breastfeeding [47]. Meanwhile, both azathioprine and tacrolimus levels are excreted in breast milk and in some cases levels are equivalent to, or even exceed that of maternal plasma and are therefore contraindicated [48].

Fertility

Fertility in women with liver disease and AIH is variable. Historically, both Kunkel and Waldenström described a typical patient as being young, predominately female with chronic liver disease and associated hypergammaglobulinaemia [3, 49]. In addition, they described common extra-hepatic features including arthralgia, rashes, fever and amennorhoea [3, 49]. More recent reports have estimated amennorhoea to affect approximately 20% of women of child-bearing age presenting with AIH [1, 2, 50–52].

The pathophysiology regarding amennorhoea and altered fertility in association with AIH is likely to be multifactorial. Firstly, AIH is associated with other endocrine abnormalities including hyperthyroidism, which in itself is widely acknowledged to affect fertility. Secondly, up to 40% of patients with AIH are cirrhotic at presentation [53]. In cirrhotic patients, disruption of the hypothalamic-pituitary axis in conjunction with disturbed estrogen metabolism occurs, leads to infertility [50]. Cundy et al. described two distinct hormonal profiles in women with cirrhosis and amennorhoea [50]. The first suggests a hypothalamically driven process with low serum gonadotrophins, oestradiol and testosterone levels in association with a low body mass index. This profile mimics those seen in patients with

secondary amennorhoea due to anorexia nervosa and suggests that chronic under-nutrition in patients with cirrhosis is likely to impact on fertility. The second is a cohort of patients with normal nutritional and gonadotrophin status. This group has higher oestradiol and testosterone levels mimicking profiles seen in patients with amennorhoea due to polycystic ovarian syndrome. Amennorhoea and infertility driven by underlying cirrhosis is largely irreversible unless liver transplantation is performed. Following transplantation, however, menstruation can recover and several reports and case series of successful pregnancies exist [35, 54].

Finally, in patients with untreated AIH who do not have underlying cirrhosis or additional endocrine abnormalities, amennorhoea can still occur. Its occurrence is thought to be rare, but no population-based studies have reported its true incidence. The exact mechanism for the secondary amennorhoea is unknown. However, once disease control is achieved, menstruation can return allowing the potential for pregnancy to occur.

Contraception

Contraception should probably be discussed with all women with AIH of childbearing age. The choice of contraception ultimately depends upon patient preference, requirement of reversibility and the nature and severity of the underlying liver disease.

Barrier methods such as diaphragms and condoms are safe both with regard to liver function and drug interactions. Condoms also provide protection from potentially harmful infections. Barrier methods should be combined with a spermicide to increase the efficacy, but despite this, failure rates when compared to other methods are reported to be between 15 and 32% [55]. Furthermore, barrier methods are frequently not the preferred choice of contraception for patients in long-term relationships.

The combined oral contraceptive pill (COCP) is the most commonly used contraceptive in many parts of the world. In patients with AIH the same contraindications as in the general population apply. These include a personal history of myocardial infarction, stroke or deep vein thrombosis. Smokers over the age of 35, migraine with focal aura and uncontrolled hypertension should not have the drug [55]. The COCP has been linked with hepatic adenomas, cholestasis and hepatic vein thrombosis both in patients with and without underlying liver disease [56]. In addition, during an episode of acute hepatitis the COCP use can worsen the underlying inflammation [56]. In chronic hepatitis the risk of the COCP is less clear, and evidence regarding its safety is lacking.

An alternative hormonal contraception, especially for women with contraindications for estrogens, is a progestin-only contraception method. They can be delivered orally, as an intramuscular injection or as an implantable device. Progesterone-based contraception appears to be safe in patients with liver disease. The liver has no progesterone receptors [57]; however, some progesterones at high doses are metabolised to compounds that may have a small effect on liver function although

by a much lesser effect than estrogens [58]. The main drawback to this choice of contraception is that progesterone can cause salt and water retention and hence should be avoided in those patients with ascites.

In view of the above comments the World Health Organisation recommend that in women with mild compensated cirrhosis or well-controlled hepatitis there is no restriction on the use of any hormonal contraceptive method. In women with severe decompensated cirrhosis, the benefits of progesterone only contraception may outweigh the risks but all other methods of hormonal contraception pose an unacceptable risk to health and should not be used. Finally, they recommend that in women who have a flare of hepatitis or acute hepatitis, progestogen only contraception may be used without restriction but estrogen containing forms should be avoided.

There are no reports on the use of intrauterine devices (IUD) in patients with AIH but they are generally regarded as safe and are used in the post-transplant setting.

In Vitro Fertilisation

Although patients with established cirrhosis have the potential to become pregnant, many women are unable to conceive naturally and the possibility and safety of in vitro fertilisation (IVF) is considered in such cases. Concern regarding hormonal changes associated with IVF which can potentially result in a flare in disease activity may worsen underlying hepatic function and precipitate decompensation. This has led to a degree of reluctance in offering IVF. The largest experience is incorporated into a review from our group and describes three patients with AIH who conceived by IVF [6]. The outcomes reported were poor, with one fetal loss at 20 weeks gestation, one severely handicapped child and only one healthy fetus. For the mothers, one patient had severe hepatic decompensation requiring premature delivery, one patient had a significant post-partum flare and although the final patient had no adverse liver-related outcomes, she miscarried at 20 weeks as described above. Isolated case reports are more favourable with successful fetal and maternal outcomes reported even in women with underlying cirrhosis [59]. In light of the discrepancy regarding safety and outcomes in the literature, more information is needed regarding IVF in AIH before conclusions can be drawn. In women with mild cirrhosis, or inactive AIH, IVF is a viable possibility providing women with a chance of pregnancy.

Conclusion

We have reviewed and appraised the available literature on AIH and pregnancy. Furthermore, we have highlighted the impact of AIH on fertility, discussed contraception options and the safety of common therapeutic regimes. We hope this chapter aids education and management of patients with AIH with regard to pregnancy.

Chapter Summary

1. Patients with autoimmune hepatitis frequently wish to get pregnant, and their clinical outcomes are generally very good, so long as liver function is preserved.
2. For patients with cirrhosis attention to risk of variceal bleeding is important.
3. Most patients have normal pregnancies with only additional bloodwork, imaging and endoscopy.

Useful Tips for Practitioners

1. Women with cirrhosis can conceive and therefore contraception and pre-conception counselling should be routine in women of child-bearing age.
2. Azathioprine is safe in pregnancy and should not be discontinued.
3. Patients are most likely to have a flare in the post-partum period and require close monitoring.
4. All women with underlying cirrhosis should have a screening endoscopy in their second trimester and consider delivery by caesarean section if large varices present.

Common Pitfalls in Practice

1. Failure to consider de novo AIH in pregnant women presenting with deranged LFT's in pregnancy.
2. Azathioprine should not be reduced in women planning or reporting pregnancy.

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Chapter 11
Treatment Side Effects and Associated Autoimmune Diseases

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Keywords Autoimmune hepatitis • Azathioprine • Combination therapy • Immunomodulators • Immunosuppressants • Prednisone

Abbreviations

6-MMP	6-Methylmercaptopurine
6-MP	6-Mercaptopurine
6-TGN	6-Thioguanine nucleotides
AIRE	Autoimmune regulator
APECED	Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy
APS	Autoimmune polyglandular syndromes
AZA	Azathioprine
CREST	Subcutaneous calcinosis, Raynaud's phenomenon, esophageal dysfunction, sclerodactyly and telangectasias
CTLA-4	Cytotoxic T lymphocyte antigen-4
HLA	Histocompatibility leukocyte antigen
IBD	Inflammatory bowel disease
NRH	Nodular regenerative hyperplasia
PBC	Primary biliary cirrhosis
PSC	Primary sclerosing cholangitis
RA	Rheumatoid arthritis
SLE	Systemic lupus erythematosus

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SS Systemic sclerosis
TNF Tumor necrosis factor
TPMT Thiopurine methyltransferase

Introduction

A wide variety of immunosuppressants and immunomodulators have been used to treat autoimmune hepatitis. As reviewed in Chaps. 6 and 7, prednisone or prednisolone, alone or in combination with azathioprine is the treatment mainstay, limited mostly by the development of drug toxicity. In that regard, combination therapy is associated with fewer side effects than prednisone monotherapy (10 versus 44%) [1]. In approximately 10% of the cases treatment with prednisone/azathioprine will fail and use of alternative therapy will be necessary. This chapter will review the most common side effects of these drugs used in the treatment of autoimmune hepatitis followed by a discussion regarding associated autoimmune diseases.

Treatment Side Effects

Prednisone/Prednisolone

Glucocorticoids like prednisone or prednisolone are potent anti-inflammatory agents which also have major metabolic effects. As such, steroids can cause a multitude of side effects which are usually related to both dosage and duration of therapy. Table 11.1 summarizes the most important side effects of steroids and other drugs used in the treatment of autoimmune hepatitis. Immunosuppression leads to decreased resistance to infections, especially, but not limited to, those bacterial in origin. In fact, it is recommended that a skin test for tuberculosis be considered prior to initiating long-term prednisone therapy. May be activate hepatitis B therefore head to be screened pre treatment. In endemic areas, consideration should be given to testing for Strongyloides infection as well and treating as appropriate prior to starting steroids. Hepatitis B reactivation should also be considered, and testing for prior exposure is recommended pre-treatment, with vaccination and/or subsequent monitoring on immunosuppression advisable.

Due to the mineralocorticoid activity, fluid retention and electrolyte imbalance can occur and induce or worsen hypertension. Patients receiving greater than 10 mg of prednisone daily are at risk for suppression of the hypothalamic–pituitary–adrenal axis leading to adrenal insufficiency. This manifests as anorexia, weight loss, lethargy, fever, and postural hypotension. Despite this potentially serious threat, it is the endocrine derangements that most often lead to treatment discontinuation. Cushingoid habitus, obesity, acne and cosmetic changes can significantly decrease compliance with therapy, and hyperglycemia may require the use of insulin [2]. Therefore, patients with brittle diabetes should be considered for combination therapy along with post-menopausal women, who are at significant risk for osteopenia and compression fractures, and also patients with obesity or with labile hypertension.

Table 11.1 Main side effects of drugs used in the treatment of autoimmune hepatitis

Drug	Side effects
Prednisone/Prednisolone	Skin thinning, purpura
	Cushingoid appearance, weight gain
	Hypertension, fluid retention, electrolyte imbalance
	Hyperglycemia
	Adrenal insufficiency
	Increased risk of infections
	Neutrophilia
	Myopathy
	Osteopenia/osteoporosis
	Cataracts formation
	Euphoria/psychosis other psychiatric symptoms
	Hepatitis B reactivation
Azathioprine/6-Mercaptopurine	GI intolerance
	Hypersensitivity
	Acute pancreatitis
	Hepatotoxicity
	Skin rash, fever
	Increased risk for infections
	Cytopenias
	Myalgias, arthralgias
	Lymphoma
Cyclosporine A	Renal insufficiency
	Hypertension
	Hyrsutism
	Gingival hyperplasia
	Hyperlipidemia
	Increased risk for infections
	Lymphoproliferative disorders
Tacrolimus	Hyperkalemia
	Renal insufficiency
	Tremors
	Hypertension, edema
	Paresthesias, neurotoxicity
	Headache
	Vivid dreams
Mycophenolate mofetil	Diarrhea
	Abdominal pain, nausea
	Cytopenias
	Alopecia
	Headache, dizziness
	Paresthesias
	Arthralgias
Budesonide	As prednisone, significantly less pronounced
	Increased risk of portal vein thrombosis seen in cirrhotics
Thioguanine nucleotides	Hypersensitivity
	Nodular regenerative hyperplasia

Other side effects of steroids include steroid myopathy, ischemic bone necrosis, increased intraocular pressure, formation of posterior subcapsular cataracts, pseudotumor cerebri, hyperlipidemia, and menstrual irregularities. Mental status changes can vary significantly, from depression to euphoria, from mild anxiety and insomnia to full blown psychosis.

Side effects are present in approximately 44% of patients on steroid monotherapy (20 mg/daily for maintenance), and can be minimized by dose reduction. Osteopenia and cataracts formation constitute an exception and can continue to progress despite minimal doses of steroids [2]. Thus, current guidelines recommend that patients at increased risk, usually those receiving ≥7.5 mg/day, be started on regular weight-bearing exercises and supplementation with vitamin D and calcium as prophylaxis of bone disease [1]. Use of bisphosphonates or other anti-resorptive drugs should be individualized. In addition, these patients on chronic steroid therapy should be monitored for bone disease with baseline and annual bone densitometry tests. Likewise, eye exams for cataracts and glaucoma should be performed on a regular basis during chronic steroid therapy.

Azathioprine/6-Mercaptopurine

Azathioprine (AZA) and 6-mercaptopurine (6-MP) are frequently used in combination with prednisone in the treatment of autoimmune hepatitis. AZA is non-enzymatically converted to 6-MP, which is then either deactivated or metabolized to an active form through a complex web of reactions. Thiopurine methyltransferase (TPMT) is the enzyme that methylates 6-MP and generates 6-methylmercaptopurine (6-MMP), an inactive metabolite. That enzyme activity is subject to significant inter-individual genetic variability, thus affecting the drugs' efficacy and tolerability. According to different polymorphisms in the TPMT gene, patients can be slow, intermediate or fast methylators. The enzyme activity will determine the proportion of 6-MP shunted toward the active metabolites, 6-thioguanine nucleotides (6-TGN), versus that inactivated in the form of 6-MMP. Slow methylators will produce more 6-TGN, potent purine antagonists which have been associated with myelotoxicity [3]. At this time, it is unclear whether measuring TPMT activity and thiopurine metabolites levels is necessary prior to starting therapy, although it could be useful in patients who fail to respond to usual therapy [4]. Another potential use would be to identify slow methylators, a subgroup of patients at particularly higher risk for myelotoxicity, among cirrhotics, who already have cytopenias at baseline.

Adverse events are observed in less than 10% of patients with autoimmune hepatitis taking AZA or 6-MP, and these adverse events can be divided into allergic and nonallergic reactions [5, 6]. As such, allergic reactions include skin rash, fever, pancreatitis, hypersensitivity and hepatitis, and are not dose-dependent. These reactions tend to occur early after initiation of AZA/6-MP, and recur with re-challenging. The nonallergic reactions are dose-dependent and relate to the

concentration of active metabolites 6-thioguanine nucleotides in the blood. These include the cytopenias, increased risk of infections and GI intolerance with nausea, vomiting and abdominal pain. Some degree of cytopenia is expected and monthly CBCs are recommended for adequate monitoring during therapy. In case of GI intolerance, dose reduction or switching from AZA to 6-MP or vice versa should be attempted prior to drug discontinuation. These GI symptoms will improve in up to 70% of patients [4, 7, 8].

Other side effects include rare teratogenicity and a potential increase in the risk of developing lymphoma. Although there are no studies involving patients with autoimmune hepatitis, data derived from large populations with inflammatory bowel disease and rheumatoid arthritis (RA) suggest a small increase in the relative risk of lymphoma [9, 10]. An association with the severity of disease as opposed to the use of AZA or 6-MP cannot be excluded [11, 12]. Finally, acute hypersensitivity can occur, albeit significantly less often than in patients with inflammatory bowel disease (2.5 vs. 19%) [13].

Cyclosporine A

Cyclosporine A is a calcineurin inhibitor that impairs the transcription of interleukin-2 and its downstream activation, thereby preventing T-cell lymphocyte activation. The use of cyclosporine has been mostly restricted to rescue therapy in adults with steroid-resistant disease or for induction of remission in children, in whom long-term use of steroids must be avoided [14, 15]. Reports in the pediatric population suggest that biochemical remission can be achieved in >90% of patients within the first 6 months, when cyclosporine A (3.3–6.5 mg/kg/day) is used as first-line therapy to induce remission in lieu of prednisone [16, 17]. Likewise, cyclosporine A (4.7–7.5 mg/kg/day) was successful in inducing remission in four pediatric patients who relapsed while on prednisone maintenance therapy and who had refused to resume high-dose steroids due to previous side effects, and in two patients who progressed to liver failure despite treatment with prednisone and azathioprine [17]. In these two patients with liver failure, cyclosporine was first administered intravenously (1 mg/kg/day), and then converted to oral formulation once the liver function improved, with levels adjusted to maintain trough levels between 200 and 250 ng/mL. Importantly, patients with severe acute hepatitis who meet minimal listing criteria for liver transplantation (MELD ≥15), as well as patients who show any signs of decompensation while on rescue therapy, should be referred for transplant evaluation.

Despite its efficacy in steroid-resistant patients, the benefit of cyclosporine is significantly limited by the toxicity profile associated with its long-term use, including hypertension, renal impairment, tremors, headaches, hirsutism, gingival hyperplasia, severe infections, hyperlipidemia and even lymphoproliferative disorders. Other less toxic alternative therapies are increasingly available.

Tacrolimus

Tacrolimus is a calcineurin inhibitor 100 times more potent than cyclosporine A, but with a more favorable toxicity profile. It has the ability to inhibit expression of the interleukin-2 receptor and impair cytotoxic T-cell proliferation. Formal experience in autoimmune hepatitis is restricted to a couple of clinical trials and a few case reports. Despite that, several hepatologists use tacrolimus in patients who fail steroids or who become steroid-dependent. Common side effects include hyperkalemia, renal insufficiency, tremors, hypertension, edema, paresthesias, vivid dreams, and headaches [18, 19]. These side effects can often be minimized by targeting a low serum level.

Mycophenolate Mofetil

Mycophenolate mofetil is an inhibitor of inosine monophosphate dehydrogenase which reduces proliferation of T-cell and B-cell lymphocytes by blocking de novo purine synthesis and impairing DNA synthesis. It represents a less toxic alternative for patients who are refractory to, or intolerant of, conventional therapy for autoimmune hepatitis; reported side effects include diarrhea, abdominal pain, nausea, cytopenias, hair loss, headaches, dizziness, paresthesias, and arthralgias [20–25]. When possible, dose reduction alleviates most of these side effects.

Budesonide

Budesonide is a potent synthetic glucocorticoid with high first-pass metabolism by the liver, which reduces its systemic bioavailability to about 10%. With that, budesonide is expected to be more effective and better tolerated than prednisone. Nevertheless, adverse events still occur, including leukocytosis, hypercholesterolemia, cushingoid habitus, acne, heartburn, weight gain and others [26, 27]. Importantly, steroid-induced diabetes [27] and bone demineralization [28] have been demonstrated even with budesonide. In a study comparing efficacy and safety of budesonide/azathioprine to prednisone/azathioprine, steroid-induced side effects were noticed in 28% of patients on the budesonide group. The dose of budesonide was 3 mg three times daily until remission, and then it was decreased to 3 mg twice daily. The number of serious adverse events was similar in both groups [29].

Finally, budesonide should only be used in noncirrhotic patients. In patients with cirrhotic-stage primary biliary cirrhosis, use of budesonide led to marked elevation of serum levels and was associated with serious adverse events. Furthermore, two of the seven patients in that study developed portal vein thrombosis in close temporal relationship with drug administration [30]. It is unclear whether patients with cirrhosis due to other etiologies, such as autoimmune hepatitis, are at similar risk.

Tioguanine Nucleotides

Active metabolites of AZA, tioguanine nucleotides (6-TG) have been advocated for use in patients with autoimmune hepatitis who were previously intolerant of AZA [31]. Prospective studies are not available and data derive from a single case series involving only three patients. Significant side effects were not reported in that series. However, among patients with inflammatory bowel disease who received 6-TG, up to 19% had an acute hypersensitive reaction and hepatic changes consistent with nodular regenerative hyperplasia were commonly described [32, 33]. Thus, 6-TG is not currently recommended for the treatment of autoimmune hepatitis outside the setting of a clinical trial.

Others

Use of other drugs including *methotrexate* and *cyclophosphamide* has been documented only in small case reports and limited data are available but plenty of side effects documented in the general literature. *Rituximab* is a chimeric anti-CD20 monoclonal antibody which depletes B-cell lymphocytes by targeting their CD20 cell surface receptor. The rationale is scientifically sound and the drug has been successfully used in one case of autoimmune hepatitis [34]. However, the interest in treating this disease with monoclonal antibodies was dampened by findings of possible induction of autoimmune hepatitis and liver failure by *infliximab*, another chimeric monoclonal antibody which has the ability to block cytotoxic T-cell lymphocytes [35].

Associated Autoimmune Diseases

Frequency

Concurrent autoimmune diseases occur in approximately 18–46% of those with type 1 autoimmune hepatitis, and in 14–34% of those with type 2 autoimmune hepatitis [36–40]. Based on this increased frequency, the International Autoimmune Hepatitis Group (IAIG) included the presence of autoimmune diseases in either the patient or their first-degree relatives as a diagnostic feature in their scoring system.

Risk Factors

It is well known that the histocompatibility leukocyte antigen (HLA) DR4 is associated with susceptibility to concurrent immune diseases in type 1 autoimmune hepatitis [41], especially if the *HLA-A11* is also present [40, 41], and that women tend to have

Table 11.2 Associated autoimmune diseases

Endocrine	Autoimmune thyroiditis Diabetes mellitus Autoimmune polyglandular syndromes
Rheumatologic	Synovitis Sjogren's syndrome Rheumatoid arthritis, Felty's syndrome Systemic sclerosis Systemic lupus erythematosus Polymyositis Juvenile arthritis
Gastrointestinal/Hepatic	Inflammatory bowel disease ± PSC Celiac disease Primary biliary cirrhosis Primary sclerosing cholangitis
Hematologic	Idiopathic thrombocytopenic purpura Hemolytic anemia Pernicious anemia
Neurologic	Multiple sclerosis (? True association)
Cutaneous	Pemphigus vulgaris Morphea Lichen planus Pityriasis lichenoides chronic Discoid lupus Alopecia Nail dystrophy Vitiligo
Renal	Membranous nephropathy Cryoglobulinemic glomerulonephritis Focal glomerulonephritis
Others	Uveitis

a higher frequency of such associated diseases [37]. Indeed, women have a higher frequency of HLA DR4 than men with type 1 autoimmune hepatitis. In addition, older patients also tend to have more concurrent autoimmune diseases, especially those rheumatic in nature [42].

Types

Multiple extra-hepatic autoimmune diseases have been described in association with autoimmune hepatitis. The nature and frequency of each condition vary significantly according to the type of autoimmune hepatitis, genetic background, geographic location, gender, and antibody profile among other possible factors. Table 11.2 shows a list of autoimmune diseases commonly associated with

autoimmune hepatitis. In the United States, thyroid disease and arthritis are the concurrent diseases most commonly associated with type 1 autoimmune hepatitis. In Brazil, on the other hand, seronegative arthritis and rheumatoid arthritis were more common. A brief discussion of extra-hepatic concurrent autoimmune diseases is provided below.

Endocrine Diseases

Autoimmune Thyroiditis

Although a true association has not been clearly demonstrated between autoimmune hepatitis and thyroiditis, this hypothesis is supported by the presence of a polymorphism in the cytotoxic T lymphocyte antigen-4 (CTLA-4) gene in both groups of patients [43]. Thyroiditis can be seen both in type 1 and in type 2 autoimmune hepatitis and appears to be quite common in North American and Italian patients with type 1 autoimmune hepatitis [44, 45] as well as English and French patients with type 2 autoimmune hepatitis [36]. Not unexpectedly, patients with autoimmune hepatitis who are older than 65 years of age appear to be at a higher risk for autoimmune thyroiditis than younger counterparts (25 vs. 5%) [44]. More frequently, patients will complain of excessive fatigue despite good control of their AIH and laboratory testing will reveal abnormalities in the thyroid profile. Both Hashimoto's and Graves' disease have been described in this scenario [46–48].

Diabetes Mellitus

The most common association is between type 1 diabetes mellitus and type 2 autoimmune hepatitis [49]. However, a case of latent autoimmune diabetes of adults has been reported [50], as well as an association of Graves' disease, type 1 diabetes mellitus and autoimmune hepatitis [51]. As diabetes mellitus can be part of an autoimmune polyglandular syndrome, these associations need to be evaluated.

Autoimmune Polyglandular Syndromes

Two types of Autoimmune Polyglandular Syndromes (APS) have been described. Type 1 is a rare autosomal-recessive disease, also known as autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), that affects juvenile patients. The gene responsible for this disease, the AIRE gene, is located on chromosome 21q22.3. APS-1 is characterized by the presence of two out of three of the

following: hypoparathyroidism, adrenocortical failure, and chronic mucocutaneous candidiasis. Several other autoimmune manifestations may develop in APECED patients, with autoimmune hepatitis occurring in up to 20% of them [52]. Type 2 APS is autosomal-dominant, occurs more frequently than type 1 and affects predominantly adult women. Potential susceptibility genes include the CTLA-4 and cytokine-related genes TNF alpha and/or PTPN22. Several distinct disease combinations can be present in APS-2, with thyroid immune disease/type 1 diabetes mellitus being the most common (41%), followed by Addison's disease/thyroid disease (14.6%) [53]. Other disease presentations include vitiligo, alopecia, hypogonadism, and pernicious anemia. Genetic variants of both type 1 and type 2 APS have been described in children with autoimmune hepatitis. If suspected, genetic analysis of the AIRE gene may be useful to confirm APS-1.

Rheumatic Diseases

Arthritis/Synovitis

This was reported by 27% of White Argentinians with type 1 autoimmune hepatitis referred for a genetic study [40]. In the United States, seronegative arthritis is also commonly described, although an accurate incidence is not available. Most studies do not methodically exclude autoimmune rheumatic diseases.

Sjogren's Syndrome

Sjogren's is an autoimmune exocrinopathy that leads to destruction of the salivary and lacrimal glands by infiltrating lymphocytes. As a result, patients develop dry eyes and dry mouth syndrome, and systemic symptoms such as fatigue and arthralgias can occur. Up to 30% of patients with Sjogren's syndrome eventually develop additional autoimmune syndromes; the liver is frequently involved [54, 55]. An earlier study from Denmark found that only 2 (6%) out of 16 patients with Sjogrens and abnormal liver tests had autoimmune hepatitis [56]. Subsequently, a smaller study from Japan, where 17 patients with Sjogrens and elevated liver tests underwent liver biopsy, eight patients (47%) were found to have autoimmune hepatitis [57]. More recently, a study from Mexico City, 44% of 95 patients with Sjogren's syndrome had abnormal liver biochemistries, and 21 (22%) of those had a well-defined liver disease. Two of the 21 had autoimmune hepatitis (9.5%), five had primary biliary cirrhosis, 11 had hepatitis C, one had hepatitis B and two had nonalcoholic fatty liver disease [55]. Thus, the reported incidence of autoimmune hepatitis in patients with Sjogren's syndrome and elevated liver tests ranges from 6 to 47%,

depending possibly on the geographic location and genetic background. Conversely, Sjogren's syndrome affects approximately 27% of patients with autoimmune hepatitis [58].

Rheumatoid Arthritis

This chronic polyarthritis is often associated with liver test abnormalities, although a specific liver disease is infrequently found. When unselected liver biopsies were performed in 117 patients with RA, nonspecific changes were the most frequent finding (43%), followed by normal biopsy (35%) and fatty changes (22%). When liver biopsies of patients with RA and abnormal liver tests were examined, 74% of the specimens were nonspecific, 13% normal and 13% with a defined chronic liver disease, of which in only one case could autoimmune hepatitis be implicated [59]. In another study evaluating liver histology of 160 patients with multiple collagen diseases including RA, the investigators failed to identify a single case of autoimmune hepatitis in association with RA [60]. Other liver diseases associated with RA are primary biliary cirrhosis (PBC), amyloidosis, and nodular regenerative hyperplasia (NRH). Felty syndrome is a rare and severe presentation of RA characterized by splenomegaly and neutropenia. NRH has been reported in association with RA, especially in the setting of Felty's syndrome. One case report of autoimmune hepatitis associated with Felty syndrome has been published [61].

Systemic Sclerosis

Systemic sclerosis (SS) is a chronic systemic disease characterized by tissue fibrosis, small blood vessel vasculopathy, and the presence of several types of auto-antibodies. Two forms are recognized: limited and diffuse cutaneous, and CREST (subcutaneous calcinosis, Raynaud's phenomenon, esophageal dysfunction, sclerodactyly and telangectasias) syndrome is a form of limited scleroderma associated with production of anti-centromere antibodies [62]. Autoimmune hepatitis has been described in association with SS only in case reports [63, 64]. As is the case in RA, SS is more often associated with PBC and NRH.

Systemic Lupus Erythematosus

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by multiorgan involvement and the presence of specific auto-antibodies. Liver involvement is not a typical feature in SLE, and when present is usually due to steatosis [65]. Cases consistent with autoimmune hepatitis, however, have been described in

up to 2.5–4.4% of patients [66]. Further, it is important to remember that in this scenario, autoimmune hepatitis can develop de novo or be triggered by medications. Findings of autoimmune hepatitis in a patient with SLE should be regarded as a second disease and treated as appropriate.

Polymyositis

This autoimmune inflammatory muscle disease has been described in association with autoimmune hepatitis only in a couple of case reports [67, 68]. Elevation of serum transaminases is also a common feature of polymyositis and this can lead to significant delay in diagnosis. The association with another autoimmune liver disease, PBC, is somewhat stronger. Elevation of serum alkaline phosphatase may be seen in polymyositis but should nevertheless trigger the clinician to investigate both diseases [66].

Gastrointestinal Diseases

Inflammatory Bowel Disease

The liver disease most commonly associated with IBD is primary sclerosing cholangitis (PSC) [69–71]. However, it is well documented that autoimmune hepatitis can also be associated with IBD. One study examined 105 patients with autoimmune hepatitis by proctoscopy and found that 17 (16%) had unsuspected findings of chronic ulcerative colitis [72]. The course of autoimmune hepatitis among patients with coexisting IBD appears no different from that seen in patients without IBD. On further evaluation, 12 of these 17 patients underwent cholangiography and features of PSC were identified in 5 (42%). Thus, in patients with autoimmune hepatitis and IBD, PSC must be thought of and appropriately excluded with magnetic resonance imaging. A series from the Mayo Clinic examined this issue from a different perspective: out of 32 patients with IBD and chronically elevated liver enzymes, only 2 (6.25%) had autoimmune hepatitis, whereas PSC was diagnosed in 25 (78%) [70]. Of note, both ulcerative colitis [73] and Crohn's disease [74] have been described in conjunction with autoimmune hepatitis.

Celiac Disease

Also known as gluten-sensitive enteropathy, celiac disease is associated with many forms of liver disease, including autoimmune hepatitis, primary biliary cirrhosis,

PSC, viral hepatitis, and nonalcoholic liver disease [75]. The prevalence of celiac disease among patients with autoimmune hepatitis appears to be 4–6.4% [76, 77], and both patients with type 1 and type 2 can be affected. The prevalence of celiac disease among pediatric patients with autoimmune hepatitis is higher, around 12.5–13.5% [78, 79]. As is frequently the case with other concurrent autoimmune diseases, celiac disease may be unmasked before or after autoimmune hepatitis is diagnosed. Liver Diseases PBC and PSC are chronic autoimmune liver diseases which can co-exist with autoimmune hepatitis in the so-called overlap syndromes – see Chapter 12 for details.

Hematologic Diseases

Idiopathic Thrombocytopenic Purpura

This condition is characterized by an isolated thrombocytopenia, with a cut-off platelet count <50,000/μL, in the absence of an implicated drug, condition or agent that is known to cause thrombocytopenia. There are several case reports describing an association with autoimmune hepatitis, particularly in conjunction with Sjogren's syndrome [80, 81]. According to a nation-wide survey in Japan, idiopathic thrombocytopenic purpura occurs in about 3% of patients with autoimmune hepatitis [80].

Autoimmune Hemolytic Anemia

This entity is characterized by the production of IgG or IgM antibodies against red blood cell surface antigens. The red blood cells are then destroyed either by activation of the complement system or by the reticuloendothelial system. The association between autoimmune hemolytic anemia and autoimmune hepatitis is rare but well documented [82, 83].

Pernicious Anemia

This occurs as a result of Vitamin B12 (cobalamin) malabsorption caused by two main factors: (1) presence of anti-intrinsic factor antibodies causing destruction of gastric intrinsic factor needed for cobalamin absorption and (2) atrophic gastritis leading to a decreased production of intrinsic factor. Chronic atrophic gastritis may

be associated with another condition called gastric carcinoid. Cases have been described of patients with autoimmune hepatitis, pernicious anemia, and atrophic gastritis with or without carcinoids [84, 85]. Of note, both pernicious anemia and autoimmune hepatitis are components of the APS-1 and thus this diagnosis should be excluded in the presence of both conditions.

Neurologic

Cases of untreated multiple sclerosis in association with autoimmune hepatitis as well as other autoimmune conditions have been reported [86–88]. Whether this represents a true association versus chance association is unclear. In addition, treatment with interferon β can trigger development of autoimmune hepatitis.

Cutaneous

A number of skin manifestations have been reported in association with autoimmune hepatitis, including pemphigus vulgaris, morphea, lichen planus, pityriasis lichenoides chronic, discoid lupus, alopecia, nail dystrophy and vitiligo [36, 89–92]. Those are uncommon and may also be part of a polyglandular syndrome.

Renal

Cases of membranous nephropathy [67, 93], cryoglobulinemic glomerulonephritis [94], and focal glomerulonephritis [95] have been described and are extremely rare.

Conclusion

To appropriately manage patients with autoimmune hepatitis, the treating physician must understand the challenges consequent upon the chronic use of immunomodulators. As adherence is extremely important to treatment success, recognizing and managing side effects that may otherwise lead to medication intolerance is essential. Likewise, a variety of concurrent autoimmune diseases can be seen in association with autoimmune hepatitis and a high index of suspicion is needed.

Chapter Summary

1. Drug toxicity may lead to dose reduction or early discontinuation of therapy. Prednisone and azathioprine are currently the mainstay of treatment for autoimmune hepatitis, but a variety of drugs are available for use as alternative therapies. This includes cyclosporine, tacrolimus, mycophenolate, and budesonide (latter only when cirrhosis about).
2. Many concurrent autoimmune conditions can be associated with autoimmune hepatitis and can affect any organ system.
3. Associated autoimmune conditions are particularly common in females and elderly patients, with thyroid disease and arthritis being the two most common concurrent diseases.

Useful Tips for Practitioners

1. Tailoring treatment according to individual patients' conditions will help minimize development adverse events. For instance, patients with osteoporosis, brittle diabetes, acne, emotional instability, obesity, hypertension, postmenopausal or elderly are poor candidates for prednisone monotherapy and would benefit from combination therapy with azathioprine. On the other hand, patients with cytopenias at baseline may not tolerate treatment with azathioprine at all.
2. Reviewing the side effects profile of each drug with the patient prior to initiating treatment will increase compliance with therapy.
3. Clinicians must have a low threshold to investigate and diagnose associated autoimmune conditions.
4. Patients with autoimmune hepatitis and multiple endocrine syndromes should be evaluated for autoimmune polyglandular syndromes and testing for genetic mutations of the AIRE gene may be helpful in that scenario.
5. Patients with autoimmune hepatitis and concomitant inflammatory bowel disease, especially in the pediatric population, should be evaluated for primary sclerosing cholangitis.

Common Pitfalls in Practice

1. Not monitoring for signs of toxicity, which should be done with routine cell counts in the case of azathioprine, and periodic eye exams and annual bone densitometry tests for those on long-term steroid therapy.
2. Not supplementing patients on long-term steroid therapy with calcium and vitamin D.
3. Delaying referral for liver transplant evaluation due to experimenting with alternative therapies for too long while patients continue to deteriorate clinically.
4. Vaccinating for Hepatitis B prior to starting stands.

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Chapter 12
Managing the Patient with Features of Overlapping Autoimmune Liver Disease

Kirsten Muri Boberg

Keywords Autoimmune hepatitis • Autoimmune liver diseases • Corticosteroids • Overlap syndromes • Primary biliary cirrhosis • Primary sclerosing cholangitis • Ursodeoxycholic acid

Introduction

For most patients within the spectrum of autoimmune liver diseases (i.e., autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC), and primary sclerosing cholangitis (PSC)), the classification into one of the primary disorders is not difficult and patients can be treated accordingly. Patients with classical AIH should receive immunosuppressive therapy according to guidelines [1, 2]. Ursodeoxycholic acid (UDCA) is recommended for patients with PBC [3–5]. In PSC, no effective medical therapy has been documented, but UDCA may also be used to some extent in this cholestatic condition [5]. Clinical experience indicates that some patients present with a combination of hepatitic and cholestatic features. These patients may be difficult to classify, or they fulfill the diagnostic criteria of one disorder but have additional features of another. Such conditions are commonly designated "overlap syndromes" [6–13]. Among these, PBC–AIH- and PSC–AIH "overlap syndromes" are most frequently described.

There are no internationally agreed criteria for the diagnosis of "overlap syndromes," and several definitions have been used in the various reports. Since there currently is no known etiopathogenetic basis for the distinction of overlaps from the classical disorders, it is questionable whether overlap conditions should be considered separate diagnostic entities [14]. Nevertheless, the management of patients with overlapping features of autoimmune liver disease may require special attention. Due to the lack of

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standardization of diagnostic criteria and the heterogeneity of patients as well as the low prevalence of these overlap conditions, randomized, controlled therapeutic trials have been impossible to perform. Treatment of PBC–AIH- and PSC–AIH overlap patients therefore is not evidence-based, but empirical and based (perhaps incorrectly) on experience in the primary conditions.

Characteristics of PBC

PBC is a chronic, cholestatic liver disease that is characterized by non-suppurative destruction of small intrahepatic bile ducts [3, 4, 15, 16]. The disease process leads to progressive scarring and ductopenia and eventually to the development of cirrhosis. PBC is usually slowly progressive over decades, but the rate of progression varies considerably among patients [4]. Approximately 95% of patients are female, typically in the age range 30–65 years at presentation [15]. More than half are asymptomatic at diagnosis of PBC. Fatigue and pruritus are the most frequently reported complaints in symptomatic cases. Biochemical tests typically reveal a cholestatic pattern with elevated serum alkaline phosphatase (ALP)- and gamma-glutamyl transpeptidase (GGT) levels [15]. Serum aminotransferase activities are normal or slightly elevated (<5× upper limit of normal (ULN)). Serum bilirubin concentration becomes elevated in later stages and is then a good marker of prognosis [3]. A positive antimitochondrial antibody (AMA) test is the serological hallmark of PBC and is found in 90–95% of patients [3, 4, 16, 17]. AMA has a high specificity (98%) for this disease. Antinuclear antibodies (ANA) and anti-smooth muscle antibodies (SMA) are present in nearly half of patients with PBC [16]. The ANAs anti-GP210 and anti-SP100 are highly specific for PBC [18]. Among immunoglobulins, in particular the IgM fraction is elevated [3, 19].

The diagnosis of PBC can be made in a patient with otherwise unexplained biochemical evidence of cholestasis (mainly based on elevated ALP levels) in combination with the presence of AMA (≥1:40) by immunofluorescence and/or AMA type M2 [4, 5]. In this situation a liver biopsy is not required for the diagnosis, but it can add information on disease activity and stage [5]. In the absence of PBC-specific antibodies, a liver biopsy is necessary for the diagnosis. Characteristic findings include non-suppurative destructive cholangitis and destruction of interlobular bile ducts [4]. A liver biopsy should also be considered in those patients with particularly high serum levels of aminotransferases and/or serum IgG levels to assess potential histologic features of AIH that might have implications for therapy [5]. Lymphocytic hepatocellular piecemeal necrosis is seen in a proportion of patients with PBC [20–22]. The 5–10% of PBC patients who are AMA-negative appear to have a disease that otherwise is identical to the AMA-positive cases [16].

Positive AMA titres, generally low and considered non-specific, have been reported in AIH patients [22, 23]. Even anti-M2 which is considered specific for PBC, has been detected in AIH [22]. Serum markers of cholestasis like elevated ALP levels, may also be present [24]. Some degree of biliary involvement may be part of the histological picture in AIH, but this is not associated with AMA [25] or other features of PBC [26]. AIH can be differentiated from PBC in the majority of cases.

Characteristics of PSC

PSC is a chronic, cholestatic liver disease with inflammation and fibrosis affecting both intra- and extrahepatic bile ducts [27]. The disease process results in irregularities and stricturing of the bile ducts. It is a disorder which progresses to cirrhosis and liver failure, although the clinical course varies considerably among patients. As opposed to AIH and PBC, there is a male preponderance in PSC with a male to female ratio of 2:1 in most populations. Patients are often young, with a median age at diagnosis between 30 and 40 years [28, 29]. Approximately half of the patients present with symptoms of the liver disease, most commonly fatigue, pruritus, jaundice, and right upper abdominal pain [28]. PSC patients characteristically have biochemical signs of cholestasis. ALP levels are typically at least three times ULN at diagnosis, but levels may fluctuate and even be normal during the disease course. GGT levels are also regularly increased. Serum aminotransferase levels are often moderately elevated (typically two to three times ULN). Serum bilirubin concentration is normal at diagnosis in up to 70% of patients [27, 28], but usually increases or fluctuates with disease progression. Both serum IgG- and IgM levels may be increased in PSC patients (in up to 61% and 45%, respectively) [27, 30]. Serum autoantibodies are also frequently detected, including ANA (8–77%), SMA (0–83%), and perinuclear anti-neutrophil cytoplasmic antibodies (pANCA) (26–94%) [31]. Liver histological changes in early-stage PSC may be very subtle, but typically consist of lymphocyte infiltration in the portal tracts and biliary epithelium, along with ductular proliferation. In later stages, bridging fibrous septa develop, while bile ducts degenerate and disappear [32]. Concentric periductal fibrosis is suggestive of PSC, but is not a regular finding. The histologic findings in PSC are not pathognomonic and can only support the diagnosis. A variable degree of interface hepatitis may also be part of the picture and will sometimes make AIH an additional diagnostic possibility.

The diagnosis of large duct PSC is confirmed by MR cholangiography showing bile duct mural irregularities and diffusely distributed multiple strictures and dilatations [33]. Both intra- and extrahepatic bile ducts are affected in the majority of cases. Being non-invasive, magnetic resonance cholangiography (MRC) is recommended as the initial cholangiographic procedure [5]. Endoscopic retrograde cholangiography (ERC) may be necessary in equivocal cases or when therapeutic endoscopic procedures are anticipated. Small duct PSC (features of PSC confined to the small bile ducts) is diagnosed in patients who present with clinical, biochemical, and histologic features compatible with PSC, but who have a normal cholangiogram [34]. PSC is associated with inflammatory bowel disease (IBD) in up to about 80% of cases.

Overlapping Autoimmune Disorders

Although AIH, PBC, and PSC all have some characteristic features, patients within each of these disorders can present with a spectrum of clinical, biochemical, serological, and histological findings. Practically all of these findings may overlap with

those of one of the other disorders, and the boundaries between the classical conditions are therefore not always distinct. Among 225 patients with autoimmune liver disease (162 type 1 AIH, 37 PBC, 26 PSC), variant forms were described in as many as 18% [35]. Overlapping features between two conditions most frequently present concomitantly, but sequential development of characteristics of two diseases has also been observed [36–43]. There are several potential explanations for the concurrence of characteristics of two disorders: (1) Two independent diseases present in a susceptible individual, (2) "Overlap syndromes" represent distinct diagnostic entities, to be differentiated from classical AIH, PBC, or PSC, (3) There is a continuum of manifestations ranging from a "pure" hepatitic to a "pure" cholestatic disorder, with overlaps represented in the middle of the spectrum, (4) There is one primary disorder that due to heterogeneity also displays characteristics of another. The latter concept has gained most support [9, 44, 45]. It is currently unknown whether there are genes or other shared pathogenetic factors that predispose to overlapping features between AIH, PBC, and PSC [46]. In clinical practice, efforts should always be made to define the primary disease and ensure that there are no external factors (e.g., medications) which may be responsible for the findings suggestive of an "overlap syndrome." Patient populations as well as criteria for classification of subgroups of patients into overlap syndromes often differ among studies, resulting in variable findings of prevalence and characteristics [22].

Patients with Overlapping Features Between PBC and AIH

The largest series describing patients with overlapping features between AIH and PBC have used definitions essentially based on either (1) a combination of diagnostic criteria of each disease [37, 47, 48] or (2) the application of the International Autoimmune Hepatitis Group (IAIHG) scoring system for the diagnosis of AIH [49, 50] or modifications thereof to patients with a known diagnosis of PBC [14, 21, 35, 51–54]. Yet, other variations of criteria have also been used [55–58]. The relevance and utility of any of these criteria have been widely discussed [12, 44, 45, 59]. A major concern regarding the extensive use of the IAIHG scoring system to select cases of both PBC–AIH- and PSC–AIH overlap, is that this system was developed to have a high specificity for the diagnosis of AIH rather than to look for similarities between conditions [59].

Chazouilleres et al. [37] defined PBC–AIH "overlap syndromes" strictly by the presence of at least two of three criteria of each disease (Table 12.1) and found that 12 (9.2%) among 130 PBC patients satisfied these criteria. Applying the same set of criteria to a group of 331 PBC patients included in a clinical trial, Joshi et al. [47] concluded that the prevalence of patients who also had features of AIH was 4.8%. Using a modified original IAIHG scoring system [49], Czaja et al. [35] found that 7 (19%) among 26 PBC patients also satisfied criteria of AIH. Corresponding results were reported by Talwalkar et al. [21], with 26 (19%) among 137 PBC patients scoring as "probable" AIH according to the revised IAIHG scoring system [50].

Table 12.1 Diagnostic criteria of PBC–AIH "overlap syndrome" [37]

PBC criteria
• Serum ALP levels at least two times ULN or serum GGT levels at least five times ULN
• A positive test for AMA
• A liver biopsy specimen showing florid bile duct lesions
AIH criteria
• Serum ALT levels at least five times ULN
• Serum IgG levels at least two times ULN or a positive test for SMA
• A liver biopsy showing moderate or severe periportal or periseptal lymphocytic piecemeal necrosis

PBC–AIH "overlap syndrome" is considered present when at least two of three criteria for both PBC and AIH are met, either simultaneously or consequtively. EASL guidelines in addition state that histologic evidence of moderate to severe lymphocytic piecemeal necrosis (interfase hepatitis) is mandatory for the diagnosis [5]. It should be noted that the relevance of using rather strict criteria can be discussed [59]

Application of the revised scoring system to a group of 138 PBC patients from different geographical regions resulted in identification of 7 (9%) overlap cases [14]. Even lower figures for the prevalence of PBC–AIH overlap by use of the revised scoring system have, however, also been reported [51, 53]. In an Italian group of patients with a diagnosis of PBC, the occurrence of PBC–AIH overlap was only 3 (2.1%) among 142 cases [53]. Among 800 Swedish PBC patients, 25 (3%) were classified as overlaps [51]. Overall, the prevalence of PBC–AIH overlap conditions varies in part due to the criteria applied for features of AIH, and the number ranges from 2 to 19% of patients with an original diagnosis of PBC. The frequency of overlaps appears to be lower among patients with an original diagnosis of AIH [59]. Only 8 (5%) among 162 AIH type 1 patients in the study by Czaja et al. [35] could be classified as an AIH–PBC variant. It should be noted that AMA positivity occasionally is observed in patients who otherwise fulfill the diagnostic criteria of AIH, but then usually in low titres and considered non-specific [22, 23].

Patients with Overlapping Features Between PSC and AIH

A variable proportion of patients with cholangiographically confirmed large duct PSC also have certain features which resemble AIH. They may have somewhat higher than expected levels of serum aminotransferases and/or immunoglobulins, positive autoantibody titres, and/or histological interface hepatitis. In most reports, PSC–AIH overlap has been defined according to the original or revised IAIHG scoring system for the diagnosis of AIH [14, 30, 35, 43, 53, 54, 60, 61]. By applying the original IAIHG scoring system, Boberg et al. [30] found that 2 (2%) of 114 PSC patients scored as "definite" AIH and 38 (33%) as "probable" AIH. By redefining patients according to the revised criteria, 2 (2%) still scored as "definite" AIH, whereas the number of "probable" AIH was reduced to 10 (9%) [50]. Kaya et al.

[60] reported similar results in a study of 211 PSC patients who according to the original scoring system scored as "definite" AIH in 2% of cases and "probable" in 19%, whereas the revised system reduced the proportion with scores in the probable range to 6%. These observations illustrate that the prevalence of overlap cases in any cohort of patients is highly sensitive to the criteria applied. Using the revised IAIHG scoring system, but requiring scores of "definite" AIH along with positive titres of autoantibodies and histological features of AIH, Floreani et al. [61] still found as many as 7 (17%) PSC–AIH overlaps among 41 PSC patients. In an international panel of 221 patients with PSC, 32 (14%) scored as AIH (4 "definite" and 28 "probable" cases) [14]. Among the largest series of PSC patients, 7–14% of cases score for features of AIH.

In a few cases considered PSC–AIH overlap, the diagnosis of AIH precedes that of PSC, often by several years [36, 43, 61]. The possibility of PSC must be considered in patients classified as AIH but presenting with one or more features typical of PSC, including cholestatic liver tests, histological evidence of bile duct injury, concomitant IBD, and poor response to therapy. A sequential development of AIH in cases of PSC may also occur [41, 43]. Cases of overlapping AIH and small duct PSC have been described [62].

PSC in childhood is more commonly associated with features of AIH than is the case in adults [63–66]. PSC was as prevalent as AIH in a study of 55 consecutive children presenting with evidence of liver disease and circulating autoantibodies [65].

Treatment of Patients with Overlap PBC–AIH

Immunosuppression markedly improves prognosis in patients with classical AIH [1, 2]. Patients who have the typical serum antibody markers and histological findings of AIH, but in combination with a cholestatic biochemical pattern or positive AMA, should also be treated as AIH unless the liver biopsy suggests PBC [67]. UDCA is the treatment of choice in classical PBC [5]. There might be a benefit of corticosteroids in PBC [68–70], but there are considerable concerns regarding side effects [67]. Recommendations on treatment of patients with PBC–AIH overlap are based on the experience in treatment of the two primary disorders and on case reports and retrospective small patient series of PBC–AIH overlap conditions.

Studies Indicating a Positive Response of PBC–AIH Overlap Conditions to a Combination of UDCA and Immunosuppressants

Several studies support a combination of UDCA and immunosuppressants. Among 11 patients with PBC–AIH overlap defined by the presence of at least two of three biochemical, serological, and histological criteria of each disease (Table 12.1), five

patients were initially treated with UDCA alone (13–15 mg/kg/d) and six with prednisolone alone (0.5 mg/kg/d, tapered when remission was obtained with ALT levels below twice ULN) [37]. After UDCA therapy for median 23 months, levels of ALP and GGT, but not ALT and IgG, decreased significantly. Two patients obtained normalization of ALP and ALT levels. Pruritus and jaundice disappeared in two of three patients who suffered from these symptoms. Liver fibrosis increased in three patients. Corticosteroid treatment with a duration of median 4 months resulted in a significant decrease in ALP, ALT, and IgG levels, but without normalization in any patient. The only symptomatic patient became asymptomatic. The total of nine patients who had persistently abnormal biochemical tests after therapy with either UDCA or prednisolone, were subsequently treated with a combination of these drugs (additional azathioprine in 5) for median 18 months. All patients then became asymptomatic and obtained near normalization of biochemical parameters. The authors concluded that a combination of UDCA and corticosteroids is required in most cases of overlap PBC–AIH to obtain complete clinical and biochemical response. Corticosteroids could, however, be suspended in two patients, and it was emphasized that some patients may remain in remission with UDCA alone.

In a subsequent report from these authors, they described the long-term follow-up (median 7.5 years) of 17 patients with PBC–UDCA overlap, identified by the same criteria as in the previous study [71]. Eleven patients received initial therapy with UDCA alone, and six were given a combination of UDCA and immunosuppressants. Immunosuppressive therapy consisted of predniso(lo)ne 0.5 mg/kg/d, tapered when ALT levels were decreased by more than 50%, with addition of azathioprine or mycophenolate mofetil in most cases. Three of the UDCA-treated patients were considered responders, with complete biochemical response in terms of AIH features (ALT <2 times ULN and IgG <16 g/l) and decreased or stable fibrosis. The remaining eight patients were non-responders with increased fibrosis in four. Seven UDCA non-responders were subsequently treated with a combination of UDCA and immunosuppressants. After follow-up for median 3 years, six among these obtained a complete biochemical response. Among those who underwent a liver biopsy, fibrosis was unchanged or decreased. Among the six patients receiving combined therapy from start, four achieved complete biochemical response, and fibrosis did not progress. Overall, fibrosis progression in non-cirrhotic patients occurred more frequently under UDCA monotherapy (4/8) than under combined therapy (0/6) ($P = 0.04$). Several important observations were thus made in this study: (1) Biochemical response occurred in only a minority of UDCA-treated patients, whereas it was the rule in those receiving combined therapy, (2) Non-responders to UDCA monotherapy responded to combined therapy, and progression of liver fibrosis was significantly more frequent in patients who received UDCA than in those given combined therapy, (3) Of note, there was a strong association between complete biochemical response of the AIH component and absence of progression of fibrosis. The authors concluded that a combination of UDCA and immunosuppressive therapy is able to induce biochemical response and to stop the progression of fibrosis in nearly all patients with strictly defined PBC–AIH "overlap syndrome" and that combined therapy appears to be superior to UDCA monotherapy [71].

In another study, 9 (75%) among 12 patients with features of PBC and AIH entered remission on a conventional corticosteroid regimen, a response that was comparable to that in patients with definite AIH with a similar follow-up [35]. The PBC–AIH patients progressed to cirrhosis less frequently than the AIH cases. Response to corticosteroids was associated with serum ALP levels less than twice ULN before treatment start. It was concluded that corticosteroids can be effective therapy in patients with features of both PBC and AIH.

In a study including 20 cases of a PBC–AIH overlap condition, 16 were treated with UDCA and steroids, and eight of these received additional azathioprine [55]. Transaminase levels fell below twice ULN in all 16 patients. In 14 among the 16 cases, both AST and ALT normalized. ALP levels normalized in the majority of patients and stayed above 1.5 times ULN in only three cases. The data supported the practice to treat PBC–AIH overlap patients with a combination of UDCA and immunosuppressants. It was underscored, however, that therapy should be individualized with more hepatitic features being a stronger indication for additional immunosuppression. In a case report on PBC–AIH overlap, cirrhosis was apparently even reversed on a combined treatment of UDCA and prednisone [72]. Combined therapy with UDCA and steroids in 15 PBC–AIH overlap cases was also associated with biochemical response in 77% of cases in a recent report [73].

The term PBC–AIH "overlap syndrome" is most commonly used to denote patients with a simultaneous occurrence of features of PBC and AIH, but consecutive presentation of these disorders has also been observed. In a review of 282 PBC patients, 5 (1.8%) patients had an initial diagnosis of AIH and developed typical PBC during follow-up, and 12 (4.3%) typical PBC patients developed AIH while on UDCA therapy [42]. In the latter group, the diagnosis of AIH was made from 6 months to 13 years after diagnosis of PBC. Ten patients were initially treated with prednisone 0.5 mg/kg/d, with maintenance therapy consisting of prednisone 10–15 mg/d ± azathioprine 1.5 mg/kg/d. Sustained remission was obtained in eight patients, whereas two had multiple relapses following short-term remission and died 7 and 8 years after diagnosis of AIH. Two patients with remission under UDCA therapy did not receive steroids. Partial or complete remission of AIH during UDCA therapy has been observed by others [74, 75] and could be used as an argument to avoid corticosteroids in patients with PBC–AIH overlap. Based on their overall experience in the above study, however, the authors concluded that it seems justified to give conventional AIH therapy to PBC patients with flare-ups of aminotransferase levels if they also fulfill the criteria of AIH [42].

Some data suggest that budesonide is a promising alternative to conventional immunosuppression to induce remission in AIH, and this drug has also been successfully used in a few patients with PBC–AIH overlap [76]. On the other hand, the addition of budesonide to UDCA for 1 year in 22 PBC patients with an incomplete response to UDCA therapy did not result in any obvious beneficial effect, but was associated with a significant progression of osteoporosis [77]. PBC–AIH overlap patients without satisfactory response to UDCA and corticosteroids have in a few cases been treated with cyclosporine A and obtained a beneficial effect [48, 78].

Studies Indicating that UDCA Alone is Sufficient Therapy in PBC–AIH Overlap Conditions

A few reports have indicated that response to UDCA therapy is similar in PBC patients with and without features of AIH. Biochemical response after 2 years of UDCA treatment and survival after 7 years of follow-up did not differ significantly in a comparison of 12 patients with PBC–AIH overlap with 159 patients with PBC treated for the same period [47]. Comparable response to UDCA in PBC–AIH overlap and PBC patients was also recorded in a study including 20 patients with PBC–AIH overlap (16 treated with UDCA and four with UDCA plus prednisolone) and 23 PBC patients (all treated with UDCA) [58]. It was suggested that UDCA is the first-line treatment, but that non-responders to UDCA may benefit from a combination of UDCA with prednisolone.

Impact of PBC–AIH Overlap on Prognosis

In a comparison of the clinical course of 26 patients with features of PBC–AIH overlap with that of 109 patients with PBC alone during 5–6 years, an association of PBC–AIH overlap with a higher risk of portal hypertension and progression to death and/or liver transplantation was suggested [79]. Only a limited number of patients overall had received treatment with UDCA. Some patients in either group had also been treated with other drugs. The authors concluded that a worse prognosis in PBC–AIH overlap could justify the risk of immunosuppressive therapy, but that a large, randomized study would be required to establish this strategy.

In a study of liver biopsies from PBC patients before and after 4 years of treatment with UDCA, the severity of lymphocytic hepatocyte piecemeal necrosis and lobular inflammation in the first biopsy was significantly associated with progression of fibrosis, giving support to the contention that features of AIH in PBC patients have a negative impact on prognosis [20]. The study further suggested that UDCA improves the bile duct destruction, but not the process leading to piecemeal necrosis and lobular inflammation which thus may need additional therapy. The severity of lymphocytic piecemeal necrosis proved to be an independent predictor of prognosis in UDCA-treated PBC patients also in another report [80].

Among ten patients with PBC–AIH, eight were given immunosuppressive therapy and two were started on UDCA [57]. 3/8 in the first group subsequently received additional UDCA and 1/2 on UDCA was given additional prednisolone. Compared with a group of 238 AIH patients, the overlap cases were significantly less likely to obtain a complete response to conventional therapy and significantly more likely to be classified as non-responders (25 vs 0.8%; $P < 0.05$). However, this did not influence the outcome in terms of survival.

Recommendations for Therapy in Patients with PBC–AIH Overlap Conditions

The lack of standardized diagnostic criteria as well as the absence of randomized, controlled data makes it difficult to provide firm guidelines for therapy in patients with overlapping features of PBC and AIH, and no clear consensus in optimal therapy for these patients exists [4]. It still seems reasonable to consider some patients within this spectrum of disease for a combined therapy with UDCA and corticosteroids. The recent European Association for the Study of the Liver (EASL) guidelines for the management of cholestatic liver diseases suggest that the diagnostic criteria of PBC–AIH "overlap syndrome" previously proposed by Chazouillères et al. [37] provide a useful diagnostic template (Table 12.1) [5]. Of note, histologic evidence of moderate to severe lymphocytic piecemeal necrosis (interface hepatitis) is mandatory for the diagnosis [5]. According to the EASL guidelines, combined therapy with UDCA and corticosteroids should be the recommended therapeutic option in patients with PBC–AIH overlap features. An alternative is to start with UDCA only and add corticosteroids if an adequate biochemical response has not been reached within a reasonable time (3 months) [5]. The presence of a PBC–AIH overlap condition might be the cause of resistance to UDCA in patients with PBC [59]. As is the case in AIH, steroid sparing immunosuppressive agents (primarily azathioprine) should be considered in patients requiring long-term immunosuppression. Since corticosteroids are associated with potential deleterious side effects (in particular osteoporosis in cholestatic disorders), it is of paramount importance to be cautious and to treat patients on an individualized basis. It should also be kept in mind that the above criteria only are supplied as guidelines and that the relevance of using rather strict criteria can be discussed [59]. As previously pointed out, the premise "at least do no harm" should be kept in mind [7]. Steroid treatment should not be prolonged if a beneficial effect cannot be documented. One option is to stop steroids after a while and observe the patient under continued therapy with UDCA only. In patients with predominant and pronounced hepatitic features, some clinicians will choose to make an initial therapeutic trial with corticosteroids alone. In end-stage liver disease, liver transplantation is the treatment of choice.

Treatment of Patients with Overlap PSC–AIH

Currently there is no effective medical therapy available in PSC. UDCA has been used in PSC patients to a certain extent, but a long-term survival benefit has not been proven [81–88]. Recently, even a higher risk for death or liver transplantation and serious adverse events in UDCA-treated patients compared with a placebo group was reported [89]. This study applied a higher UDCA-dose (28–30 mg/kg/d) than previously used, so a direct toxic effect of high-dose UDCA may be a possibility [89, 90]. This observation has led to a higher awareness about potential detrimental effects

of UDCA in PSC and warnings against its use [91]. No studies have supported a definite role of various immunosuppressive agents in PSC [92], although favorable effects in some patients have been suggested with [93] or without [94] combination with UDCA. Still, it is possible that such drugs can have a positive effect in the subgroup of PSC patients with concurrent features of AIH. As is the case in PBC–AIH overlap conditions, no randomized, controlled therapeutic trials have been performed in patients with overlapping features between PSC and AIH. Treatment therefore is not evidence-based, and therapeutic traditions may vary between centers.

Studies of Immunosuppression ± UDCA in Patients with PSC–AIH Overlap Conditions

There are several reports on patients with PSC–AIH overlap who have been treated with corticosteroids or a combination of corticosteroids and azathioprine. In an early case report in 1992, a combination of prednisone (40 mg/d, tapered to 10 mg/d) and azathioprine (50 mg/d, increased to 150 mg/d) resulted in significant clinical improvement and near normalization of serum transaminase levels from an initial elevation of about ten times ULN [95]. In a small series of three patients, all appeared to benefit from a combination of steroids, azathioprine, and UDCA [96]. The authors commented that it is important to distinguish PSC–AIH overlap patients from ordinary PSC, since immunosuppressive treatment can completely suppress the hepatocellular inflammatory component of this condition. A corresponding experience was reported in another five patients who all demonstrated a marked clinical and biochemical response to prednisolone and azathioprine [97]. Four of these patients had relapses during subsequent reduction or withdrawal of therapy. Histological improvement was also noted in these four cases, except for progression of the biliary lesions. Normalization of biochemical parameters was noted in a case report of a patient with combined hepatitic and cholestatic features and cholangiographic findings consistent with PSC [98]. Among four PSC–AIH patients treated with steroids in another study, partial response was recorded in three cases and deterioration in one [60]. A positive biochemical response to cyclosporine in a patient with overlapping features between PSC and AIH has also been observed [99].

Impact of PSC–AIH on Prognosis

In a study comparing the results of corticosteroid therapy in variant syndromes of autoimmune liver disease, remission was less common in patients with a PSC–AIH overlap condition than in patients with definite AIH or overlap PBC–AIH [35]. Among nine PSC–AIH patients, only two (22%) obtained remission. Overall, results in this subgroup were less favorable. The patients with PSC–AIH overlap died of liver failure or required liver transplantation significantly more frequently than did patients with classical AIH.

Biochemical response to immunosuppressive therapy was obtained in all of nine patients with overlapping features of PSC and AIH in another study [43]. Three patients achieved long-term remission, but three needed liver transplantation after 4 months, and 7 and 9 years, respectively. A therapeutic effect of immunosuppression in PSC–AIH overlap conditions inferior to that regularly obtained in classical AIH was supported, and the authors speculated that immunosuppressive treatment does not influence the PSC component of the overlap condition. During the course of their disease, six patients also received UDCA therapy with resulting slight biochemical improvement in some cases.

In a prospective study of seven patients with AIH–PSC overlap syndrome treated with prednisolone (initial dose 0.5 mg/kg/d, tapered to 10–15 mg/d) and azathioprine (initial dose of 2 mg/kg/d, maintenance dose of 50–75 mg/d) plus UDCA (15–20 mg/kg/d), a significant reduction in serum AST levels was obtained over a 5-year course [61]. A reduction in ALT levels was also observed, but this was not significant. Serum levels of ALP and GGT did not change significantly. Among the 34 classical PSC patients receiving UDCA therapy only and followed in the same study, no significant changes in biochemical parameters occurred. The Mayo score prognostic index did not change significantly in the overlap cases, in contrast to a significant increase (sign of disease progression) observed in the classical PSC group. Liver transplantation was carried out in 1/7 cases with overlap and 6/34 cases with classical PSC. Cholangiocarcinomas (five cases) and deaths (nine cases) only occurred in the latter group. Notably, the survival among the PSC–AIH overlap cases appeared to be better than that in classical PSC patients.

In a comparison of 16 patients with PSC–AIH overlap syndrome with ten PBC–AIH- and 238 AIH patients on similar immunosuppressive therapy, the PSC–AIH cases had a significantly reduced survival (hazard ratio 2.08 and 2.14, respectively), despite the fact that the majority had a good initial response [57].

In a retrospective study of PSC patients treated with corticosteroids, it was concluded that a long-term beneficial effect on disease progression may be obtained in a subgroup of patients [94]. The subgroup classified as responders had features similar to patients otherwise denoted PSC–AIH overlap cases. The group was characterized by having significantly higher serum levels of transaminases and bilirubin, but lower ALP levels, at treatment start than non-responders. They also tended to be younger. More patients among responders than non-responders had positive scores for histological features of AIH, although the difference was not statistically significant.

Conclusion: Recommendations for Therapy in Patients with PSC–AIH Overlap Conditions

Considerations regarding the managing of patients with overlapping features of PSC and AIH are in line with those in PBC–AIH overlap conditions outlined above, except that the role of UDCA is questionable. Recently both EASL [5] and American Association for the Study of Liver Diseases (AASLD) [91] guidelines recommended

the use of immunosuppressive therapy in patients with a PSC–AIH overlap. In the EASL guidelines, UDCA is recommended along with immunosuppressive therapy, whereas the AASLD only recommends corticosteroids and other immunosuppressive agents. In our center, we select for such therapy PSC patients who present with markedly increased serum aminotransferase levels (usually at least five times ULN) with or without elevated IgG concentration, in combination with a liver biopsy with clear features of AIH. We usually start with prednisolone 45 mg/d, tapering the dose like in AIH. Azathioprine is added as a steroid sparing agent according to guidelines for AIH. However, treatment is not evidence-based and as in PBC, there are concerns about side effects particular skeletal. If normalization of serum aminotransferase levels is not easily obtained, we tend to accept higher levels during follow-up than in AIH. PSC–AIH patients are candidates for liver transplantation in end-stage liver disease.

Chapter Summary

1. Randomized, controlled therapeutic trials in patients who present with overlapping features between the autoimmune liver diseases (i.e., PBC–AIH- and PSC–AIH "overlap" conditions) are impossible to conduct due to the lack of standardized diagnostic criteria, patient heterogeneity, and the low prevalence of such cases. Treatment recommendations therefore are not evidence-based.
2. Efforts should always be made to define the primary disorder (PBC, PSC, or AIH), and appropriate treatment should be given accordingly.
3. Addition of immunosuppressive therapy (corticosteroids ± azathioprine) should be considered in patients with overlapping conditions between PBC- or PSC and AIH. Treatment must be individualized, with high attention to potential side effects (in particular osteoporosis).

Useful Tips for Practitioners

1. In patients with PBC or PSC and disproportionally elevated serum aminotransferase activities and/or IgG concentration, a liver biopsy should be considered for assessment of histological features of AIH.
2. The possibility of PSC must be considered in patients classified as AIH but presenting with one or more features typical of PSC (cholestatic liver tests, histological evidence of bile duct injury, concomitant inflammatory bowel disease, poor response to immunosuppressive therapy).
3. Immunosuppressive therapy in patients with overlapping features between PBC or PSC and AIH should not be prolonged if a beneficial effect cannot be documented.
4. Liver transplantation is the treatment of choice in patients with end-stage liver disease.

Common Pitfalls

1. Biopsy interpretation in the absence of clinical correlation can be misleading.
2. Cirrhosis can give changes to the biliary tree on imaging that mimics PSC.
3. Overlap features should be considered carefully and slowly with a longitudinal perspective taken; treatment decisions rarely need to be rushed.

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Chapter 13
Autoimmune Hepatitis: A Look Toward the Future

Gideon M. Hirschfield

Over the last 50–60 years, autoimmune hepatitis as a complex disease has evolved in many ways, most notably for the now excellent outcomes for patients. When thinking about the future of any disease, and its management, it is sometimes helpful to frame one's thoughts about what patients are asking in clinic, in particular which questions you as the clinician find hardest to answer. Patients ask many things, but frequent questions I am faced with which I find variably hard to answer definitively include:

(a) Why did I get this disease?
(b) Will it recur if I stop treatment?
(c) Why aren't there more specific treatments with fewer side effects?
(d) Will my family get this, or another associated autoimmune disease?
(e) Are the long-term side effects of treatment worth the risk if I feel so well?
(f) If I need a liver transplant will the disease come back?

Although imperfect, the knowledge base we presently have does allow us to start to answer these questions. We hope that this textbook has gone some way to consolidating these, and other many day-to-day issues, faced by clinicians. We have attempted to cover the biology of the disease and its associated autoantibodies, as well as the clinical presentation in adults and children. Therapies for AIH, albeit apparently simple, require careful thought before initiation. Our authors describe the present varied treatment guidelines, including when to stop and when to consider more novel agents. Finally, we touch upon the contentious issue of overlap syndromes, which causes so much angst and confusion for clinicians and patients.

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Overall, despite this concise but thorough look at this disease, it will be clear to the reader that regardless of our advances, AIH remains enigmatic and without a cure. Managing autoimmune liver disease, in particular AIH, will for the foreseeable future remain a traditional balance of science and art, until such time as we have truly representative descriptions of the disease and its triggers. Varied clinical, laboratory, histologic, and treatment responses suggest that while the disease carries one name, in reality it is likely a collection of many subtypes of disease. Looking to the future, accurate phenotyping of patients seems key to unlocking this substructure, as that approach has the greatest potential to allow investigators to unscramble the triggering etiologies, alongside the individual predisposition and inherent responses to liver injury and its treatment. We will need to move beyond the traditional type 1 and 2 descriptors and provide means of classifying patients with greater precision. Collaborative efforts are essential for such a process to be successful, given the relative rarity of the disease. The tools for such studies already exist and will need to harness both human and animal material. Genomics, whole genome sequencing, proteomics, and metabolomics are all hypothesis-free approaches that may come close to distinguishing the complex genetic, protein, and cellular changes in patients with AIH. Because it is nearly impossible to investigate patients before disease is clinically apparent or identified, mouse models will remain an essential translational tool to test and develop disease hypotheses. Already there are exciting discoveries to suggest that regulation of T cell function may be amiss in AIH, and may be potentially a target for therapy. Alongside this idea are discoveries in the related autoimmune diseases, primary biliary cirrhosis, and primary sclerosing cholangitis, which are defining the genetic architecture of disease predisposition.

The goal of twenty-first century medicine remains to provide personalized care that is disease-specific, and which maximizes treatment efficacy while minimizing side effects. With this in mind hopefully this textbook will look very different if written again by the next generation of clinicians in 50 years.

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