Hiromasa Ohira *Editor*

Autoimmune Liver Diseases

Perspectives from Japan

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Preface

The liver is a unique organ that can induce immune tolerance but can also be affected by autoimmune disorders. For this book, we invited leading Japanese scientists in the field to outline recent advances in autoimmune hepatitis (AIH) and primary biliary cirrhosis (PBC), the two major autoimmune liver diseases caused by immune disorders. It is our great pleasure to have the opportunity to introduce to the world's readers the current clinical approaches in treating AIH and PBC in Japan as well as various clinical challenges and research results.

Recent epidemiological studies on AIH have raised questions about the pathogenesis, medical therapy, and treatment outcomes of the condition. In response to this trend, new approaches for analyzing and interpreting its etiology and pathology, including new animal models, have been introduced. Although most Japanese cases of AIH are responsive to steroid therapy, some cases undergo a relapse of inflammation during steroid reduction. Furthermore, some cases even present acute hepatitis-like manifestations in the form of severe hepatitis or acute liver failure (fulminant hepatitis, late-onset liver failure), which can be treatment-refractory. Recent studies have revealed that the pathogenesis of acute hepatitis-like manifestations includes two phases: the acute exacerbation phase and the acute hepatitis phase. Patients in the acute hepatitis phase may have low serum IgG levels and negative or low autoantibody titers, making diagnosis difficult. Attention has also been paid to the treatment of mild and elderly cases, including cases in which steroid treatment can be discontinued, cases in which there is an overlap with nonalcoholic steatohepatitis (NASH), and cases of IgG4-related AIH.

Concerning PBC, a study group of the Ministry of Health and Welfare (currently The Ministry of Health, Labour and Welfare) has been conducting an epidemiological study since 1980 and has created one of the world's largest databases including more than 8,500 cases of PBC thus far. Moreover, taking advantage of the homogeneous racial population of Japan, prognosis prediction by analyzing SNPs and autoantibodies, such as anti-gp210 and anti-centromere antibodies, has become increasingly feasible. The recent introduction of genome-wide association study (GWAS) has also contributed to the generation of research findings that are more relevant to the pathogenesis of the disease. There has also been an

accumulation of data on the effectiveness of bezafibrate. New pathological staging/activity grading criteria and clinical practice guidelines have also been established. The world's first case of adult living-donor liver transplantation was performed in Japan in a patient with PBC. Unlike in Western countries, most cases of PBC undergo living-donor liver transplantation as the first-line treatment in Japan, with favorable outcomes.

I hope that this book will be helpful in facilitating clinical and research activities on autoimmune liver diseases. Finally, I would like to thank all of the authors for their contributions as well as Springer Japan for their efforts in publishing this book.

Fukushima, Japan Hiromasa Ohira

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Part I
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Chapter 1
Pathogenesis of Autoimmune Hepatitis

Hiroki Takahashi

Abstract Autoimmune hepatitis (AIH) is a chronic inflammatory liver disease and its pathogenesis is characterized by an autoimmune reaction to hepatocytes. Initiation of the disease can be related to both environmental and genetic factors, and AIH is likely to develop in people with predisposed genetic backgrounds who have been exposed to conducive environmental factors, such as viral infections or drugs. In autoimmune diseases, autoantigens stimulate autoreactive cytotoxic T cells and induce a pathological autoimmune reaction; however, AIH-specific autoantigens and mechanisms of onset remain unknown. Under normal physiological conditions, autoimmune reactions are negatively regulated by immunological tolerance and the loss of tolerance is an important component in the pathogenesis of AIH. The loss of tolerance may be caused by abnormalities of regulatory cells such as Foxp3-positive $CD4^+$ T cells, which participate in the maintenance of tolerance. Additionally, the interaction among several kinds of immune cells (dendritic cell, T cell, B cell, natural killer cell, and natural killer T cell), hepatocytes, and non-parenchymal cells (Kupffer cell, hepatic stellate cell, and sinusoidal endothelial cell) may also participate in the pathogenesis. Identification of the autoantigens as well as the cellular interactions involved is critical for a complete understanding of the pathogenesis of AIH.

Keywords Autoantigen • Autoimmune hepatitis • Effector cells • Pathogenesis • Regulatory T cells

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1.1 Introduction

1.1.1 Overview of the Pathogenesis of Autoimmune Hepatitis

Autoimmune hepatitis (AIH) is a chronic inflammatory liver disease that is characterized by an autoimmune reaction to hepatocytes. Although its pathogenesis has been extensively studied for a long time, the detailed mechanisms are unknown and many questions remain (Fig. 1.1).

1. What are the *genetic factors* that relate to the susceptibility and disease progression of AIH?
2. What are the *environmental factors* that relate to the initiation of AIH?
3. How does the *innate immune response* relate to the pathogenesis of AIH?
4. How do antigen-presenting cells, such as dendritic cells, activate autoantigen-specific T cells?
5. What is the disease-specific *autoantigen*?
6. How are the autoantigens released from hepatocytes and captured by antigen-presenting cells?
7. How is the *immune tolerance* of autoreactive T cells broken?

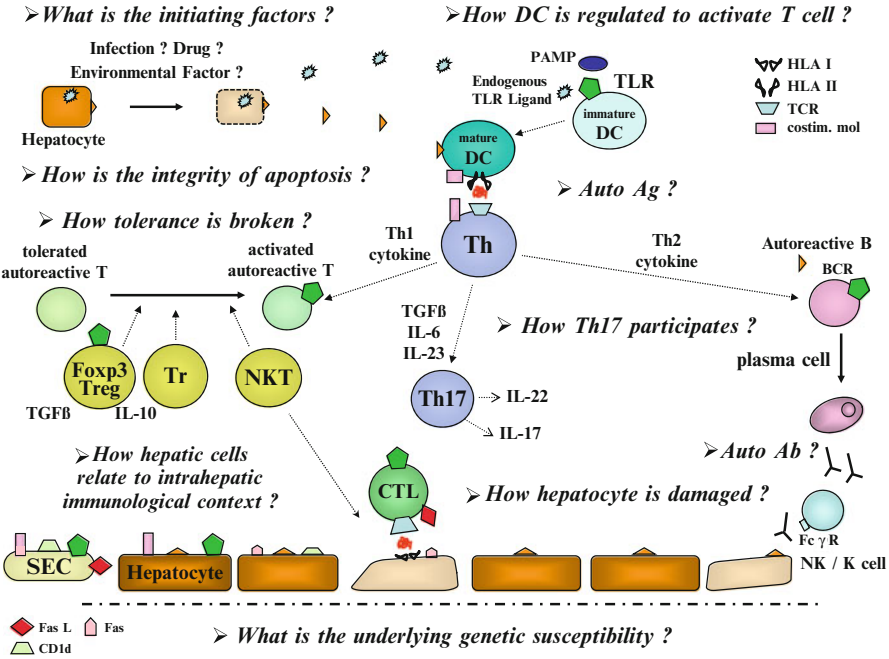

Fig. 1.1 Unsolved questions in the pathogenesis of AIH

8. Do abnormalities of frequency and function of *regulatory T cells* exist? And how do these cells relate to the loss of the immune tolerance to autoreactive T cells?
9. How do effector cells damage hepatocytes?
10. How do effector cells migrate into the liver?
11. How are autohepatocytes damaged by effector cells?
12. How do non-parenchymal cells participate in the pathogenesis of AIH?
13. Do *autoantibodies* participate in hepatocyte damage?

1.1.2 The Events and Factors That Relate to the Pathogenesis of AIH

The pathogenesis of AIH is considered to follow for sequential events.

1.1.2.1 Initiation of Disease

The first event is the initiation of disease and both environmental and genetic factors may participate. AIH may develop when people with a disease-specific genetic background are exposed to conducive environmental factors such as viral infections or drug.

1.1.2.2 Appearance of an Autoantigen and the Development of Autoimmune Reaction

The second event is the autoimmune response to an autoantigen on hepatocytes, including the production of autoantibody. The appearance of autoantigen is considered the most important event in the pathogenesis of AIH; unfortunately, the AIH-specific autoantigen has not been identified and the underlying mechanism of its appearance has not been analyzed. Currently, this is the biggest challenge in the research of the pathogenesis of AIH.

Under normal physiological conditions, autoimmune reactions are negatively regulated by immunological tolerance. The loss of immunological tolerance occurs in AIH by unknown mechanisms. Recent progress in basic immunology research has revealed that immunological tolerance is maintained by regulatory T cells such as Foxp3-positive CD4 T cells (Tregs). It is possible that abnormalities in the number and/or function of regulatory cells may result in the loss of immunological tolerance in AIH.

1.1.2.3 Damage to Hepatocytes

The third sequential event is the actual damage to hepatocytes mediated by effector cells. Autoantigen-specific *cytotoxic T cells* (CTLs) are considered primary effector cells that damage hepatocytes. However, recent studies of another organ-specific autoimmune disease such as rheumatic arthritis indicate that other effector cells, such as Th17 cells and NKT cells, can also participate in the pathogenesis of disease.

1.1.2.4 Maintenance of Chronic Inflammation

Finally, the last step is the maintenance of chronic intrahepatic inflammation. Several factors are involved in chronic inflammation including endogenous danger molecules, which are derived from dying cells and considered to stimulate innate cells. Nonetheless, the precise mechanisms controlling the chronic autoreactive response in AIH remain unknown.

1.2 Environmental and Genetic Factors Related to the Initiation of AIH

AIH is considered initiated when people with predisposed genetic backgrounds are exposed to conducive environmental factors, such as viral infections or drugs.

1.2.1 Environmental Factors

The most well-described environmental factors related to the initiation of AIH are viral infections and drugs (Table 1.1).

1.2.1.1 Viruses

The viruses reported to initiate the development of AIH includes measles virus, hepatitis A virus, Epstein–Barr (EB) virus, herpes virus, and cytomegalovirus [1].

1.2.1.2 Drugs

Several drugs including oxyphenisatin, methyldopa, nitrofurantoin, diclofenac, interferon, pemoline, and minocycline have all been reported to initiate the

Table 1.1 Environmental factors that initiate AIH

Virus
Hepatitis A virus
EB virus
Herpes virus
Hepatitis B virus
HIV
Drugs
Statin
Atorvastatin, simvastatin
Others
Oxyphenisatin, methyldopa, nitrofurantoin, diclofenac
Interferon, pemoline, minocycline

development of AIH. Recently, several cases of patients who developed AIH after taking statins, such as atorvastatin and simvastatin, have also been reported [2].

1.2.1.3 How Do Environmental Factors Initiate AIH?

The mechanisms by which environmental factors initiate AIH remain largely unknown. There is speculation that interactions between drug metabolites or element of virus and the hepatocyte membrane create a neo-antigen, which becomes the autoantigen driving AIH. Furthermore, these metabolites or particles could stimulate immune cells via *toll-like receptors* (TLR) or intranuclear receptors signaling and induce immune activation. Such antigen-nonspecific immune activation may contribute to the induction of the antigen-specific autoimmune response.

1.2.2 Genetic Background

Genetic factors are considered to contribute to the susceptibility of AIH and disease progression as well as response to therapy, and human leukocyte antigen (HLA) has long been studied in this context. Recently, single nucleotide polymorphisms (*SNPs*) have also been investigated in known potential disease-specific susceptibility genes as well as genome-wide association study (GWAS), which analyzes SNPs throughout the genome to identify disease-specific susceptibility genes.

1.2.2.1 HLA

The most well-described disease-specific susceptibility gene is HLA. *HLA-DR4* (DRB1*0405) is a disease-specific susceptibility gene that is observed in approximately 60 % of Japanese patients with AIH [3]. In addition to disease susceptibility, it is clear that HLA-DR alleles relate to prognosis and response to therapy.

Table 1.2 SNPs as susceptibility gene of AIH

CTLA-4 (exon 1 + 49)
Yes: UK, China
No: Brazil, Germany, Japan
TNF-α (promoter −308)
Yes: UK (DRB1*0301)
No: Brazil, China
VDR (exon 2)
Yes: Germany, China

DRB1*04 relates to prognosis, and, compared with patients with other HLA-DR alleles, DRB1*13 which is frequently observed in patients who are negative for DRB1*04 positively correlates with response to therapy in those patients carrying the DRB1*13 allele and who do not relapse during maintenance therapy.

Interestingly, ethnic differences between Japanese and Caucasian patients with AIH are observed in the HLA genotype. In Caucasian patients, HLA-DR3 (DRB1*0301) is most frequently observed, followed by DR4 (DRB1*0401). HLA-DR3 is not observed in Japanese patients. There is also a difference in susceptible age and disease activity between patients with DR3 and patients with DR4 among Caucasian patients. Patients with DR3 are young and have severe disease activity, whereas those with DR4 are middle-aged or elderly and have mild disease activity. Because Japanese patients with DR4 are middle-aged or elderly and have mild disease activity, the effect of HLA-DR4 as a susceptibility gene may reach beyond ethnic differences.

1.2.2.2 SNPs

Genetic polymorphisms have also been studied as candidates of disease-specific susceptibility in AIH. SNPs within particular transcripts such as *cytotoxic T lymphocyte-associated antigen-4* (*CTLA-4*) on chromosome 2 [4], tumor necrosis factor-α (TNF-α) on chromosome 7 [5], and *1,25-dihydroxyvitamin D receptor* on chromosome 14 [6] have been reported to confer disease-specific susceptibility in patients with AIH. SNP within CTLA-4 has also been reported to confer susceptibility to other autoimmune liver diseases, including primary biliary cirrhosis (PBC). However, the results of SNP studies differ from countries and are still controversial (Table 1.2).

1.2.2.3 Genome-Wide Association Study (GWAS)

GWAS, which analyzes SNPs throughout the genome, is performed to identify disease-specific susceptibility genes in several autoimmune diseases. In PBC, GWAS of Japanese patients identified SNPs in several molecules, in the immunological pathway as disease-specific susceptibility genes [7]. GWAS of Japanese

Table 1.3 Epigenetic changes

Methylation of DNA
Modification of histone protein
Substantively change of messenger RNA by microRNA

patients with AIH is currently in progress and is expected to yield new and valuable information on the mechanism of this disease.

Thus the influence of SNPs on the function of a molecule is an important point to remember. When disease-specific SNPs are identified, it will be critical to analyze how the genetic change influences the expression or function of the protein to completely understand how SNPs participate in the pathogenesis of diseases.

1.2.3 Epigenetic Changes

While the importance of environmental factors that initiate autoimmune diseases has been recognized, the underlying mechanisms remained unknown for a long time.

Recently, epigenetic changes such as DNA methylation, histone modification, and substantive changes in messenger RNA by microRNAs, which alter gene expression and/or function without modifying the base sequence, have been linked to environmental factors (Table 1.3). Presumably, an autoimmune response may be the result of epigenetic changes, which are induced by environmental factors, in genes related to immune function in immune cells or non-parenchymal cells. Analysis of epigenetic changes in immune cells and non-parenchymal cells will give us new knowledge regarding the interaction between environmental and genetic factors that participate in the pathogenesis of AIH.

1.3 The Role of Innate Immune Response in the Pathogenesis of AIH

Recent advances in basic immunology have revealed the mechanisms of innate immune response that recognize pathogen-associated molecule patterns (PAMP) using pattern-recognizing receptors (PRRs) such as TLRs on immune cells.

The innate immune response mainly participates in infectious immunity such as antibacterial and antiviral responses. Innate immune responses also participate in inflammatory responses in the absence of infection. For example, damage-associated molecular patterns (DAMPs) such as nucleic acid or uric acid, which are released from necrotic cells, also stimulate the innate response by binding to TLRs expressed on immune cells, thereby inducing inflammatory responses through the activation of the inflammasome pathway or cytokine production.

Table 1.4 How innate immune response participates to the pathogenesis of AIH

Does it participate in the initiation phase?
Does it participate in the chronic inflammatory phase?

Although detailed mechanisms remain unknown, such phenomenon may participate in the initiation phase and/or chronic inflammatory phase of AIH (Table 1.4).

1.4 The Role of Acquired Immunity in the Pathogenesis of AIH

Generally, the primary pathogenesis of autoimmune diseases is the autoantigen-specific acquired immune response. There are several essential concepts that need to be understood in the autoantigen-specific acquired immune response in AIH.

1.4.1 What Is an Autoantigen?

In type II AIH, which is rarely found in Japanese patients, CYP2D6 has been identified as the disease-specific autoantigen [8]. Furthermore, the epitopes that are recognized by autoreactive T cells have also been identified [8]. In contrast, the disease-specific autoantigen of type I AIH, which is frequently found in Japanese patients, has not yet been identified. In fact, the disease-specific autoantibody of type I AIH is an antinuclear antibody, which likely recognizes several antigens.

1.4.2 How Is the Autoantigen Revealed?

The mechanisms of the appearance of autoantigen also remain unknown (Fig. 1.2).

1.4.2.1 *Neo-antigen* Induced by Initiation Factors

It is hypothesized that the interaction between drug metabolites or viral particles and the hepatocyte membrane leads to the formation of a neo-antigen, which then becomes the autoantigen.

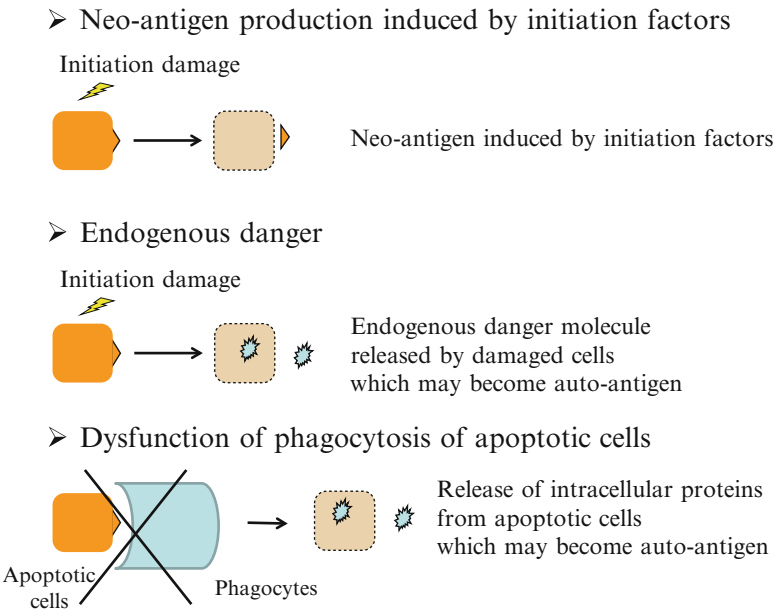

Fig. 1.2 How does an autoantigen appear?

1.4.2.2 Endogenous Danger Molecules

Intracellular proteins released by damaged cells may work as "endogenous danger molecules" and become autoantigen.

1.4.2.3 Dysfunction of Phagocytosis of Apoptotic Cells by Phagocytes

Another possibility is that dysfunction of phagocytosis of apoptotic cells by macrophages may lead to release of intracellular proteins that may become autoantigens.

1.4.3 How Autoreactive T Cells Activated?

1.4.3.1 Tolerogenic Intrahepatic Dendritic Cells in Steady State

The liver is a unique organ that has the ability to induce immune tolerance. Intrahepatic *dendritic cells* (*DCs*) exist in an immature condition and induce immune tolerance as tolerogenic DCs in physiological condition [9]. Efficacy of

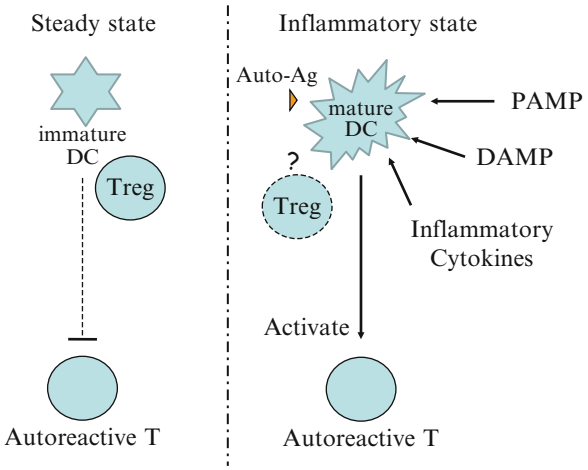

Fig. 1.3 How are intrahepatic DCs activated?

tolerance induction by intrahepatic DCs compared with DCs that exist in other organs has also been reported. There is a possibility that regulatory T cells such as Foxp3-positive regulatory T cells (Tregs) cooperate to maintain intrahepatic immune tolerance (Fig. 1.3).

1.4.3.2 Activated DCs Activate Autoreactive T Cells in Inflammatory State

Once intrahepatic inflammation occurs, intrahepatic DCs are activated and stimulate T cells by presenting antigen (Fig. 1.3). Although the detailed mechanisms controlling the activation of intrahepatic DCs remain unknown, there is the possibility that PAMPs or DAMPs, which appear in intrahepatic inflammatory condition, stimulate a conversion from tolerogenic DCs to powerful antigen-presenting cells. Inflammatory cytokines such as IFN-γ or IL-12, which are produced in inflammatory conditions, may also activate DCs. Activated DCs may phagocytose the autoantigen released by hepatocytes and present it to autoreactive T cells. The reports that demonstrated higher expression of activated *co-stimulatory molecule* B7-H1 on intrahepatic DCs in patients with AIH compared with healthy control support the activated state of intrahepatic DCs [10]. Dysfunction of Tregs, which will be discussed later, may also contribute to the activation of DCs.

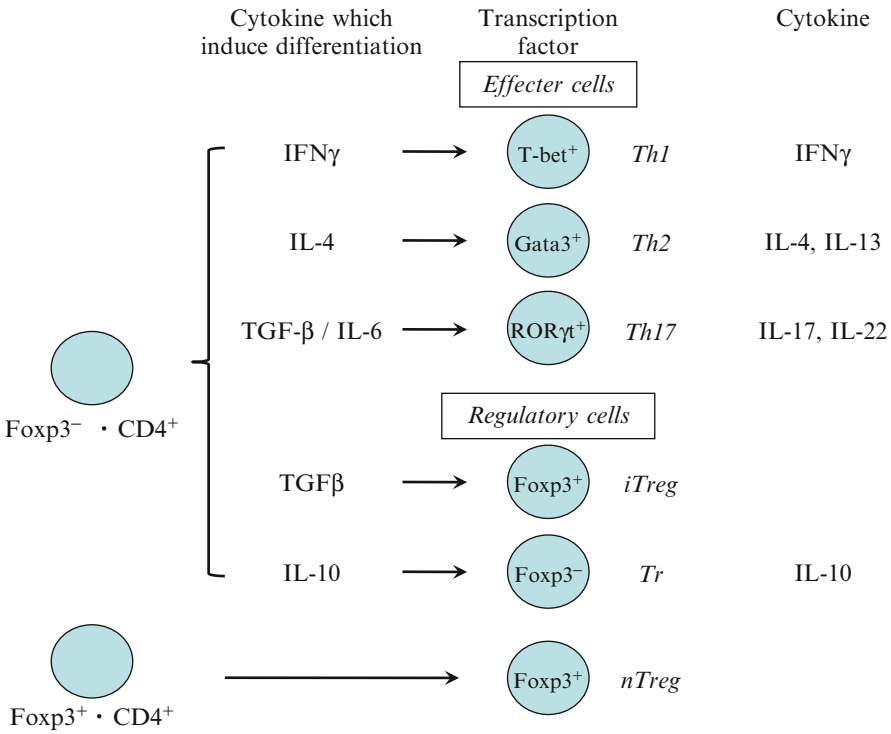

Fig. 1.4 The differentiation of CD4-positive T-cell subsets

1.4.4 What Are the Effector Cells That Damage Hepatocytes?

1.4.4.1 Cytotoxic T Cells

Because the intrahepatic infiltration of CD8-positive T cells is frequently observed in patients with AIH, CD8-positive cytotoxic T cells (CTLs) are thought to participate in damage of hepatocytes. CTLs are activated by inflammatory cytokines such as IFN-γ or IL-12, which are produced in inflammatory conditions.

1.4.4.2 Th17 Cells

Several effector and regulatory cells have been identified to be subsets of differentiated CD4-positive T cells. The cytokines that induce each subset and the transcription factors that regulate their differentiation have also been identified (Fig. 1.4). Recent reports of other organ-specific autoimmune diseases have revealed that Th17 cells, which are a newly identified CD4-positive T-cell subset

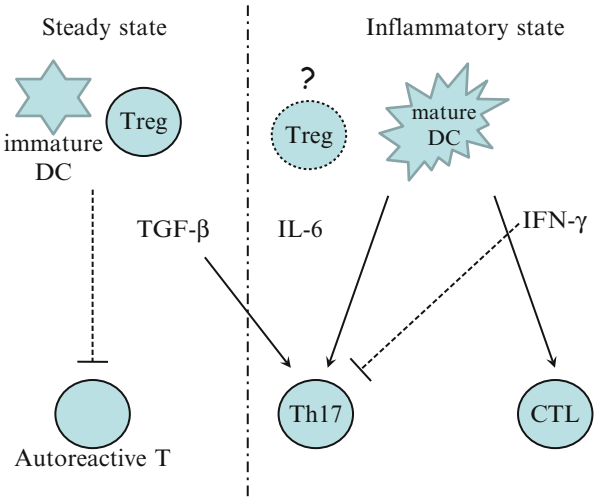

Fig. 1.5 Mechanisms of the induction of CTLs or Th17 cells

that produce IL-17, also work as effector cells. In AIH, increases in serum levels of IL-17 as well as the frequency of intrahepatic Th17 cells have been reported [11]. However, it is not clear whether Th17 cells work as effector cells in the pathogenesis of AIH.

Whether CTLs and Th17 cells work with each other as effector cells in AIH also remains unclear. Interestingly, CTLs are induced by *IFN-γ*, and Th17 cells, which are induced by *TGF-β* and *IL-6*, are inhibited by IFN-γ (Fig. 1.5). These findings indicate that the intrahepatic cytokines profile might regulate the balance of CTLs and Th17 cells. However, this hypothesis requires further investigation.

Another interesting characteristic of Th17 cells is their ability to produce IL-22. Because IL-22 has a protective role for hepatocytes in a *concanavalin A-induced mouse hepatitis* model [12], it would be interesting to investigate how IL-22 and IL-17 produced by Th17 cells relate to the pathogenesis of AIH.

1.4.4.3 Natural Killer T Cells

Invariant natural killer T (iNKT) cells are unique because they express the T-cell receptor (TCR) as well as NK-cell receptor and are constitutively distributed among several organs, including the liver where iNKT cells represent >50 % of the intrahepatic lymphocyte population. Because iNKT cells produce Th1 and Th2 cytokines during certain immune responses, they may act as effector or regulatory cells in the pathogenesis of autoimmune diseases.

iNKT cells work as effector cells to damage hepatocytes in a concanavalin A-induced mouse hepatitis model. However, it remains unknown whether iNKT cells also work as effecter cells in human AIH.

1.5 Regulatory T Cells in the Pathogenesis of AIH

In general, autoreactive T cells are eliminated in the thymus. However, some autoreactive T cells escape elimination and exist in the peripheral blood of healthy people. Such autoreactive T cells are suppressed by regulatory T cells such as Foxp3-positive regulatory T cells (Tregs) or Tr cells, which produce IL-10, in peripheral blood (Fig. 1.6). This mechanism is called "peripheral tolerance," which protects against autoimmune diseases under normal physiological conditions. However, if the number or function of regulatory cells altered, negative regulation of autoreactive T cells may be lost, thereby leading to development of autoimmune diseases. In fact, a decrease in the number of Tregs and dysfunction of Tregs has been reported in several autoimmune diseases.

1.5.1 Frequency and Function of Tregs

In AIH, a decrease in the number of peripheral blood Tregs at diagnosis compared with healthy controls has been reported [13]. Interestingly, recovery of the number of Tregs during remission has also been reported [14]. Furthermore, a loss of suppressive function of CD8-positive cells at diagnosis as well as recovery during remission has been reported [15]. These results indicate that a decrease in the number of Tregs and dysfunction of Tregs at diagnosis correlates with the onset of AIH.

These data remain controversial because another study reported that Tregs in AIH were fully functional and not reduced in number [16]. Further study is needed to resolve the role of Tregs in AIH.

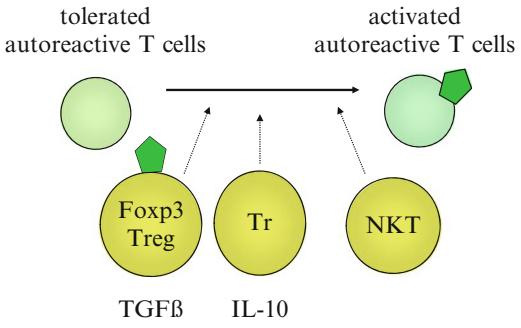

Fig. 1.6 Regulatory cell subsets that maintain peripheral tolerance

1.5.2 Natural Tregs and Inducible Tregs

There are two subsets of Tregs, *natural Tregs* (nTregs; differentiated in the thymus) and *inducible Tregs* (iTregs; induced from naïve CD4-positive, Foxp3-negative T cells), in peripheral blood or an organ in the context of inflammation. TGF-β is considered an important factor for inducing iTregs.

Because iTregs are induced in the context of inflammation, it is possible that they suppress inflammation. In an experimental mouse AIH model, which was established by injecting a fusion cell of DCs and well-differentiated hepatoma cells, an increased expression of Foxp3-positive iTregs was observed in the inflamed liver [17]. Furthermore, inflammation decreased after the expression of iTregs, suggesting that iTregs induced in the inflammatory phase work as negative regulators of inflammation [17].

However, a recent study revealed that both the expression of Foxp3 and the induction of a specific *DNA methylation pattern* are required for the regulatory function of Tregs [18]. Although nTregs have both properties and demonstrate regulatory functions, some iTregs do not have the Treg-specific DNA methylation pattern although they express Foxp3. Such iTregs do not demonstrate regulatory function. These findings indicate that iTregs, which become Foxp3 positive in the context of inflammation, possibly cannot function as a true "Treg" because they do not have the Treg-specific DNA methylation pattern. Thus, great care should be taken to carefully evaluate the significance of iTregs in the inflamed liver.

1.5.3 Balance of Tregs and Th17 Cells

Recent reports have revealed that a shift in the quantitative balance of Tregs and Th17 cells, resulting in a predominance of Th17 cells, is observed in PBC [19]. Interestingly, TGF-β is required for the differentiation of both Tregs and Th17 cells. However, IL-6 is required for the differentiation of Th17 cells but not for that of Tregs (Fig. 1.7). These observations indicate that the balance of Tregs and Th17 cells may have a role in the pathogenesis of AIH primary by regulating the dominance of effector cells and regulatory cells and that the cytokine profile may prescribe this balance.

1.6 Intrahepatic Immunological Status

Recent reports suggest that immune cells, hepatocytes, and non-parenchymal cells (*Kupffer cell*, *hepatic stellate cell*, and *sinusoidal endothelial cell*) in the inflamed liver participate in the pathogenesis of AIH by expressing several important functional molecules and interacting with lymphocyte (Fig. 1.8).

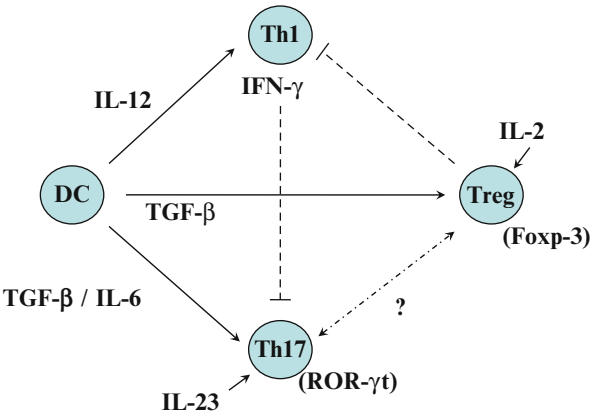

Fig. 1.7 Cross talk between Th1 cells, Th 17 cells, and Tregs

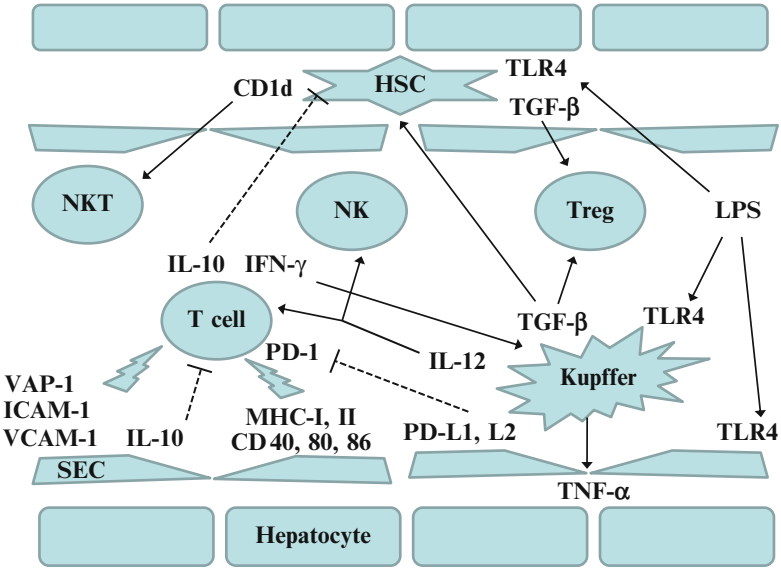

Fig. 1.8 Interaction between immune cells and non-parenchymal cells, which promote intrahepatic immunological status that participates in the pathogenesis of AIH

1.6.1 Recruitment of Lymphocytes

When sinusoidal endothelial cells express adhesion molecules such as *ICAM-1* and *VCAM-1*, which are induced by inflammatory cytokines present in the inflamed liver, the ligands of these molecules expressed by lymphocytes could attach to sinusoidal endothelial cells and migrate into the liver from peripheral blood. Because the expression of adhesion molecules is induced by inflammatory

cytokines such as IL-12, such phenomenon may occur in the inflamed liver. In addition, inflammatory cytokines may also induce the production of *chemokines* by intrahepatic immune cells or non-parenchymal cells, which participate in the recruitment of lymphocytes. Because inflammatory cytokines also induce the expression of chemokine receptors, lymphocytes could migrate easily into the site of inflammation. Therefore, to understand the mechanisms of intrahepatic recruitment of lymphocytes, it is important to analyze which *non-parenchymal cells* express which adhesion molecules as well as which cells produce which chemokines in the inflamed liver. Moreover, because a particular subset of lymphocytes responds to specific adhesion molecules and chemokines, it is also important to know the mechanisms of lymphocyte subset-specific intrahepatic recruitment.

1.6.2 Interactions of Non-parenchymal Cells, Hepatocytes, and Immune Cells

In addition to recruitment of lymphocytes, non-parenchymal cells and hepatocytes may influence the function of immune cells. For example, when non-parenchymal cells and hepatocytes express the ligand of co-stimulatory molecules, they can regulate the function of immune cells by interacting with co-stimulatory molecules expressed on the immune cells. If non-parenchymal cells and hepatocytes express B7-2 or PD-L1, which are the ligands of inhibitory co-stimulatory molecules CTLA-4 and *PD-1*, they can inhibit activated intrahepatic lymphocytes and regulate inflammation. In AIH, an increased expression of PD-1 on intrahepatic T cells and PD-L1 on Kupffer cells and sinusoidal endothelial cells has been reported [20]. The same phenomenon was also observed in type C chronic viral hepatitis. Because the expression of inhibitory co-stimulatory molecules and their ligands is induced by inflammatory cytokines, it is possible that an increased expression of inhibitory co-stimulatory molecules and their ligands, in the context of inflammation, may protect against excessive inflammation. However, it is incorrect to make such an assumption based on the analysis of only one pathway of co-stimulatory molecules because many types of co-stimulatory molecules and their ligands have been recently identified. In addition, the influence of interactions between non-parenchymal cells, hepatocytes, and immune cells through co-stimulatory molecules might be very complicated because there are two types of co-stimulatory molecules. One type can activate immune cells, whereas the other has an inhibitory function. Thus, it may be very important to comprehensively analyze these effects.

1.6.3 Interaction of Immune Cells

Several types of immune cells such as T cells, NK cells, NKT cells, Tregs, and Kupffer cells exist in the liver. Indeed, the liver has a high frequency of cells involved in innate immunity, such as NKT cells and NK cells, compared with other organs. Therefore, it is possible that there is interaction between immune cells in the inflamed liver, for example, Kupffer cells may activate T cells, NK cells, or Tregs by producing *IL-12* or TGF-β.

Many types of cells and factors participate in promoting intrahepatic immunological status, which directly contributes to the pathogenesis of AIH. Methods must be developed to evaluate the interaction of several cells and molecules.

1.7 Conclusion

As this review clearly points out, there are a lot of unanswered questions regarding the pathogenesis of AIH. At this juncture, perhaps the most important step is to identify the autoantigen. In addition, development of methods is needed for further assessing the role of multiple cell types and their interactions in the pathogenesis of AIH.

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Chapter 2
Models of Autoimmune Hepatitis

Norihiko Watanabe and Aki Ikeda

Abstract Several murine models of autoimmune hepatitis (AIH) have been described in the course of investigating the immune mechanisms involved in the development of AIH. However, those models have lacked the characteristics of human AIH, such as hypergammaglobulinemia and production of circulating autoantibodies. In contrast, we have developed mouse models of spontaneous AIH with hypergammaglobulinemia and ANA production. Immune dysregulation by a concurrent loss of naturally arising regulatory T cells and PD-1-mediated signaling induces different phenotypes of AIH in mice with different genetic backgrounds. Using these models, we have shown that the spleen is the induction site for AIH, that follicular helper T cells constitute the T-cell subset responsible for induction, and that the CCR6–CCL20 axis is crucial for the migration of dysregulated T cells from the spleen into the liver. As fatal AIH progresses, the CXCR3–CXCL9 axis is crucial for the migration of T helper 1 cells and effector $CD8^+$ T cells into the liver, causing fatal damage. Dendritic-cell-derived IL-18 is critical for differentiation of CXCR3-expressing Th1 cells and $CD8^+$ effector T cells in the spleen. In addition, we have found some clues that should help in overcoming the therapeutic insufficiency of corticosteroids for AIH patients.

Keywords Autoimmune liver disease • Chemokines • Cytokines • Pathogenesis • T-cell response

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2.1 Introduction

Autoimmune hepatitis (AIH) is an immune-mediated disorder in the liver that is characterized by the histological findings of mononuclear cell infiltration invading the parenchyma, ranging from piecemeal necrosis to submassive lobular necrosis without bile duct destruction in the portal area [1, 2]. In addition, the serologic hallmarks of AIH are elevated gamma globulins and the production of a variety of characteristic circulating autoantibodies (autoAbs), including antinuclear antibodies (ANAs) [1, 2]. There is little evidence that autoAbs play a part in the pathogenesis of AIH; liver-infiltrating T cells are considered to be the primary disease mediators of inflammatory liver damage in AIH [3, 4]. However, it had been unclear how an aberrant regulation of autoreactive T cells against liver antigens is initiated in vivo and how the dysregulated autoreactive T cells trigger the development of AIH. In addition, it had been uncertain how characteristic B-cell activation, including B-cell mediated autoimmunity, is associated with its development.

Several murine models of AIH have been described in the course of investigating the immune mechanisms in the development of AIH [4]. In those models, concanavalin A (Con A)-induced acute hepatic injury, associated with activation of natural killer T cells and T cells, has been extensively examined as an experimental model of human AIH [4–6]. However, Con A-induced acute hepatic injury does not produce circulating autoAbs; a single intravenous injection of Con A into BALB/c mice induces rapid injury of hepatocytes, with a striking increase in plasma transaminase levels within 24 h [5, 7]. Thus, this model is not likely to provide clues for understanding the molecular mechanisms that trigger AIH development.

Recently, we have developed mouse models of spontaneous AIH [8, 9]. Using these models, we have identified induction sites, responsible T-cell subsets, and key molecules for induction of AIH and described the mechanisms involved in the progression to fatal AIH [10–13]. In addition, we have found some clues to revealing varied clinical manifestations of AIH and overcoming the therapeutic insufficiency of corticosteroids for AIH patients [9].

2.2 A New Mouse Model of Spontaneous AIH

Naturally arising Foxp3$^+$ regulatory T (Treg) cells are critical in maintaining immunologic self-tolerance and in regulating a variety of pathological and physiological immune responses [14]. In patients with AIH, Treg cells are reduced numerically and functionally, and Foxp3 expression of Treg cells is lower than in normal subjects [15, 16]. However, loss-of-function mutations in the gene encoding Foxp3 in mice and humans and severe Treg-cell depletion by neonatal thymectomy (NTx) of normal mice result in the development of organ-specific autoimmune

diseases without inducing AIH [14, 17, 18]. Therefore, it had been unclear whether dysfunction of Treg cells is primarily involved in the development of AIH.

Programmed cell death 1 (PD-1), an immunoreceptor belonging to the CD28/cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) family, provides negative co-stimulation to antigen stimulation [19, 20]. One of the PD-1 ligands, the PD-L1 molecule, is constitutively expressed in the liver [19, 20]. In the absence of PD-1-mediated signaling, the local $CD8^+$ T-cell response to adenovirus infection in the liver was enhanced [21]. Mice deficient in PD-L1 displayed increased constitutive accumulation of activated $CD8^+$ T cells in the liver [22]. PD-1-deficient mice (PD-$1^{-/-}$ mice) have been shown to develop several organ-specific autoimmune diseases in mice from different genetic backgrounds [19, 20]. However, it had been unclear whether PD-1 deficiency can trigger the development of AIH [19, 20]. We have examined whether immune dysregulation by concurrent loss of Treg cells and PD-1-mediated signaling triggers AIH. We used BALB/c PD-$1^{-/-}$ mice thymectomized 3 days after birth (BALB/c–NTx–PD-$1^{-/-}$ mice) in which the number of naturally arising Treg cells was severely reduced [8]. We found that because of the massive destruction of the parenchyma of the liver, BALB/c–NTx–PD-$1^{-/-}$ mice started to die as early as 2 weeks of age, with most dying by 4 weeks [8].

Three-week-old PD-$1^{-/-}$ mice and NTx mice on a BALB/c background develop mild autoimmune gastritis; however, they do not show any inflammation of the liver [8]. In contrast, 3-week-old BALB/c–NTx–PD-$1^{-/-}$ mice develop not only gastritis but also fatal hepatitis without any inflammation of other organs. In these mice, hepatitis is characterized by a massive degeneration of hepatocytes and severe mononuclear cell infiltration in the portal area and parenchyma without bile duct destruction. In 3-week-old BALB/c–NTx–PD-$1^{-/-}$ mice, serum concentrations of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and total bilirubin are markedly increased. In addition, the diluted sera from these mice strongly stain nuclei of hepatocytes in normal BALB/c mice with a speckled pattern. These data suggest that immune dysregulation by a concurrent loss of naturally arising Treg cells and PD-1-mediated signaling can induce the development of fatal AIH.

2.3 Induction Sites and Responsible T-Cell Subsets for AIH

In BALB/c–NTx–PD-$1^{-/-}$ mice, monitoring of histological findings of the liver from 1 to 3 weeks indicates that the mononuclear cell infiltration predominantly in the portal area of the liver started at 2 weeks [8]. These mononuclear cell infiltrations rapidly progressed and were followed by massive destruction of the parenchyma of the liver. In 3-week-old BALB/c–NTx–PD-$1^{-/-}$ mice with severely infiltrating mononuclear cells in the liver, $CD3^+$ T cells predominantly infiltrated the liver. These infiltrating cells were mainly $CD8^+$ T cells and, to a lesser extent, $CD4^+$ T cells. However, depletion of $CD4^+$ T cells by anti-CD4 monoclonal antibodies

(mAbs) in BALB/c–NTx–PD-$1^{-/-}$ mice inhibited not only the infiltration of $CD4^+$ T cells but also that of $CD8^+$ T cells in the liver, resulting in suppression of fatal AIH [10]. In contrast, although depletion of $CD8^+$ T cells suppressed fatal AIH, it allowed $CD4^+$ T cells to infiltrate the liver [10]. These data suggest that both $CD4^+$ and $CD8^+$ T cells are indispensable for the development of fatal AIH and that the recruitment of $CD8^+$ T cells in the liver is regulated by $CD4^+$ T cells.

When the $CD4^+$ T-cell infiltration into the liver is started at 2 weeks of age, the spleen but not draining lymph nodes became enlarged in BALB/c–NTx–PD-$1^{-/-}$ mice [10]. Neonatal splenectomy in these mice suppressed mononuclear infiltration in the liver, suppressing fatal AIH. In addition, transfer of splenic $CD4^+$ T-cell cells from BALB/c–NTx–PD-$1^{-/-}$ mice into BALB/c–RAG$2^{-/-}$-recipient mice induced the development of hepatitis. These data suggest that the spleen is an induction site for AIH and that splenic $CD4^+$ T cells are responsible for induction of fatal AIH.

In the spleen of 2-week-old BALB/c–NTx–PD-$1^{-/-}$ mice, most of the $CD4^+$ T cells are preferentially localized within $B220^+$ B-cell follicles, whereas $CD8^+$ T cells are mainly localized outside the follicles [10]. Interestingly, in the spleen of these mice, B-cell follicles with $CD4^+$ T-cell accumulation autonomously develop germinal centers (GCs). The rapid accumulation of $CD4^+$ T cells in the follicles with GC formation depends on concurrent loss of naturally arising Treg cells and PD-1-mediated signaling, because neither PD-$1^{-/-}$ mice nor NTx mice at 2 weeks of age exhibit any of these phenotypes. In addition, transfer of Treg cells from either normal BALB/c or PD-$1^{-/-}$ mice into BALB/c–NTx–PD-$1^{-/-}$ mice suppressed GC formations in the spleen. On the other hand, concomitant administration of blocking mAbs to PD-L1 and PD-L2 induced an accumulation of $CD4^+$ T cells in the follicles and development of GCs in the spleen of BALB/c–NTx mice at 2 weeks.

Follicular helper T (T_{FH}) cells are a recently defined effector T-cell subset that constitutes a powerful helper function in B cells to form GCs [23, 24]. T_{FH} cells arise from activated T cells that express Bcl6, a master transcription factor for T_{FH}-cell differentiation. Differentiated T_{FH} cells express interleukin (IL)-21, IL-21 receptor (IL-21R), inducible costimulator (ICOS), CXC chemokine receptor (CXCR)5, and PD-1 [23, 24]. Indeed, accumulated $CD4^+$ T cells in the follicles of the spleen in BALB/c–NTx–PD-$1^{-/-}$ mice show increased mRNA expression of Bcl-6 and IL-21, and detectable protein expressions of Bcl-6, IL-21, ICOS, and CXCR5, displaying the molecular signature of T_{FH} cells [10].

2.4 Key Molecules for Induction of AIH

IL-21 and ICOS are indispensable for T_{FH}-cell generation and helper function in B cells [23, 24]. In BALB/c–NTx–PD-$1^{-/-}$ mice, either injections of blocking mAbs to ICOS or IL-21 suppressed GC formation in the spleen and accumulation of $CD4^+$ T cells in the follicles as well as development of fatal AIH, including hypergammaglobulinemia and ANA production [10]. This finding suggests a link between

generation of T_{FH} cells and induction of AIH. In addition, IL-21 has the potential to modulate the activity of $CD8^+$ T cells, and other immune and nonimmune cells, in vivo [25]. In BALB/c–NTx–PD-$1^{-/-}$ mice, IL-21 is suggested as a key cytokine for not only T_{FH} generation but also activation of $CD8^+$ T cells in AIH development [10].

After triggering inflammation, activated T cells express specific chemokine receptors in induction sites, such as draining lymph nodes. Chemokine ligands expressed by target tissues recruit those cells from induction sites and coordinate inflammatory responses. CC chemokine receptor (CCR)6 is expressed on T helper (Th)1 cells, Th17 cells, Treg cells, and human T cells that produce both IL-17 and interferon (IFN)-γ [26–29]. It is vital to the initiation of cell migration to target tissues expressing specific CCR6 ligand (CC chemokine ligand 20: CCL20) [26–29]. In BALB/c–NTx–PD-$1^{-/-}$ mice, CCR6-expressing $CD4^+$ T cells predominantly increase at 2 weeks only in the spleen and liver, not in mesenteric lymph nodes [10]. Splenic $CCR6^+CD4^+$ T cells in BALB/c–NTx–PD-$1^{-/-}$ mice contain a $CXCR5^+CCR6^+$ population, suggesting that these T cells retain the molecular signature of T_{FH} cells. In addition to $CD4^+$ T cells, CCR6-expressing $CD8^+$ T cells predominantly increase in the spleen and liver in the induction phase of AIH. Moreover, in the induction phase of AIH, gene expression of CCL20 is elevated in the liver. In BALB/c–NTx–PD-$1^{-/-}$ mice, neutralizing CCL20 by blocking anti-CCL20 mAbs induced further accumulation of T_{FH} cells in the GC^+ follicles in the spleen and inhibited infiltration of $CD4^+$ and $CD8^+$ T cells into the liver, suppressing fatal AIH. These data suggest that in the induction phase of AIH, the CCR6–CCL20 axis is crucial for migration of dysregulated T_{FH} cells and activated $CD8^+$ T cells from the spleen into the liver (Fig. 2.1).

2.5 Mechanisms Involved in the Progression to Fatal AIH

Experimental autoimmune encephalomyelitis (EAE) is a $CD4^+$ T-cell-mediated disease of the central nervous system. In EAE, Th17 cells migrate via the CCR6–CCL20 axis, triggering inflammation in the induction phase, whereas Th1 cells are mainly involved in inflamed lesions in the central nervous system during active progression [29]. In addition, T_{FH}-like cells are transiently generated during IL-12-mediating Th1 cell differentiation [30]. In mice infected with *Toxoplasma gondii*, an obligate intracellular parasite, T_{FH}-like cells were generated 7 days after infection, the proportion of T_{FH}-like cells declined, and IFN-γ producing Th1 cells increased at day 15. In BALB/c–NTx–PD-$1^{-/-}$ mice, AIH induction was started at 2 weeks of age by infiltration of dysregulated T_{FH} cells in the spleen [10]. Within 7 days of induction, the mononuclear cell infiltrations rapidly progressed, resulting in massive destruction of the parenchyma of the liver. In 3-week-old mice, expression of Th1 lineage-specific transcription factor T-bet, together with IFN-γ, tumor necrosis factor (TNF)-α, and CXCR3 was upregulated in inflamed liver tissues, suggesting that Th1-type inflammation is involved in the fatal progression

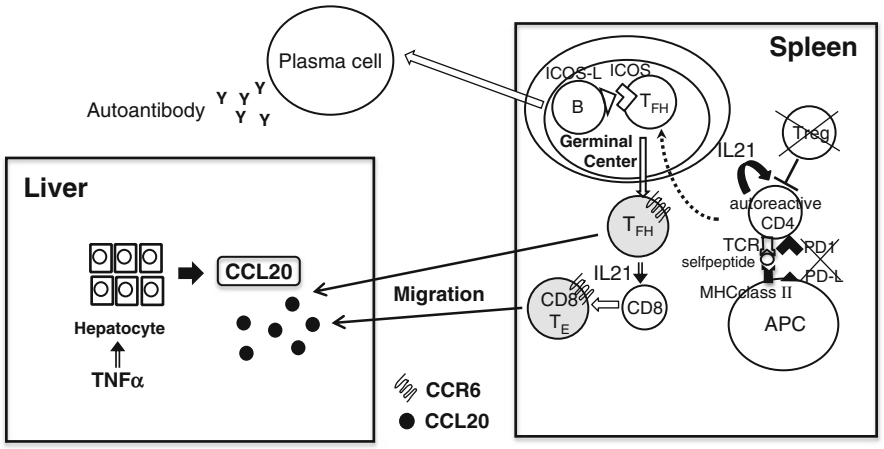

Fig. 2.1 The model of mechanistic links of cytokines and chemokines in the induction phase of AIH. In the induction of AIH in NTx–PD-$1^{-/-}$ mice, a concurrent loss of naturally arising Treg cells and PD-1-mediated signaling induces dysregulated generation of follicular helper T (T_{FH}) cells. IL-21 and ICOS are indispensable for dysregulated T_{FH}-cell generation, germinal center formation in B-cell follicles in the spleen, hypergammaglobulinemia, and ANA production. IL-21 can also induce activation of $CD8^+$ T cells (effector T cells: T_E) in the spleen. In the induction phase, T_{FH} and T_E cells express CCR6, and TNF-α induces CCL20 expression in hepatocytes. The CCR6–CCL20 axis is crucial for migration of dysregulated T_{FH} and T_E cells from the spleen into the liver

of AIH [13]. In summary, in the development of fatal AIH, different types of T cells are critically involved at different time points in both the induction and the fatal progression of AIH.

2.6 Key Molecules Involved in the Progression to Fatal AIH

In contrast to the induction phase, CXCR3-expressing $CD8^+$ T cells predominantly increased only in the spleen and liver but not in mesenteric lymph nodes in 3-week-old BALB/c–NTx–PD-$1^{-/-}$ mice [13]. In addition to $CD8^+$ T cells, CXCR3-expressing $CD4^+$ T cells predominantly increased in the spleen and liver [10]. CXCR3-expressing T cells can be guided by three ligands—CXC chemokine ligand (CXCL)9, CXCL10, and CXCL11—and expression of these CXCR3 ligands in the inflamed tissues determines inflamed tissue-specific infiltration of CXCR3-expressing T cells in various immunoinflammatory settings [31–34]. Although all three can bind to the common receptor CXCR3, differences have been reported in the kinetics and the tissue-/cell-type expression patterns of these chemokine genes and their proteins during infection or inflammatory responses [35–38]. Studies using CXCL9- or CXCL10-deficient mice have shown the nonredundant function

of these chemokines in various immunoinflammatory settings, including a hepatitis B virus transgenic mouse model and a liver injury model [35–38]. Severely inflamed livers of 3-week-old BALB/c–NTx–PD-$1^{-/-}$ mice showed markedly elevated expression of CXCL9 but not of CXCL10 and CXCL11 [13]. The serum level of CXCL9 was also elevated. In addition, administering anti-CXCL9 but not anti-CXCL10 suppressed fatal AIH. These data suggest that in the progression phase of fatal AIH, the CXCR3–CXCL9 axis is crucial for the migration of Th1 cells and effector $CD8^+$ T cells into the liver [13].

IL-12 is decisive in the development of Th1 subsets [39]. A recent study showed that IL-12 can trigger naïve T cells to transitionally differentiate into T cells with features of T_{FH} and Th1 cells [30]. However, in BALB/c–NTx–PD-$1^{-/-}$ mice, neutralizing IL-12p40 did not suppress the development of fatal AIH [10, 13]. In addition, although IFN-γ has been shown to be essential for IL-12-induced Th1 differentiation [40], neutralizing it did not suppress the development of AIH [11]. These data suggest that IL-12 is not exclusively involved in differentiation into T cells with features of Th1 cells in the progression of fatal AIH.

Serum levels of IL-18 increased in patients with AIH and fatal hepatitis [41, 42]. In humans, IL-18 produced by dendritic cells (DCs) promoted Th1 induction [43]. IL-18 stimulated Th1-mediated immune responses and activated Th1 cells, which highly express functional IL-18 receptor, producing large amounts of IFN-γ [44, 45]. In addition, in an atopic dermatitis mouse model, IL-18 induced differentiation of Th1-like cells that expressed IFN-γ and CXCR3 [46]. Indeed, in BALB/c–NTx–PD-$1^{-/-}$ mice, serum levels of IL-18 but not IL-1β were elevated, and IL-18 elevation gradually increased through the progression of AIH [13]. IL-18 is known to be produced by various types of immune cells and epithelial cells [44, 45]. In BALB/c–NTx–PD-$1^{-/-}$ mice, splenic and hepatic DCs increased IL-18 mRNA expression and isolated splenic DCs secreted IL-18 but not IL-1β [13].

IL-18 signals through the IL-18 receptor (IL-18R) complex, and IL-18R contains the heterodimer IL-18Rα and IL-18Rβ subunits. The IL-18Rα subunit is responsible for extracellular binding of IL-18, whereas the IL-18Rβ subunit is nonbinding but confers high affinity binding for the ligand and is responsible for biological signals [44, 45]. Administering anti-IL-18Rβ but not anti-IL-1β suppressed the progression of fatal AIH [13]. Neutralizing IL-18-mediated signaling reduced the number of $CXCR3^+$ cells in splenic $CD4^+$ and $CD8^+$ T cells and suppressed expression of T-bet, IFN-γ, TNF-α, and IL-18Rα in splenic $CD4^+$ T cells. In addition, although injecting anti-IL-18Rβ mAb induced enlargement of PNA^+ GC in $B220^+$ follicles, it reduced total immunoglobulin and ANA in the Th1-dependent IgG2a subclass. These data suggest that DC-derived IL-18 is involved in the differentiation of $CD4^+$T cells into Th1 cells and $CD8^+$ T cells into effector T cells, respectively, in the spleen and that IL-18-mediated signaling is critical for the progression to fatal AIH (Fig. 2.2).

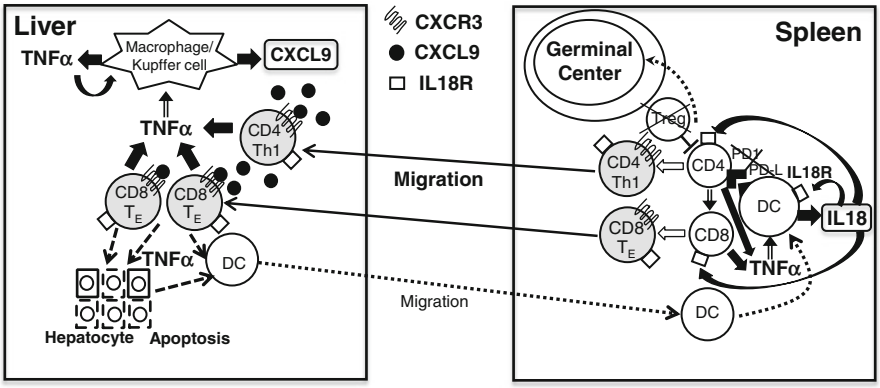

Fig. 2.2 The model of mechanistic links of cytokines and chemokines in the progression phase of NTx–PD-1$^{-/-}$ mice. In the progression of fatal AIH in BALB/c–NTx–PD-1$^{-/-}$ mice, DC-derived IL-18 is critical for differentiating CXCR3-expressing Th1 cells (Th1) and CD8$^+$ effector T cells (T_E) in the spleen. CXCL9 production by hepatic macrophages/Kupffer cells triggers migration of these T cells into the liver. CXCR3-expressing T_E and, to a lesser extent, Th1 infiltrate the liver; T_E triggers the fatal destruction of the liver. TNF-α secreted in paracrine/autocrine fashion induces uncontrollable CXCL9 expression in the liver. TNF-α may be involved in the maturation of DCs and may induce apoptosis of hepatocytes

2.7 Roles of the Inflammatory Cytokines IFN-γ and TNF-α in the Development of AIH

AIH-bearing BALB/c–NTx–PD-1$^{-/-}$ mice show markedly increased levels of mRNA expression of IFN-γ and TNF-α in the inflamed liver [13]. Infiltrating T cells in the liver have predominant production capacity of IFN-γ and TNF-α [8]. On the other hand, in 1-week-old BALB/c–NTx–PD-1$^{-/-}$ mice before the induction of AIH, the serum level of IFN-γ but not TNF-α was elevated [11, 12]. In 2-week-old mice in the induction phase of AIH, serum levels of TNF-α markedly increased, and these levels were maintained during AIH progression. In contrast, the elevated serum level of IFN-γ at 1 week of age gradually decreased during AIH progression. These data suggest that IFN-γ and TNF-α play different roles in the process of AIH development [11, 12].

In BALB/c–NTx–PD-1$^{-/-}$ mice, neutralizing IFN-γ did not suppress the development of fatal AIH as mentioned above, but rather exacerbated inflammation of the liver [11]. IFN-γ acts as a critical proinflammatory mediator in autoimmune processes, whereas it exerts regulatory functions to limit tissue damage associated with inflammation [47–49]. In BALB/c–NTx–PD-1$^{-/-}$ mice, neutralization of IFN-γ did not induce any aberrant differentiation of T cells in the inflamed liver and enhanced proliferation of T cells rather than reduction of apoptotic T cells, resulting in effector T-cell expansion and exacerbated T-cell infiltration [11]. Although IFN-γ generally acts as a critical proinflammatory mediator, it exerts

regulatory functions to limit tissue damage associated with inflammation of AIH in progress.

In contrast to neutralization of IFN-γ, neutralizing anti-TNF-α prevented the induction of fatal AIH [12]. Administering anti-TNF-α did not prevent splenic T-cell activation in the induction phase of AIH, but suppressed hepatic CCL20 expression in the hepatocytes. In contrast, administering anti-CCL20 suppressed fatal AIH but not elevated serum levels of TNF-α. In addition, stimulation by recombinant (r)TNF-α upregulated CCL20 expression in hepatocytes both in vivo and ex vivo. These findings suggest that TNF-α is critically involved in the induction of fatal AIH through upregulation of hepatic CCL20 expression, which allows migration of dysregulated splenic T cells [12].

In contrast, in AIH progression of BALB/c–NTx–PD-1$^{-/-}$ mice, the majority of CXCL9-expressing cells in the inflamed liver were macrophages/Kupffer cells [13]. Although IFN-γ can mediate the induction of CXCR3 ligands [31, 34], in BALB/c–PD-1$^{-/-}$ mice, injections of rTNF-α upregulated hepatic expression of both CXCL9 and CXCL10 and sustained CXCL9 upregulation [13]. Interestingly, neutralizing serum levels of TNF-α in AIH progression did not suppress hepatic CXCL9 expression and allowed fatal progression of AIH [12, 13]. After migration of TNF-α-producing activated T cells into the liver, TNF-α was produced by several immune cell types, including hepatic macrophages/Kupffer cells in BALB/c–NTx–PD-1$^{-/-}$ mice [13]. TNF-α-dependent upregulation of CXCL9 expression may be induced by hepatic macrophages/Kupffer cells in an autocrine fashion and/or by activated T cells in a paracrine fashion, resulting in uncontrollable CXCL9 expression under anti-TNF-α monotherapy (Fig. 2.2).

2.8 The Same Disruptions in Immune Regulation Produce Different Phenotypes of AIH

In patients, clinical manifestations of AIH vary, ranging from non-symptomatic mild chronic hepatitis to fulminant hepatic failure [1, 2]. It is unclear whether varied clinical manifestations of AIH result from the same immune dysregulation. In AIH patients, the disease manifestation has been associated with specific alleles of the major histocompatibility complex [50–52]. To examine whether different genetic backgrounds induce milder disease manifestation in NTx–PD-1$^{-/-}$ mice, NTx was performed in PD-1$^{-/-}$ mice that had been backcrossed onto the C57BL/6 background for 11 generations [9]. Four-week-old C57BL/6–NTx–PD-1$^{-/-}$ mice develop mononuclear cell infiltrations, predominantly in the portal area in the liver [9]. In 8-week-old mice, these infiltrations progress interface hepatitis without bile duct destruction. The infiltration is sustained in older mice, resulting in bridging fibrosis. These findings are associated with increased serum levels of AST and ALT. C57BL/6–NTx–PD-1$^{-/-}$ mice older than 4 weeks also show hypergammaglobulinemia and significantly increased production of ANAs. In addition, some of

the AIH-bearing C57BL/6–NTx–PD-$1^{-/-}$ mice, with lesser frequency, manifest other organ-specific autoimmunities such as sialadenitis, as do patients with chronic AIH. These findings suggest that human chronic AIH and AIH developed in C57BL/6–NTx–PD-$1^{-/-}$ mice share characteristic components of the disease [9].

In addition, as described in BALB/c–NTx–PD-$1^{-/-}$ mice, chronic AIH-bearing C57BL/6–NTx–PD-$1^{-/-}$ mice autonomously develop accumulation of T_{FH} cells and GC formation in the B-cell follicles of the spleen [9]. The spleen is the induction site of AIH, and splenic $CD4^+$ T cells directly trigger chronic AIH similar to fatal AIH [9]. These findings suggest that immune dysregulation by a concurrent loss of naturally arising Treg cells and PD-1-mediated signaling can induce different phenotypes of AIH in mice with different genetic backgrounds [8, 9].

Analysis of the T-cell receptor (TCR) repertoire in several autoimmune diseases has shown antigen-driven clonal expansion of autoreactive T cells in the target organs [53–57]. In patients with AIH, analyses of the TCR repertoire have shown skewing of V_β chain usage, suggesting oligoclonal expansion of liver-infiltrating T cells [58, 59]. In addition, liver-infiltrating LKM-1-specific CD4+ T-cell clones have shown a restricted TCR V_β repertoire [60]. In chronic AIH-bearing C57BL/6–NTx–PD-$1^{-/-}$ mice, effector T cells infiltrated into the liver exhibited clonal TCR V_β usage [9]. In contrast, in the case of fatal AIH-bearing BALB/c–NTx–PD-$1^{-/-}$ mice, effector T cells infiltrated into the liver showed more abundant clonal TCR V_β usage [9]. These data suggest that mono- or oligoclonal expansion of infiltrating effector $CD4^+$ T cells in the liver may be relevant to the proliferation of autoreactive $CD4^+$ T cells induced by autoantigen-presenting DCs in the spleen.

2.9 Splenectomy Overcomes Corticosteroid Insufficiency and Reduced Severity of AIH

Corticosteroid administration is the first-line therapy for patients with AIH. The majority initially respond well to corticosteroids, alone or in combination with azathioprine [1, 2, 52]. After initial remission, maintenance therapy is continued for years. However, long-term treatment is discontinued in some patients because of drug-related side effects [61, 62]. Half of AIH patients with remission relapse within 6 months after corticosteroid withdrawal, and multiple relapses are associated with a poor prognosis [63, 64]. Even when liver inflammation disappears completely, 13 % of those patients eventually relapse [65]. To find clues to overcoming the therapeutic insufficiency of corticosteroids, preclinical animal models for detailed examination are needed.

Intraperitoneal injections of dexamethasone (DEX) in BALB/c–NTx–PD-$1^{-/-}$ mice from 1 day after thymectomy suppressed dysregulated T_{FH} cells in the spleen and prevented the development of fatal AIH [9]. In contrast, although therapeutic injections of DEX after the development of AIH resolved liver inflammation and resulted in a significantly increased survival rate, dysregulated T_{FH} cells remained

in the spleen, and discontinuing DEX therapy induced a relapse of fatal AIH due to residual splenic dysregulated T_{FH} cells [9]. Notably, either splenectomy after the development of AIH or splenectomy following the DEX therapy suppressed fatal AIH. In addition, in C57BL/6–NTx–PD-$1^{-/-}$ mice, both DEX injections and splenectomy also reduced liver inflammation of chronic AIH. These findings suggest that splenectomy overcomes corticosteroid insufficiency in the murine models and prolongs the remission of AIH.

2.10 Conclusion

We have developed mouse models of spontaneous AIH [8–13]. Immune dysregulation by a concurrent loss of naturally arising Treg cells and PD-1-mediated signaling produced different phenotypes of AIH in mice with different genetic backgrounds. Using these models, we have identified induction sites, responsible T-cell subsets, and key molecules for induction of AIH and have accounted for mechanisms involved in the progression to fatal AIH. In addition, we have found some clues to overcoming therapeutic insufficiency of corticosteroids for AIH in humans.

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Potential Conflict of Interest Nothing to report.

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Abbreviations

autoAbs	Autoantibodies
AIH	Autoimmune hepatitis
ANAs	Antinuclear antibodies
ALT	Alanine aminotransferase
AST	Aminotransferase
BALB/c–NTx–PD-$1^{-/-}$ mice	BALB/c PD-$1^{-/-}$ mice thymectomized 3 days after birth
CCL	CC chemokine ligand
CCR	CC chemokine receptor

Con A	Concanavalin A
CTLA-4	Cytotoxic T lymphocyte-associated antigen 4
CXCL	CXC chemokine ligand
CXCR	CXC chemokine receptor
DCs	Dendritic cells
DEX	Dexamethasone
EAE	Experimental autoimmune encephalomyelitis
GCs	Germinal centers
ICOS	Inducible costimulator
IFN	Interferon
IL	Interleukin
IL-18R	IL-18 receptor
IL-21R	IL-21 receptor
mAbs	Monoclonal antibodies
NTx	Neonatal thymectomy
PD-1	Programmed cell death 1
PD-$1^{-/-}$ mice	PD-1-deficient mice
r	Recombinant
TCR	T-cell receptor
T_{FH}	Follicular helper T
Th	T helper
TNF	Tumor necrosis factor
Treg	Regulatory T

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Chapter 3
Epidemiology and Natural History in Japan

Kaname Yoshizawa, Akihiro Matsumoto, and Takeji Umemura

Abstract Although the exact incidence and prevalence of autoimmune hepatitis (AIH) in Japan remain unknown, several nationwide surveys have shed light on the clinical features of this disease. The number of patients with AIH has risen since it was established as a disease entity and its diagnostic criteria have become better understood. AIH showing acute presentation has also increased, with some patients exhibiting histological features of acute hepatitis. A number of patients with AIH progress to fulminant hepatic failure, whose prognosis is poorer than hepatic failure from other causes. Early European studies demonstrated low 5- and 10-year survival rates for untreated AIH. However, the long-term prognosis of AIH is significantly improved if immunosuppressive therapy is successful. Multiple relapses have been shown to contribute to a poor outcome and development of hepatocellular carcinoma (HCC). Therefore, it is considered essential to minimize relapses by continuing immunosuppressive therapy indefinitely in patients with AIH.

Keywords Epidemiology • Fulminant hepatic failure • Hepatocellular carcinoma • Long-term prognosis

3.1 Introduction

Autoimmune hepatitis (AIH) was originally thought to be rare in Japan. However, data of incidence or prevalence on AIH in Japan was scarce. AIH might have been underdiagnosed, because the surveys were mainly carried out in tertiary referral centers, and may have overlooked mild or atypical AIH. The epidemiology and clinical features of AIH in Japan have since been reviewed by several reports.

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3.2 Epidemiology

Nationwide surveys of AIH by means of a questionnaire were conducted several times by the Intractable Diseases Study Group organized by the Ministry of Health (Labor) and Welfare of Japan [1–4]. The most recent study conducted in 2008 disclosed several key clinical features of AIH in Japan [4]. One thousand and fifty-six patients who were diagnosed as having AIH from 2006 to 2008 were collected from 153 of 462 hospitals to which a questionnaire was sent. The male-to-female ratio was 1:6, with the proportion of men increasing from previous surveys [1–4] (Table 3.1). The mean age at diagnosis was 59.9 years and widely distributed from under 10 to over 80 years of age. It was also higher compared with earlier results, as nearly 30 % of patients were over 70 years old. Approximately 95 % of patients were positive for antinuclear antibody (ANA) [3, 4]. Nine of 79 patients were positive for anti-LKM-1 antibody [3]. Of them, one patient was positive for anti-HCV antibody, while the remaining eight were also positive for ANA, indicating type 2 AIH to be rare in Japan. HLA serotype was also studied in 219 patients in the survey, which revealed that 60 % of patients were positive for the HLA-DR4 serotype. This result was lower than previous studies that had reported an incidence of 70–90 % [3, 5–8]. The current survey also described that more than 30 % of patients had serum IgG levels of less than 2,000 mg/dL and that 10.9 % showed histological features of acute hepatitis [4]. IgG levels of current survey were lower than previous surveys, because hypergammaglobulinemia (>2.0 g/dL) was one of the essential diagnostic criteria of AIH in first and second nationwide surveys [1, 2]. Atypical AIH that includes acute hepatitis has increased in recent years [4, 9, 10]. Taken together, AIH might have indeed been underdiagnosed in Japan and not sufficiently surveyed for incidence and prevalence.

The only nationwide study on the incidence of AIH to date has come from Denmark, with population of over 5,600,000 in which the incidence rate was 1.68 per 100,000 individuals [11]. Other reports have come from specific areas within countries. In Europe, the mean annual incidence of AIH was found to be 1.07 to 1.9

Table 3.1 Characteristics of AIH patients in nationwide surveys

Report	Monna et al. [1]	Onji et al. [2]	Toda et al. [3]	Abe et al. [4]
Survey periods	1975–1982	1975–1990	1994–1995	2006–2008
Case number	253	866	496	1,056
Male:female	1:12.0	1:9.5	1:7.0	1:6.0
Mean age	Nd[a]	47.8	50.8	59.9
Acute hepatitis[b] (%)	Nd	Nd	Nd	10.9
Liver cirrhosis (%)	Nd	14.1	12.3	6.4
Mean IgG (mg/dL)	3,657/3,396[c]	3,589	3,242	2,399
HLA-DR4 (%)	Nd	Nd	78	60

[a]Not described
[b]Histological features
[c]Lupoid hepatitis (LE cell+)/lupoid type (LE cell−)

per 100,000 individuals, with a point prevalence ranging from 11.6 to 18.9 per 100,000 people [12–14]. A survey from Israel showed an annual incidence of 0.67 per 100,000 people, with a point prevalence similar to that of Europe of 11.0 per 100,000 individuals [15]. However, a higher prevalence of AIH was uncovered among Alaskan natives, Mongoloid ancestry to be as high as 42.9 per 100,000 people [16]. A survey of the incidence and prevalence of AIH is thus sorely needed in Japan.

3.3 Natural History and Clinical Outcomes

3.3.1 Natural History

The natural history and prognosis of untreated AIH were reported in the 1970s in Europe, in which immunosuppressive therapy considerably reduced the mortality [17–19]. Ten-year survival rate was significantly improved in the corticosteroid-treated group (63 %) compared with that in the control group (27 %) in a controlled prospective trial [19]. This study also revealed that AST and serum albumin levels of untreated patients fluctuated during the natural courses. No such studies have been undertaken in Japan.

3.3.2 Clinical Course and Prognosis

The clinical manifestations of AIH patients are diverse and range from asymptomatic to fulminant hepatic failure. Whereas some cases are diagnosed due to fatigue, anorexia, jaundice, and even ascites or encephalopathy, others are detected by chance alone. AIH often has a chronic and fluctuating disease course, and approximately 70 % of patients already have chronic hepatitis and 10–20 % already have cirrhosis at presentation [4, 8]. The number of AIH patients presenting with acute symptoms has increased as well [20]. AIH with acute presentation includes acute histological hepatitis in 10–20 % of cases along with acute exacerbation of preexisting chronic disease [4, 9, 10, 20].

We earlier reported the clinical findings of 226 patients of Japanese ethnicity who were diagnosed as having probable or definite AIH between 1974 and 2010 [8]. Although some patients died from fulminant hepatic failure within a short period of time, most responded well to immunosuppressive therapy and achieved remission.

3.3.2.1 Fulminant Hepatic Failure

Study of the etiology and prognosis of fulminant hepatitis (FH) and late-onset hepatic failure (LOHF) between 2004 and 2009 in Japan revealed the frequencies of FH and LOHF caused by AIH to be 8.3 % (38/460 patients) and 32.1 % (9/28), respectively [21]. The report also described a particularly low survival rate of AIH patients, namely 32.4 % (9/28) for FH and 12.5 % (1/8) for LOHF, as compared with hepatic failure from other etiologies. In our study, 11 (5.9 %) of 226 patients died from fulminant hepatic failure due to poor treatment outcome within several weeks of diagnosis [8]. Three other patients died from hepatic failure after they discontinued prednisolone treatment without prior consultation with the doctor. Meanwhile, Yamamoto et al. and Intractable Liver and Biliary Diseases Study Group of Japan reported that 11 (11 %) of 96 AIH patients with acute presentation reached fatal outcomes: nine led to death and two required liver transplantation [17].

3.3.2.2 Long-Term Outcome

Prognosis. We previously reported on the survival of 203 AIH patients successfully treated with immunosuppressive therapy compared with that of the general population in Japan [8]. Two hundred patients achieved remission within 3 months of prednisolone treatment. The remaining three patients entered remission within 6 months after the addition of azathioprine, which was administered to a total of nine patients because of a poor response or adverse reactions to prednisolone. Immunosuppressive therapy was successfully withdrawn without relapse for more than 1 year in 13 patients (6.4 %). In the remaining patients, prednisolone was continued at 2.5–10.0 mg daily doses until the study endpoint. Forty-eight patients (23.6 %) experienced at least one relapse during tapering or after cessation of immunosuppressive therapy. Twenty-seven patients (13.3 %) experienced relapses two times or more. The causes of liver-related death in these patients were HCC in nine patients (3.0 %) and hepatic failure in one (0.5 %) [8]. Onji et al. also reported in the second nationwide survey that the causes of liver-related death of AIH patients were hepatic failure in 20 patients (3.2 %) and GI tract bleeding and HCC in 3 (0.5 %) each [2]. With treatment, the overall survival of our AIH patients was similar to that of the general population in Japan (Fig. 3.1) [8]. However, the prognosis of AIH with two or more relapses was significantly poorer than that of remission or a single relapse (Fig. 3.2). In multivariate analysis, the prognosis of patients with multiple relapses was identified as the only risk factor associated with liver-related death. These findings imply that the long-term outcome of even severe AIH is good if adequate therapy is maintained over an extended period. In support of this, Miyake et al. showed that the prognosis of Japanese AIH patients was favorable if transaminases were sustained at normal levels [22], while Hino et al. confirmed the importance of achieving a good response to immunosuppressive

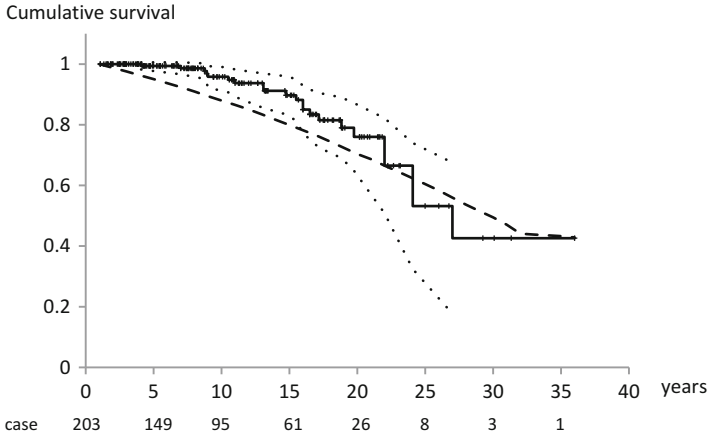

Fig. 3.1 Cumulative overall survival curve (*solid line*) and its 95 % confidence interval (*dotted lines*) for patients with AIH. The estimated survival of the age- and sex-matched Japanese general population is represented by a *broken line*. The survival of patients is similar to that of the general population in Japan

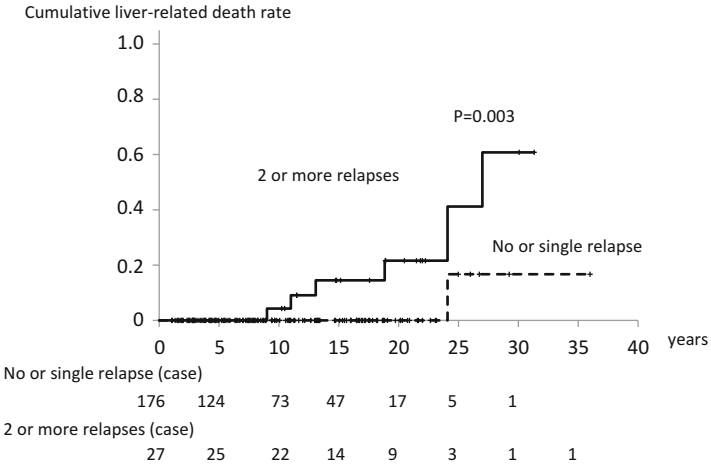

Fig. 3.2 Cumulative liver-related death rates of patients who experienced no or a single relapse (*broken line*) and those with two or more relapses (*solid line*). The prognosis of patients with repeated relapses is poorer than that of patients with remission or a single relapse ($p = 0.003$)

therapies at treatment onset [23]. Kanzler et al. also observed that the survival of German AIH patients followed over a long time course did not differ noticeably from the age- and sex-matched normal German population [24]. On the other hand, Hoeroldt et al. reported the long-term mortality of AIH patients to be higher than that of the general population in England [25].

In our study, only 6.4 % of patients were successfully withdrawn from prednisolone treatment (mean cessation time: 38.4 months; range, 29–52 months) with sustained remission [8]. Similarly, Kanzler et al. described that long-term or lifelong immunosuppressive therapy was needed in most cases and that only 6.8 % of patients achieved sustained remission without the need for continued treatment [24].

Complication with Autoimmune Disorders. A nationwide survey in 2008 revealed that autoimmune disorders were present in 26.9 % of AIH patients: 9.2 % were complicated with chronic thyroiditis, 7.2 % had Sjögren's syndrome, 2.8 % had rheumatoid arthritis, and 1.9 % had primary biliary cirrhosis [4].

Hepatocellular Carcinoma (HCC). Results of a nationwide questionnaire showed that HCC developed in 5.1 % (250/4,869) of patients with AIH in Japan [26]. Afterwards, a secondary survey clarified the clinical features of AIH patients with HCC: mean age at diagnosis was 69 years, the male-to-female ratio was 1:5.7, the mean period form AIH diagnosis to the detection of HCC was 8 years, and the prevalence of liver cirrhosis was high (77.9 %). Migita et al. reported that 7 (3.6 %) of 193 AIH patients developed HCC over a mean follow-up period of 8.0 years [27]. They revealed that cirrhosis at presentation was a risk factor for HCC development. Similarly, Hino-Arinaga et al. revealed that 3.3 % of patients with AIH developed HCC and uncovered that cirrhosis at AIH diagnosis and abnormal ALT at final observation were independently associated with HCC onset [28]. Watanabe et al. performed a systematic literature review of 38 HCC cases associated with AIH in Japan and showed that 58.1 % (18/31) of HCC patients had cirrhosis [29]. In our study, eight AIH patients (3.9 %) developed hepatic malignancy over a mean period of 14.5 years (range: 9–21 years) of the follow-up [8]. Liver histology at presentation demonstrated liver cirrhosis in two patients, F3 histological stage in five, and F1 histological stage in one. Six patients experienced multiple relapses. Histological or clinical stages worsened in three subjects, reaching F4 from F3 in two patients and liver cirrhosis (no histology) from F1 in one. Five (62.5 %) of the eight HCC patients exhibited liver cirrhosis at the time of HCC diagnosis. In Caucasian countries, Yeoman et al. have reported that 15 (6.2 %) of 243 patients with AIH developed HCC, for which cirrhosis at presentation was a risk factor [30]. Wong et al. disclosed that the risk of developing HCC among AIH patients with cirrhosis was 1.9 % per year [31]. Cumulatively, these data show that liver cirrhosis is a risk factor for developing HCC in AIH patients. Careful observation is therefore needed to detect HCC at an early stage, especially in patients with cirrhosis.

Adverse Effects of Immunosuppressive Therapy. According to our study of 203 patients with AIH, the adverse effects of long-term immunosuppressive therapy included steroid-related osteoporosis in 25 (12.5 %) patients, diabetes mellitus in 20 (10.3 %), fatty liver change in 19 (9.4 %), cataract in 5 (2.5 %), and compression fracture of the spine, cerebral bleeding, gastric ulcer, and psychiatric problems in 1 (0.5 %) each [8]. Such adverse effects were mild to moderate and were controllable by medication; only one patient each experienced severe side effects of cerebral bleeding or necrosis of the femoral head.

3.4 Conclusion

Nationwide surveys have made progress in revealing the clinical features of AIH in Japan. The number of patients with AIH has increased since the disease entity was defined and its diagnostic criteria have become better understood. AIH showing acute presentation has also increased in incidence, and some patients exhibit histological features of acute hepatitis. Yet other cases of AIH progress to fulminant hepatic failure, for which the prognosis of fulminant AIH is poorer than that of liver failure from other causes. However, the long-term prognosis of AIH is favorable if immunosuppressive therapy is promptly begun and successful. As repeated relapses lead to a poor prognosis that includes HCC, it is deemed important to minimize relapses by continuing maintenance doses of immunosuppressive therapy over the lifetime of the patients.

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Chapter 4
Histological Findings of Autoimmune Hepatitis

Kenichi Harada

Abstract Histology of autoimmune hepatitis (AIH), chronic active hepatitis, is characterized by portal inflammation with interface hepatitis. Although the basic histology of AIH is similar to that of virus-related chronic hepatitis, hepatitic changes are usually prominent in AIH compared with chronic viral hepatitis. Clinicopathological diagnosis of AIH requires exclusion of other causes of liver disease, including hepatitis virus, alcohol, drugs, metabolic disorders, and other autoimmune diseases. At present, some criteria systems considering clinicopathological findings are proposed to categorize patients as having either "definite" or "probably/atypical" AIH. Among the pathological items of a simplified AIH scoring system of the International AIH Group, in addition to evident chronic hepatitis with interface hepatitis and hepatic rosette formation, emperipolesis, indicating the close immunological interaction of lymphocytes and hepatocytes, is noted but is sometimes difficult to evaluate. In addition to classical AIH, showing chronic active hepatitis, some AIH patients show a clinically acute hepatitis-like clinical course. These patients have mostly acute exacerbation from chronic active AIH, but acute-onset AIH cases, which histologically exhibit diffuse lobular hepatitis and/or confluent necrosis including perivenular zonal necrosis (zone 3 necrosis, centrizonal necrosis), are also encountered.

Keywords Autoimmune hepatitis • Centrizonal necrosis • Chronic active hepatitis • Emperipolesis • Rosette formation

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4.1 Introduction

Chronic hepatitis is defined as an inflammatory disease of the liver that lasts for more than 6 months. Histologically, chronic active hepatitis accompanies interface hepatitis (formerly termed piecemeal necrosis). As an etiology, viral hepatitis (hepatitis B virus, hepatitis C virus, etc.), metabolic disease (nonalcoholic fatty liver disease (NAFLD), Wilson's disease, etc.), toxic agents and drugs (alcoholic liver disease, amiodarone, etc.), and autoimmune disease (autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC), and sarcoidosis) have been described. In particular, AIH preferably affects middle-aged women and is characterized by chronic active hepatitis related to autoimmune-mediated and continuous hepatocellular damage. Usually, it can be readily distinguished from the other two major autoimmune biliary diseases, PBC and PSC, but overlap syndromes exist. However, AIH lacks pathognomonic features and histological manifestations are observed in acute and chronic liver diseases of diverse causes.

4.2 Basic Histology of AIH

Basic histology of AIH includes chronic active hepatitis characterized by portal inflammation with interface hepatitis, lymphoplasmacytic infiltration and follicle-like aggregation of lymphocytes in portal tracts (Figs. 4.1 and 4.2), fibrous enlargement of portal tracts with fibrous septa formation, hepatocellular damage and necrosis/apoptosis around portal tracts with the destruction of limiting plates (interface hepatitis) (Fig. 4.2), parenchymal necroinflammatory changes including focal necrosis (lobular hepatitis) (Fig. 4.3), and sinusoidal lymphocytic infiltration.

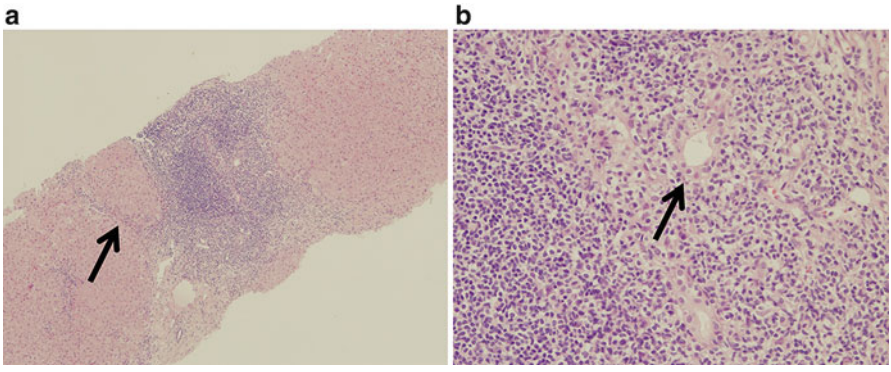

Fig. 4.1 Typical autoimmune hepatitis (AIH) showing chronic active hepatitis. Enlargement of portal tracts and bridging formation (**a**, *arrow*) are observed. In inflamed portal tracts, bile duct damage is observed (**b**, *arrow*)

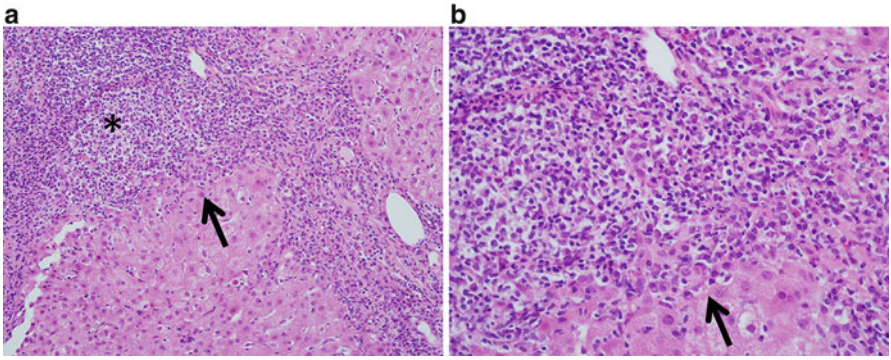

Fig. 4.2 Typical autoimmune hepatitis (AIH) showing chronic active hepatitis. In enlarged portal tracts, severe inflammatory cell infiltration and follicle-like aggregation (*) are observed (**a**). Interface hepatitis is also prominent (*arrows* in **a** and **b**). Inflammatory cells consist of lymphocytes and plasma cells (**b**). **b** is a higher magnification of **a**

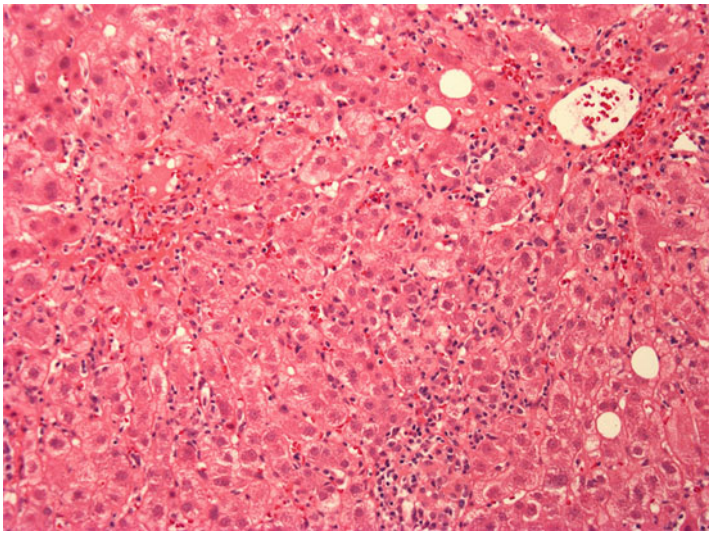

Fig. 4.3 Parenchymal change in autoimmune hepatitis (AIH) showing chronic active hepatitis. In parenchyma, many focal necroses are observed. This lobular hepatitis is prominent in AIH

In particular, interface hepatitis is a pathogenic hallmark of chronic active AIH and is prominent during disease flares. These histologies are similar to those observed in chronic active hepatitis caused by viral infection and are not specific for AIH. Interface hepatitis may occur even in biliary diseases such as PBC. However, compared with those observed in chronic viral hepatitis, the hepatitic changes that are prominent in typical AIH include the following: infiltration and

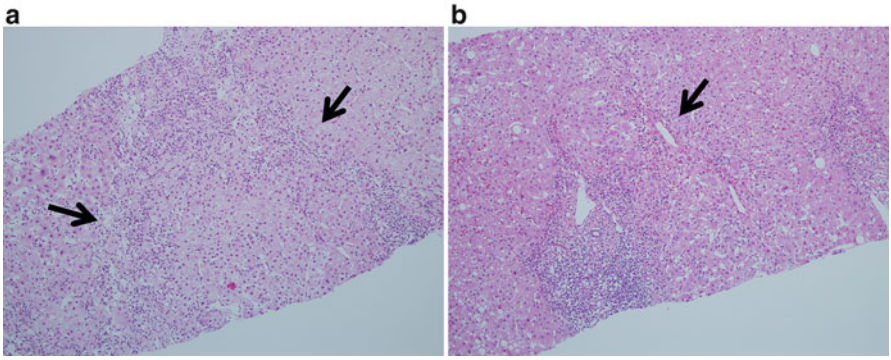

Fig. 4.4 Confluent necrosis. Bridging necrosis (**a**, *arrow*) and perivenular zonal necrosis (**b**) are observed in AIH cases

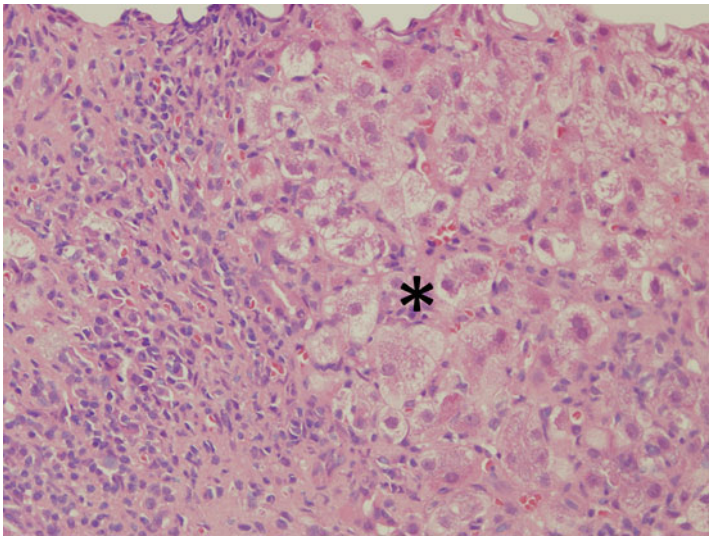

Fig. 4.5 Ballooning degeneration of hepatocytes (*) is observed in the periportal area in autoimmune hepatitis (AIH)

accumulation of marked plasma cells in portal tracts, confluent necrosis (bridging and zonal necrosis) (Fig. 4.4), ballooning degeneration of hepatocytes (Fig. 4.5) and many acidophilic bodies (apoptosis of hepatocytes) (Fig. 4.6), rosette formation of hepatocytes (Fig. 4.7), and pigmented macrophages (pigment-laden or ceroid-laden macrophages) (Fig. 4.8). As for the predominant plasma cell infiltration, this feature does not occur in all patients with AIH [1]. Its presence supports the diagnosis of AIH because it is a finding that is more common in AIH (66 %) than

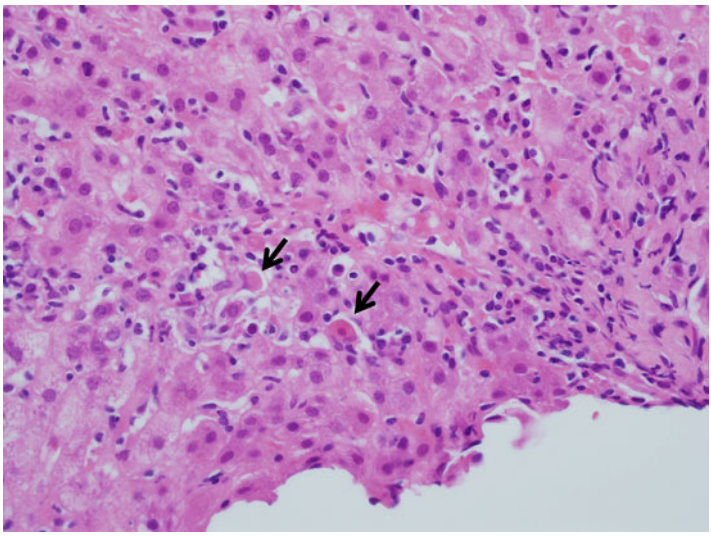

Fig. 4.6 The presence of acidophilic bodies indicating the apoptosis of hepatocytes (*arrows*) reflects the marked parenchymal hepatitis in autoimmune hepatitis (AIH)

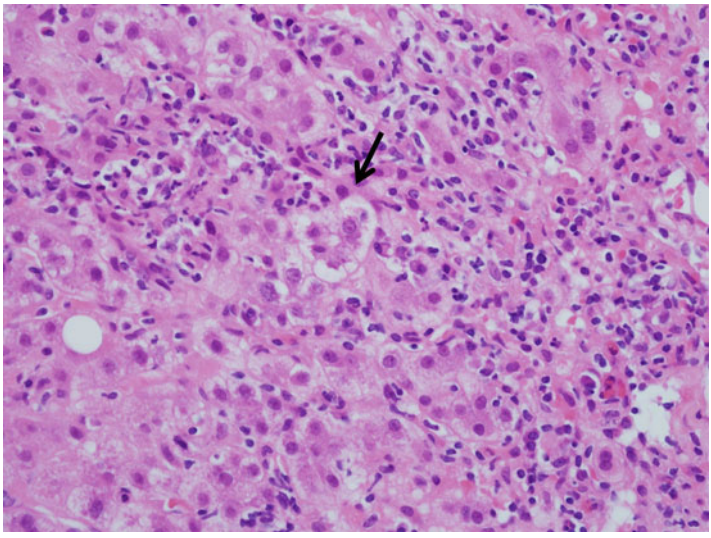

Fig. 4.7 Hepatocyte rosette formation (*arrow*) around a severe interface hepatitis area

in chronic hepatitis B (40 %) or chronic hepatitis C (21 %). In severe cases and acute exacerbation of AIH, giant cell formation of hepatocytes (giant syncytial multinucleated hepatocytes) (Fig. 4.9), broad hepatocellular collapse (Fig. 4.10), and multiple confluent necrosis consisting zonal and bridging necrosis are observed.

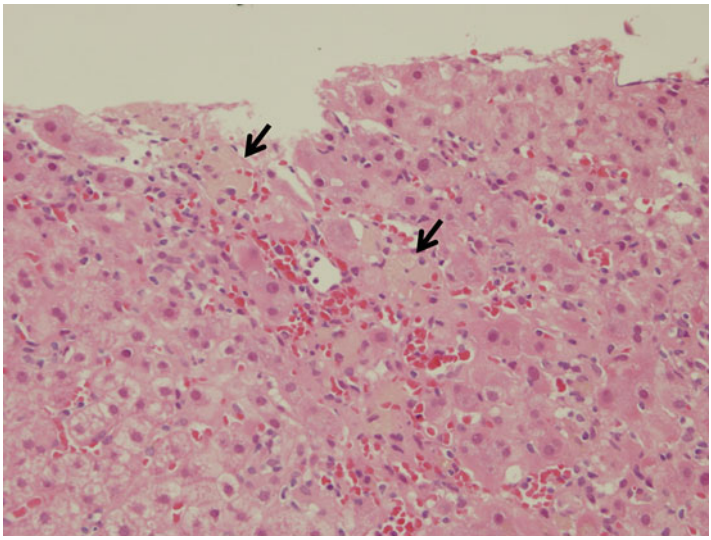

Fig. 4.8 Pigmented macrophages (*arrows*) are scattered in a necrotic area of autoimmune hepatitis (AIH)

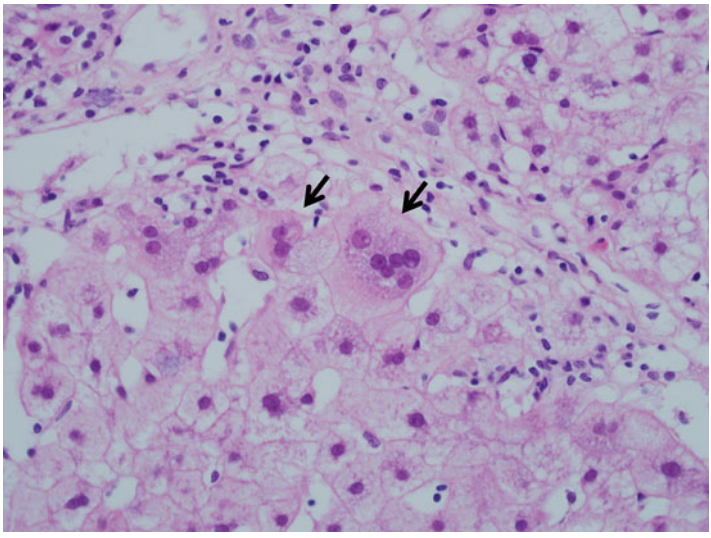

Fig. 4.9 Giant syncytial multinucleated hepatocytes (*arrows*) are present in some autoimmune hepatitis (AIH) cases

Cases of fulminant hepatitis, histologically showing submassive and massive necrosis, are also present. In addition to severe lobular necrosis including massive hepatocyte necrosis and dropout, regeneration of hepatocytes may be present and mimic parenchymal nodules of established cirrhosis in the recovery phase of fulminant AIH.

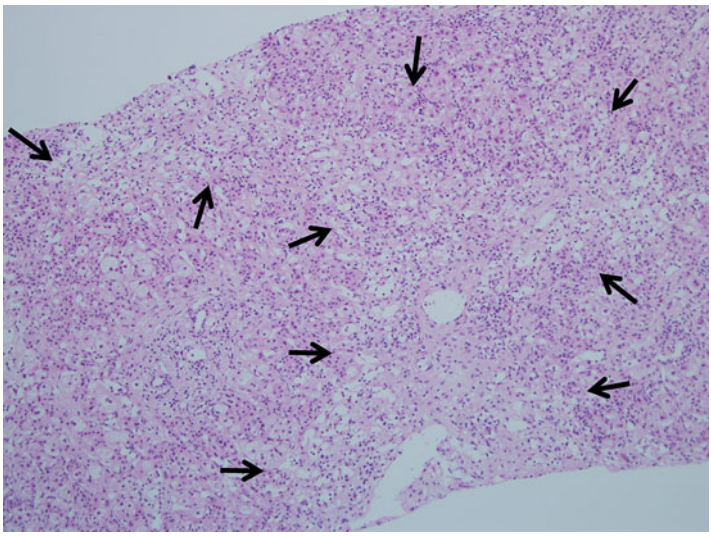

Fig. 4.10 Broad collapse of hepatocytes (*arrows*) in acute exacerbation of autoimmune hepatitis (AIH)

4.3 Pathogenesis of AIH from the Aspect of Pathogenic and Regulatory Helper T Cells

Several studies, including animal model studies, have been reported for the pathogenesis of AIH. It is postulated that an environmental agent, either a drug, virus, or other agent, appears to trigger a T cell-mediated cascade directed against hepatocellular antigens in genetically predisposed individuals to cause AIH. Immunohistochemically $CD8^+$ T cells are a dominant subset of lymphocytes observed within the area of interface hepatitis and $CD4^+$ T cells predominate within the portal tracts [2]. $CD4^+$ helper T cells are essential regulators of immune responses and inflammatory diseases. Immunoreactivity to intra- and extracellular antigens is regulated mainly by two different types of memory $CD4^+$ helper T cells, i.e., Th1 and Th2 cells, which are principally distinguished by their production of different cytokines and their ability to induce either cellular (Th1) or humoral (Th2) immune reactions. The advancement of the understanding of polarized Th1 and Th2 cells in human diseases suggests that the balance between these two subsets is altered in autoimmune disorders; organ-specific autoimmune diseases, including AIH, are mainly mediated by Th1 cells, whereas the Th2 subset predominates in systemic autoimmune disorders [3–5]. Immunohistochemically, Th1 and Th2 cells are easily distinguishable by the transcription factors T-box expressed in T cells (T-bet) and GATA-binding protein-3 (GATA-3), respectively, in addition to Th1-type cytokines (IL-2 and IFN-γ) and Th2-type cytokines (IL4, IL10, and IL13). In fact, many T-bet-positive lymphocytes infiltrate the portal tracts and

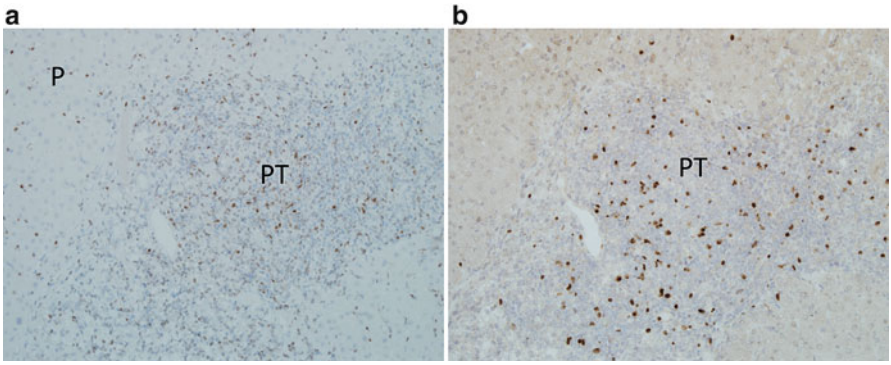

Fig. 4.11 Immunohistochemistry for T-bet (**a**) and Foxp3 (**b**). Many T-bet-positive Th1-type T cells are observed in portal tracts (PT) and parenchyma (P) (**a**). Foxp3-positive Treg cells are scattered in PTs (**b**)

parenchyma (Fig. 4.11), whereas GATA-3-positive cells are scarce. Of late, a third pathogenic type, Th17 cells, and their association with the chronic inflammation present in autoimmune diseases via the production of the proinflammatory cytokines IL-17, IL-22, and TNF-α, have been noted [6–9]. Th17 cells are elevated in the circulation and liver of patients with AIH and contribute to autoimmunity against hepatocytes by inducing the secretion of IL-6 by these cells [10]. Both natural Tregs (nTregs, $Foxp3^+CD25^+$Tregs), which originate in $CD4^+$ T cells in the thymus, and induced Tregs (iTregs, $Foxp3^+$Tregs), which develop in the periphery, can play a role of dominant immunosuppression on effector T cells and antigen-presenting cells. Patients with AIH have a reduced number and function of $CD4^+CD25^+FOXP3^+$Tregs [11, 12]. However, a controversial study has reported that the frequency and function of circulating Tregs is not impaired in AIH (Fig. 4.11) [13].

4.4 Pathological Diagnosis: Histological Components of the AIH Diagnostic Scoring System

The clinicopathological diagnosis of AIH requires the exclusion of other causes of liver disease, including viral hepatitis, alcohol and drug abuse, metabolic disorders (NAFLD and NASH), and other autoimmune diseases. In particular, the pathological differentiation of AIH from chronic viral hepatitis and the presence of AIH superimposed on HCV-infected patients are difficult or impossible in most cases. Pathologically, the histological difference between AIH and chronic viral hepatitis depends on the relative evaluation of several findings regarding chronic active hepatitis. Therefore, some systems of criteria that take into consideration clinicopathological findings have been proposed to categorize patients as having either

"definite" or "probably/atypical" AIH. The criteria of the Intractable Hepatobiliary Disease Study Group in Japan (2013) [14] include the following: (1) exclusion of other causes of liver disease, (2) positivity for the antinuclear antibody (ANA) and/or antismooth muscle antibody, (3) increased IgG level (>1.10 times the upper normal limit), (4) interface hepatitis and plasma cell infiltration in liver tissues, and (5) marked efficacy of steroid therapy. Typical AIH is defined as the presence of 1) and another three items among 2)–5), and the atypical cases are defined as those exhibiting (1) and another one or two item(s) among (2)–(5). In addition to these Japanese AIH criteria, the AIH scoring system of the International AIH Group (IAIHG) is useful. At present, modified criteria (1999) [15] and simplified criteria (2008) [16] are used. The former consist of many items and are complex; however, it is possible to adequately distinguish AIH from other liver diseases, particularly primary biliary diseases, such as PBC and PSC, and chronic viral hepatitis. Pathological items consist of interface hepatitis (+3), predominantly lymphoplasmacytic infiltrate (+1), rosette of liver cells (+1), none of the above (−5), biliary changes (−3), and other changes (−3), which make up score 5 in full score 29. The most important point is that biliary changes and other changes suggestive of other hepatobiliary diseases, including PBC and PSC, and a different etiology, respectively, provide negative points toward the accurate identification of AIH alone. "Biliary changes" refers to bile duct changes that are typical of PBC or PSC (i.e., granulomatous cholangitis or severe concentric periductal fibrosis with ductopenia established in an adequate biopsy specimen) and/or a substantial periportal ductular reaction with copper/copper-associated protein accumulation (Fig. 4.12). The deposition of copper reflects chronic cholestasis [17, 18], and orcein staining is very useful to detect the deposition of copper-binding proteins. This deposition in the early stage of chronic liver diseases suggests cholestatic liver diseases, such as PBC, PSC, and Wilson's disease; however, in advanced liver diseases, including cirrhosis, this deposition in hepatocytes is usually observed, regardless of etiology. Pathologists, therefore, have to evaluate orcein staining results with caution to avoid overdiagnosing biliary diseases. In contrast, the simplified criteria [16] have been proposed for the rapid diagnosis and treatment for AIH and are useful to nonspecialized and specialized hepatologists. Regarding the pathological items in these criteria, three categories are defined for grading histology and give out a score of 0–2 in full score 8: atypical histology (0 points), histology compatible with AIH (1 point), and typical histology (2 points). In addition to evident hepatitis as a necessary condition, interface hepatitis, lymphocytic/lymphoplasmacytic infiltrates in portal tracts and extends into the lobule, emperipolesis, and hepatic rosette formation are regarded as typical for the diagnosis of AIH. To be considered typical, each of the three features of typical AIH histology has to be present. Compatible features are a picture of chronic hepatitis with lymphocytic infiltration without all the features that are considered typical. Histology is considered atypical when signs of another diagnosis, such as steatohepatitis, are present. These findings reflect chronic hepatitis with severe activities; however, it is impossible to establish a definite diagnosis of AIH on the basis of these findings because they are not specific to AIH. Because atypical AIH

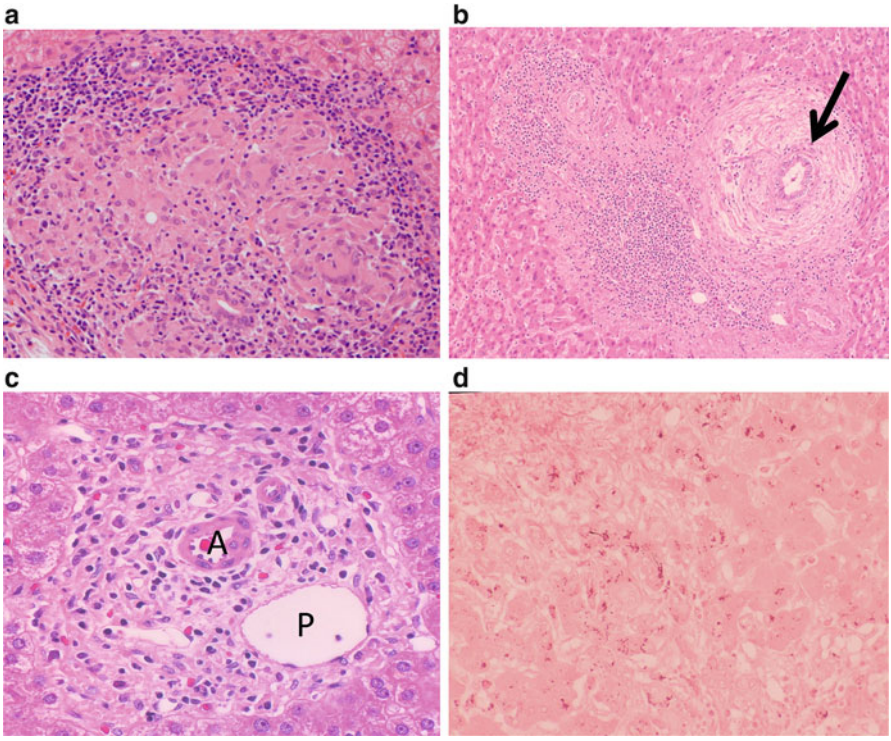

Fig. 4.12 Biliary changes raised by modified criteria (1999) of the autoimmune hepatitis (AIH) scoring system. (**a**) Granulomatous cholangitis in primary biliary cirrhosis. (**b**) Severe concentric periductal fibrosis in primary sclerosing cholangitis. (**c**): Ductopenia. The portal vein (P) and artery (A) are found, but the bile duct is missing. (**d**) Orcein staining. Copper-binding proteins are scattered in hepatocytes

cases, such as acute-onset and excavation AIH, are probably ruled out as being non-AIH when using the simplified criteria, the modified criteria (1999) should be applied in these cases. Moreover, steatohepatitis is considered as a disease that is difficult to differentiate from AIH based on these criteria, and ANA is detected in approximately one third of cases of NASH and NAFLD [19, 20], although pathological differentiation is relatively easy on liver biopsy.

Among the histological findings of the simplified IAIHG criteria, emperipolesis is unfamiliar in the hepatology field but is pathologically well known as a characteristic of the Rosai–Dorfman disease (sinus histiocytosis with massive lymphadenopathy). Emperipolesis is an active penetration by one cell into and through a larger cell and is immunologically the strongest pattern of cell-to-cell contact (Fig. 4.13). Although lymphocytes are frequently found in close contact with hepatocytes and bile ducts in various hepatobiliary diseases, the presence of emperipolesis indicates the close immunological interaction of immune competent cells (lymphocytes) and target cells (hepatocytes) in AIH. In addition to rosette

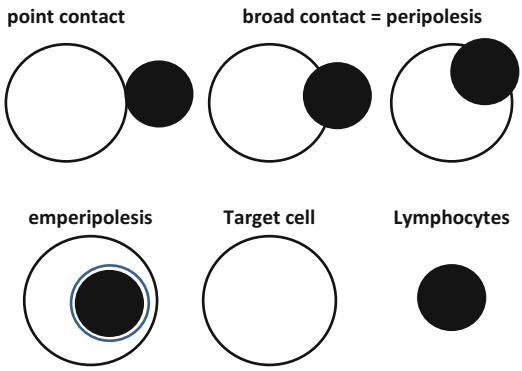

Fig. 4.13 Immunological contact pattern between target cells and lymphocytes. Emperipolesis is a unique feature of penetration of lymphocytes into hepatocytes, and the tightest pattern of target cells (hepatocytes) and effector cells (lymphocytes)

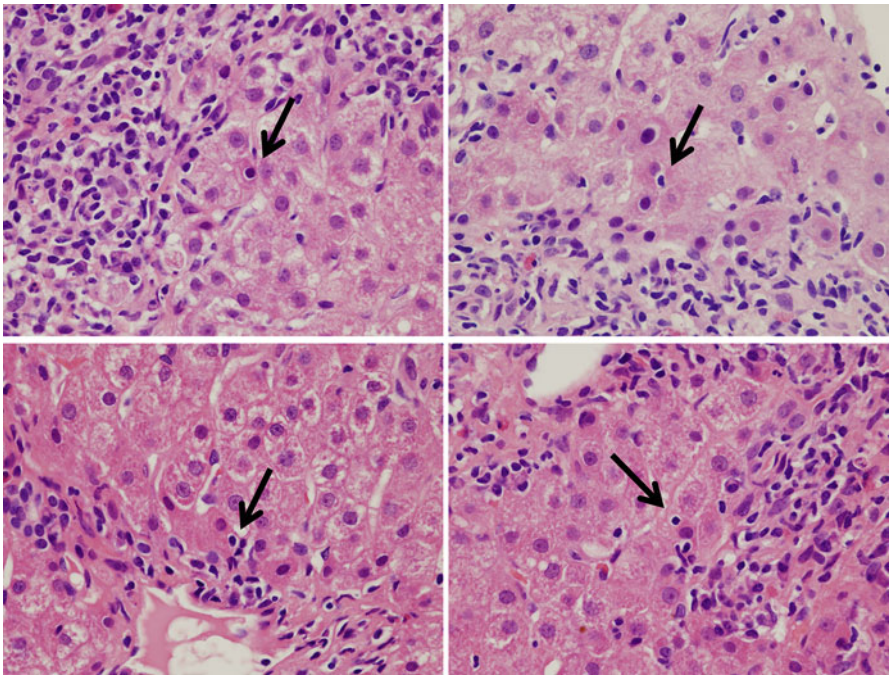

Fig. 4.14 Emperipolesis in autoimmune hepatitis (AIH). Emperipolesis is observed around the interface area. Although *arrows* indicate emperipolesis, all *arrows* are possibly hard to evaluate as emperipolesis

formation of hepatocytes, this emperipolesis is frequently observed in hepatocytes around the interface hepatitis of AIH with severe hepatitic changes (Fig. 4.14). At present, emperipolesis is noted as a pathological finding and is included in the simplified criteria of AIH. However, this finding was primarily reported in the field of hepatology as a histological finding of HBV-related chronic viral hepatitis [21]

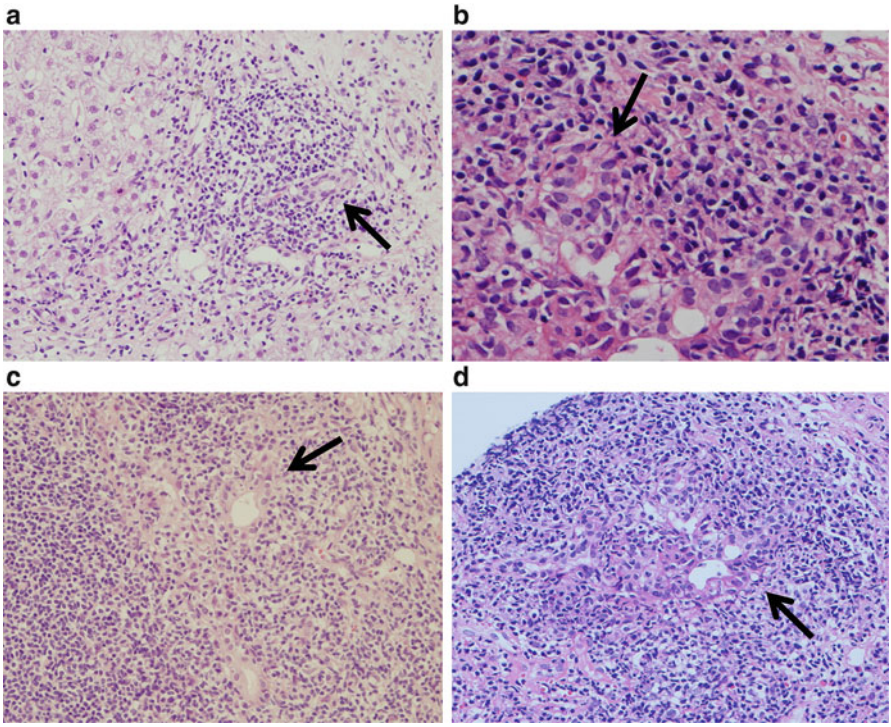

Fig. 4.15 Bile duct damage (hepatitic bile duct injury) in autoimmune hepatitis (AIH). *Arrows* denote the damaged interlobular bile ducts at various degrees (**a**–**d**). In particular, bile ducts in **c** and **d** show destructive changes resembling chronic nonsuppurative destructive cholangitis (CNSDC) of primary biliary cirrhosis (PBC)

and is found in other hepatitic diseases with chronic active hepatitis and AIH. In practice, in the establishment of a pathological diagnosis using HE staining, it is always difficult to distinguish emperipolesis from apoptotic body-laden macrophages and to differentiate whether lymphocytes are located inside or outside of hepatocytes. Because the presence or absence of emperipolesis greatly affects the score of simplified AIH criteria (1 in a full score of 8) [16], the survey of emperipolesis is a heavy burden for pathologists.

Compared with the histology of chronic viral hepatitis, several of the findings that indicate the possibility of AIH described above are observed in AIH, but all these findings are not observed in needle liver specimens. However, the presence of highly active hepatitis is necessary for the pathological diagnosis of pretreated AIH cases, and chronic hepatitis with broad hepatocellular necrosis should be suspected as AIH. Bile duct damages are thought to be a histological characteristic of PBC and PSC. However, bile duct damage is often observed in AIH with severe portal inflammation (Fig. 4.15). This bile duct damage is called hepatitic duct lesion or hepatitis-associated bile duct damage and is often observed in chronic active

hepatitis, including AIH and chronic viral hepatitis (in particular, HCV-related disease) (Fig. 4.15). These bile duct damages sometimes accompany destructive changes (up to 12 % of biopsies) [1] and resemble chronic nonsuppurative destruction cholangitis (CNSDC) of PBC (Fig. 4.15). The observation of bile duct lesions alone cannot be used to differentiate AIH from PBC [22]. However, the bile duct loss found in biliary diseases such as PBC and PSC is rarely observed in AIH.

4.5 Histological Staging and Grading System

Liver biopsy provides information regarding the staging of fibrosis and the degree of hepatic inflammation as well as the diagnosis of AIH. However, there is no scoring system that reflects the unique histological features of AIH. Regarding the staging and grading systems for AIH, four systems, such as those described by Batts and Ludwig [23] and Scheuer (Table 4.1) [24], the French Metavir system [25], and the modified histological activity index (Table 4.2) [26] for chronic viral hepatitis, are diverted. In Japan, the New Inuyama Classification [27] is diverted as a grading and staging system that reflects activity and fibrosis, although this originally should be applied to chronic viral hepatitis. In this classification, the degree of necroinflammatory change (grading or activity system) is classified into the following four categories that take into consideration portal inflammation including interface hepatitis and parenchymal inflammation: A0 (minimal: no or minimal necroinflammatory change), A1 (mild: mild necroinflammatory change), A2 (moderate: moderate necroinflammatory change), and A3 (severe: marked necroinflammatory change including confluent necrosis, such as zonal and bridging necrosis). A staging score has been developed to reflect the extent of portal fibrosis. Fibrosis stages are as follows: F0 (no fibrosis: no or minimal portal fibrosis), F1 (mild fibrosis: as above, with portal fibrous enlargement), F2 (moderate fibrosis: as above, with bridging fibrosis), F3 (severe fibrosis: as above, with lobular disarray), and F4 (cirrhosis).

Table 4.1 Scheuer classification for grading and staging of chronic hepatitis

Grade	Portal/periportal activity	Lobular activity
0	None	None
1	Portal inflammation	Inflammation but no necrosis
2	Mild piecemeal necrosis	Focal necrosis or acidophil bodies
3	Moderate piecemeal necrosis	Severe focal cell damage
4	Severe piecemeal necrosis	Damage includes bridging necrosis
Stage fibrosis		
0	None	
1	Enlarged, fibrotic portal tracts	
2	Periportal or portal–portal septa, but intact architecture	
3	Fibrosis with architectural distortion, but no obvious cirrhosis	
4	Probable or definite cirrhosis	

Table 4.2 Ishak modified hepatic activity index (HAI) for scoring of necroinflammatory activity and staging in chronic hepatitis

Necroinflammatory scores	
(A) Periportal or periseptal interface hepatitis (piecemeal necrosis)	
Absent	0
Mild (focal, few portal areas)	1
Mild/moderate (focal, most portal areas)	2
Moderate (continuous around <50 % of tracts or septa)	3
Severe (continuous around >50 % of tracts or septa)	4
(B) Confluent necrosis	
Absent	0
Focal confluent necrosis	1
Zone 3 necrosis in some areas	2
Zone 3 necrosis in most areas	3
Zone 3 necrosis + occasional portal–central (P–C) bridging	4
Zone 3 necrosis + multiple P–C bridging	5
Panacinar or multiacinar necrosis	6
(C) Focal (spotty) lytic necrosis, apoptosis, and focal inflammation	
Absent	0
One focus or less per ×10 objective	1
Two to four foci per ×10 objective	2
Five to ten foci per ×10 objective	3
More than ten foci per ×10 objective	4
(D) Portal inflammation	
Absent	0
Mild, some or all portal areas	1
Moderate, some or all portal areas	2
Moderate/marked, all portal areas	3
Marked, all portal areas	4
Staging	
No fibrosis	0
Portal fibrosis, with or without short fibrous septa	1
Fibrous septa	2
Transition to cirrhosis	3
Cirrhosis, probable or definite	4

4.6 Liver Cirrhosis

Cirrhosis is the terminal stage of AIH. However, at the diagnosis of AIH, 6.4 % of AIH cases in Japan have already progressed to cirrhosis [28]. Moreover, cirrhosis of AIH is a risk factor for hepatocellular carcinoma, although its incidence is lower than that observed in hepatitis virus-related cirrhosis [29]. In general, cirrhosis is thought to be an irreversible terminal stage, regardless of etiology. However, in cases that exhibit great clinical improvement after immunosuppressive treatment, fibrosis and cirrhosis regression, as well as hepatocellular regeneration, result in the disappearance of the remnant of cirrhosis. In contrast, AIH-related cirrhosis,

although inactive, is thought to be caused by a burnt-out process without specific laboratory findings and absent disease activity. Therefore, as in the preceding diseases of cryptogenic cirrhosis, AIH and nonalcoholic steatohepatitis are usually at the top of the list.

4.7 Variations of AIH

4.7.1 Acute AIH

Some AIH patients show a clinically acute hepatitis-like clinical course. These AIH patients have mostly acute exacerbation from chronic active AIH (Fig. 4.16), but acute-onset or fulminant AIH cases with diffuse and severe hepatocellular damage without definite chronicity, such as fibrosis and preceding liver dysfunction, have also been reported (Figs. 4.17 and 4.18) [30]. These acute AIH cases have higher serum bilirubin, transaminase, and γ-GTP compared with ordinary chronic AIH. In contrast, the serum levels of IgG and γ-globulin and the titer of autoantibodies are not generally high. Therefore, it is difficult to diagnose acute AIH using the international criteria as mentioned above. Liver biopsy is useful for the diagnosis of acute AIH. There is portal inflammation and diffuse lobular necroinflammation (Fig. 4.17). Perivenular zonal necrosis and bridging necrosis among portal tracts and central veins, and rarely periportal zonal necrosis, may accompany lobular disarray (Figs. 4.18 and 4.19) [31–33]. In some cases, zonal necrosis (zone 3 necrosis, centrozonal necrosis) located around the central vein, similar to a characteristic of drug-induced liver injury, is prominent (Fig. 4.20) [34, 35]. Zone 3 necrosis has not been formally included in the histological features of AIH but is thought to be a characteristic feature of acute-onset AIH.

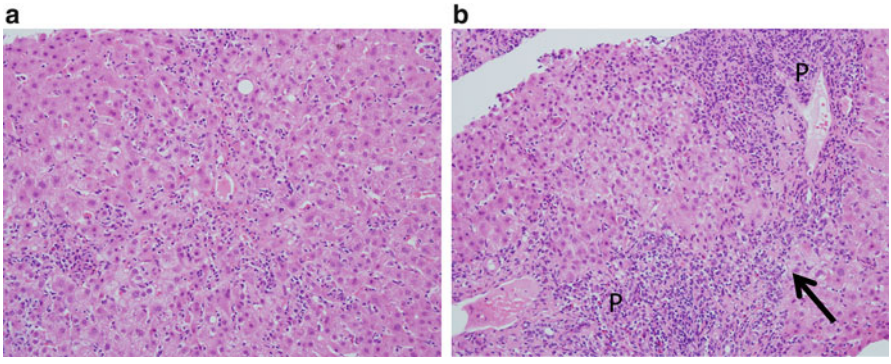

Fig. 4.16 Acute exacerbation of autoimmune hepatitis (AIH). (**a**) Many focal necroses are diffusely seen in parenchyma. (**b**) Bridging necrosis (*arrow*) is observed between enlarged portal tracts with inflammation (P)

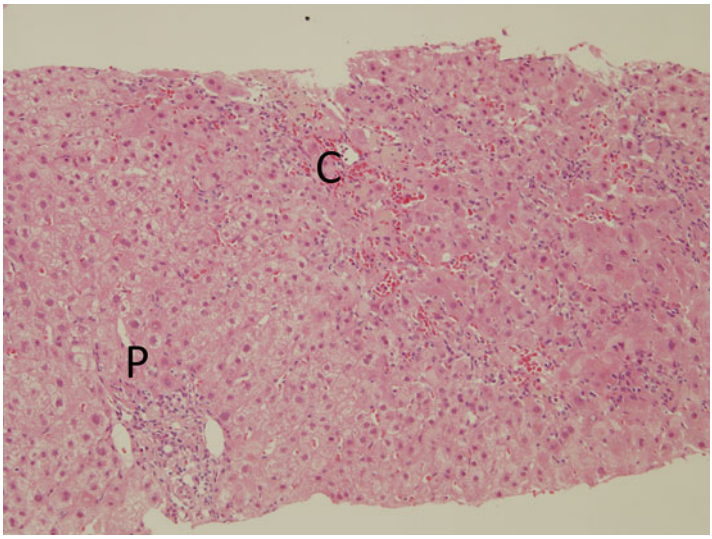

Fig. 4.17 Acute-onset autoimmune hepatitis (AIH) case. In parenchyma, many focal necroses and pigmented macrophages are scattered and accumulate around the central vein (C). Portal tracts (P) are almost preserved

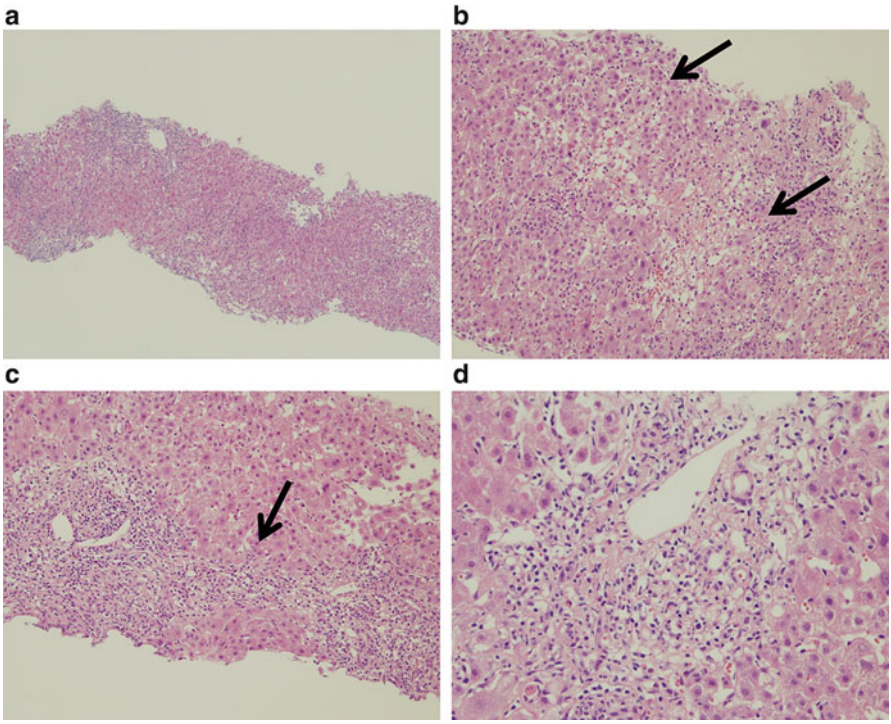

Fig. 4.18 Acute-onset autoimmune hepatitis (AIH) case without preceding liver dysfunction. (**a**) Lower magnification. A diffuse inflammatory change is observed. (**b**) Perivenular zonal necrosis is observed (*arrows*). (**c**) Bridging necrosis is seen between portal tracts. (**d**) Although mild inflammation and edema are observed in portal tracts, reticulin staining shows no distinct fibrous enlargement

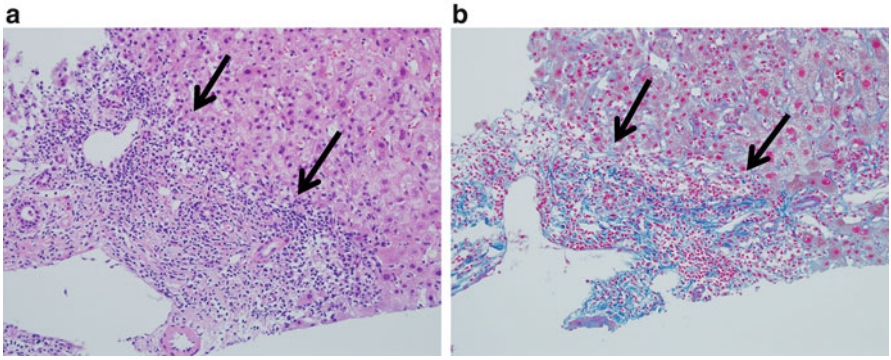

Fig. 4.19 Acute-onset autoimmune hepatitis (AIH) case without preceding liver dysfunction and drug intake. Antinuclear antibody is positive (1280×). In addition to diffuse lobular hepatitis, periportal zonal necrosis is observed (*arrows*, **a**). Azan–Mallory staining indicates an absence of fibrosis in the periportal zonal area (*arrows*, **b**)

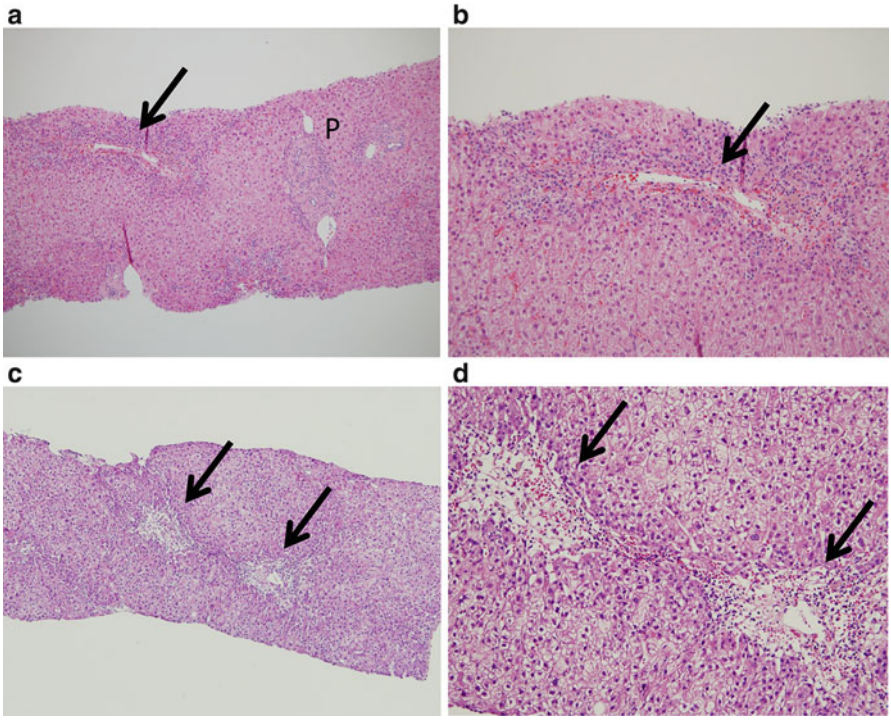

Fig. 4.20 Two cases of acute-onset autoimmune hepatitis (AIH) showing zone 3 necrosis. **a** and **b**: Perivenular zonal necrosis with hemorrhage is observed but portal inflammation is minimal (P). **c** and **d**: Perivenular zonal necrosis resembling hepatocellular necrosis of drug-induced liver injury is observed

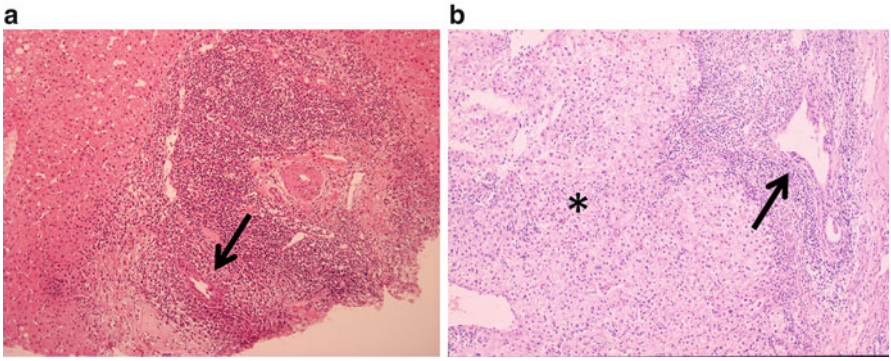

Fig. 4.21 Two cases of primary biliary cirrhosis–autoimmune hepatitis (PBC–AIH) overlap syndrome (hepatitic form of PBC). In addition to chronic nonsuppurative destruction cholangitis (CNSDC) (*arrows*), marked portal inflammation is observed. In the case on the right (**b**), interface hepatitis and lobular hepatitis (*) are also prominent

4.7.2 PBC–AIH Overlap Syndrome

AIH and PBC may simultaneously or metachronously coexist in some patients, which is designated as PBC–AIH overlap syndrome. Previous studies suggest that combination therapy of ursodeoxycholic acid (UDCA) and corticosteroids may be effective in these cases. Although its pathogenesis has been discussed for a long time, according to the statements of IAIHG, this overlap syndrome has been regarded as a subtype of PBC with the feature of AIH-like severe hepatitic change [36] and corresponds to the disease group that has been formerly called hepatitic form of PBC. Although AIH-like features are dominant in liver histology, distinct PBC features, such as CNSDC and bile duct loss, are also observed (Fig. 4.21).

Chazouilleres's criteria [37] (Paris criteria) have been used as the diagnostic criteria for this overlap syndrome, and the simplified AIH system of the IAIHG has been used as the criteria for the AIH feature in PBC. Steroid therapy (PSL) is recommended in addition to UDCA for cases that are considered to be PBC–AIH overlap syndrome. The Intractable Hepatobiliary Disease Study Group in Japan (2011) recommends PSL in addition to UDCA for cases that are considered to be PBC–AIH overlap syndrome and simultaneously meet the two following criteria: (1) diagnosis of PBC using the criteria of the Intractable Hepatobiliary Disease Study Group in Japan (2010) and (2) diagnosis of probable/definite AIH using IAIHG simplified criteria (2008). Regarding liver histology, hepatitic activity (HA) scores in the PBC grading/staging system should be used as follows: 0 points for HA score 0 or 1, 1 point for HA score 2, and 2 points for HA score 3 [18, 38, 39]. Because the presence of emperipolesis, which is not familiar to clinicians and even pathologists, is not required to survey the disease, pathological evaluation becomes relative easy.

4.8 Conclusion

In the diagnosis and management of AIH, liver biopsy is an essential element, because individual histological, serological, and clinical features are not specific for the diagnosis of AIH. Histological examination of liver biopsies helps exclude other potential causes of liver disease and identify variant syndromes. Therefore, AIH is thought to be a clinicopathological entity, and the communication between pathologists and clinicians is crucial in AIH.

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Chapter 5
Diagnosis of Autoimmune Hepatitis

Mikio Zeniya

Abstract Because no specific clinical index has been established, the first step in the diagnosis of autoimmune hepatitis (AIH) is to exclude factors for already known hepatic disorders. As strategies to establish the diagnosis, various diagnostic criteria, guidelines, and scoring systems have been proposed. Because a recently conducted nationwide survey on AIH in Japan has revealed clinical pathological conditions that are different from those previously known, new diagnostic criteria have been issued. This section reviews the diagnosis of AIH mainly on the basis of the new Japanese diagnostic criteria.

Keywords Autoimmune hepatitis (AIH) • Diagnosis • Diagnostic criteria

5.1 Introduction

Because there is no specific clinical index useful for the diagnosis of autoimmune hepatitis (AIH), it is, in principle, diagnosed by excluding the causes of already known hepatic disorders. Needless to say, several clinical findings are considered as classic features of AIH. They include positivity for autoantibodies such as serum antinuclear antibody (ANA) and anti-smooth muscle antibody (ASMA); elevated serum immunoglobulin (Ig)G level; histologically proven interface hepatitis in the portal area, especially plasma cell infiltration; and formation of liver cell rosettes. However, none of them is sufficient for AIH specificity. In these circumstances, many diagnostic criteria for AIH have been proposed. In Japan, the Autoimmune Hepatitis Study Group, a subgroup of the Intractable Hepatobiliary Disease Study Group of the Ministry of Health, Labor and Welfare, issued diagnostic criteria in 1996 in the "research study on intractable hepato-biliary disease," supported by

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Health and Labor Science Research Grants for Research on Intractable Diseases, and the criteria were revised on the basis of the results of the latest nationwide statistics in 2012. This section reviews the diagnosis of AIH mainly on these diagnostic criteria.

5.2 The Present Status of the Diagnostic Guidelines

At present, a method widely used in clinical practice is the AIH scoring system, which was proposed by the International Autoimmune Hepatitis Group (IAIHG) in 1998 [1]. Compared with the Japanese diagnostic criteria containing only descriptive indices, the AIH scoring system is superior in allowing quantitative assessment using numerical values to determine the certainty of diagnosis and can be considered to have been suited to the demands of clinicians. The Japanese criteria issued in 1999 specify that the 1998 revised AIH scoring system should be used as reference for the diagnosis of AIH. It is important to understand the fact that this scoring system was not developed for clinical diagnosis but rather to include and stratify cases presenting with pathological conditions of AIH in clinical studies. Subsequently, the clinical usefulness of the system was confirmed by many additional studies, and it has been widely used for diagnosis because of the excellent specificity and detection sensitivity of the system. However, the scoring system is not without problems. Calculation of scores according to the system requires many clinical indices, and they are not always immediately available in clinical practice. To solve these problems, the IAIHG proposed the simplified scoring system in which the number of indices required for the diagnosis was reduced to four [2]. This scoring system is simple and has been reported to be extremely useful for establishing a diagnosis and determining therapeutic interventions in typical cases. However, a diagnosis based on the scoring system is inadequate in atypical cases, and it has also been revealed that the system has a problem with detection sensitivity.

The previously described Autoimmune Hepatitis Study Group, subgroup of the Intractable Hepatobiliary Disease Study Group (led by Prof. Tsubouchi, Kagoshima University), started a nationwide survey on AIH in Japan in 2008 and registered 1,056 patients [3]. The analysis of these registered patients revealed that the clinical pictures of the autoimmune disease had changed in Japan compared with the similar nationwide statistics in 1996 [4]. Although it is not clear whether these changes are changes in pathological conditions themselves or are attributable to environmental factors including diagnostic ability, it is a fact that cases in which AIH is difficult to diagnose using the conventional criteria are increasing. Although this fact was taken into consideration, new criteria and guidelines for the diagnosis of AIH were developed. In the following subsections of this section, the results of the latest nationwide survey and the new diagnostic criteria are reviewed.

5.3 The New Diagnostic Criteria

Box 1 shows the new diagnostic criteria. The main revisions and cautions are reviewed as follows.

In AIH, for which no specific clinical diagnostic index has been established, exclusion of already known hepatic disorders still remains important. Although the differential diagnosis of viral hepatic disorders has been further facilitated by the advance in genetic diagnosis of viruses, the diagnosis of hepatic disorders caused by unknown viruses is still difficult. Thus, exclusion of infection with hepatitis virus, cytomegalovirus, and Epstein–Barr virus is an appropriate method at present. Although the guidelines issued by the IAIHG and the American Association for the Study of Liver Diseases (AASLD) indicate that autoantibodies that have been considered to be the clinical features of AIH in the past, such as ANA and ASMA, should be measured by the indirect immunofluorescence (IF) method using fresh frozen liver tissue of rodents, this method is performed at only a few research facilities in Japan. ANA is measured by using human epithelial (HEp-2) cells, an established cell line, in most cases. However, the IAIHG recommends detection by using frozen liver tissue [5], and the AASLD guidelines also contain similar descriptions. Based on a study conducted by the authors, the correlation between measurement using frozen liver tissue and that using HEp-2 cells is extremely high. While the present status of laboratory test in Japan and consistency of results were taken into consideration, the new criteria specify that autoantibodies should be measured by the indirect IF method using HEp-2 cells. In recent years, the measurement of autoantibodies has commonly been performed by enzyme-linked immunosorbent assay (ELISA) using solid-phase correspondent antigens. For ANA, ELISA is also used in daily clinical practice. While ELISA is performed using MESACUP Protein Kinase Assay kit (BML International) in most cases in Japan, antigens used for ELISA are prepared for testing for collagen diseases such as systemic lupus erythematosus. In fact, the authors revealed that when serum samples of AIH patients were examined by the MESACUP kit and the indirect IF method using HEp-2 cells, 36.7 % of the samples were false-negative by the MESACUP kit. QUANTA Lite (INOVA Diagnostics, Inc.), which is frequently used in Europe and the United States, is superior to the MESACUP kit in detection sensitivity because nuclear HEp-2 cell extract antigens are added to QUANTA Lite. However, this kit still gives false-negative results in 20 % of samples compared with the indirect IF method. While these circumstances are taken into consideration, the new criteria specify that ANA in samples from AIH patients should be measured by the indirect IF method using HEp-2 cells.

In the past, serum levels of γ-globulin and IgG were markedly elevated in AIH, and an IgG level of 2.0 g/dL or higher was used as an index for the diagnosis. Although the survey in 1997 showed that IgG levels exceeded 2.0 g/dL in 73.5 % of the patients, the latest nationwide survey showed a marked decrease in the proportion of patients with that IgG level to 35 %. This might have been attributable to the revision of the IgG measuring method, and in many current cases, the diagnosis is

difficult to establish by the old criteria. Thus, based on the results of the nationwide survey and the scoring system developed by the IAIHG, the index for the diagnosis was changed to at least 1.1 times higher than the standard value in the new criteria.

As for differentiation from drug-induced liver injury (DILI), which is the most important problem in differential diagnosis, the new criteria specify that the DILI diagnostic scale [6] and its manual proposed in the Japan Digestive Disease Week in 2004 should be used as references. How to handle the problem has been clarified. Meanwhile, Miyake et al. [7] reported that the measurement of anti-programmed cell death-1 antibody in blood early after the onset is useful for the differentiation between DILI and AIH.

The clinical fact of excellent responses to corticosteroid (CS) therapy is a feature of AIH. Although the assessment of this responsiveness can be a therapeutic diagnosis, as described later, atypical AIH such as the acute-onset type is difficult to diagnose in more than a few patients. While this present status is taken into consideration, the responsiveness to CS has been added as one of the diagnostic criteria. This is another feature of the new criteria. Early therapeutic interventions resulting from this addition are expected to prevent exacerbation of AIH and lead to improved prognosis.

For diagnosis, exclusion of the causes other than diagnostic criterion one is necessary. Patients fulfilling three criteria from diagnostic criterion two to five are to be diagnosed as having typical AIH, and those fulfilling one or two criteria are to be diagnosed as having atypical AIH. To examine the validity of the new diagnostic criteria, accumulation of cases in which the diagnosis is established by these criteria is needed.

One of the features of the new diagnostic criteria is the addition of the severity assessment criteria to determine severity at the time of diagnosis. Although this severity classification had been presented in the past, it was not commonly accepted and rarely applied in clinical practice. It is greatly expected that the addition of this severity classification to the new diagnostic criteria will lead to the application of appropriate treatment for patients with severe disease. Meanwhile, it is also important to understand that the diagnosis of severe AIH does not always indicate exacerbation but only identifies patients with a possibility of exacerbation. To prevent overtreatment, assessment by following the clinical course is also important.

Box 1. New Diagnostic Criteria for Japanese AIH Patients [1]

I. Introduction

Autoimmune hepatitis (AIH) is an inflammatory liver disease of unknown etiology that occurs predominantly in middle-aged to elderly women. Genetic susceptibility and autoimmune pathogenesis have been reported to be associated with both onset and progression of the disease process.

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AIH is characterized clinically by the presence of autoantibodies, 2, especially antinuclear antibody (ANA) or anti-smooth muscle antibody (ASMA), and hypergammaglobulinemia. AIH presents as acute or chronic hepatitis, and some patients are diagnosed by the occasional detection of laboratory abnormalities in liver function test (elevation of aspartate aminotransferase (AST) or alanine aminotransferase (ALT)) without any symptoms. Acute hepatitis phase of AIH sometimes lacks these characteristic features of AIH. These patients have a risk to progress to hepatic failure.

In the majority of patients, the response to corticosteroid treatment is excellent and resulting in normalization of serum transaminases. On the other hand, corticosteroid treatment shows insufficient response in patients who delay the treatment commencement and have severe liver injury. In addition, some patients show resistance to corticosteroid treatment.

The characteristic histological feature is chronic hepatitis with interface hepatitis and predominantly lymphoplasmacytic infiltrate in portal tracts. In addition, severe focal necrosis, zonal necrosis, or bridging necrosis is usually seen. The formation of liver cell rosettes is often present in severe cases. The patients with severe portal infiltrate sometimes show the bile duct damage, but ductopenia is rarely seen. Some patients have liver cirrhosis and/or hepatocellular carcinoma at initial presentation.

In addition to these characteristic features, exclusion of other known specific causes of liver injury, such as virus infection, exposure to drug, and hepatic steatosis, is a prerequisite for making a diagnosis of AIH. The diagnostic criteria proposed by the International Autoimmune Hepatitis Group (IAIHG) are available to confirm a diagnosis of AIH. The simplified diagnostic criteria are helpful to decide the indication for corticosteroid treatment.

Note

1. In Japan, HLA-DR4 allele is strongly associated with AIH.
2. A measurement of liver–kidney microsome antibody type 1 (LKM-1) is necessary for proper diagnosis in patients who are seronegative for ANA and ASMA. ANA screening should be performed by indirect immunofluorescent techniques using human epithelial (HEp-2) cell lines.

II. Diagnosis

#1. Exclusion of other known specific causes of liver injury
#2. Presence of serum antinuclear antibody (ANA) or anti-smooth muscle antibody (ASMA)

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#3. High serum immunoglobulin G levels (>1.1 times upper normal limit)
#4. Histological features such as interface hepatitis and/or plasma cell infiltrationin to portal area
#5. Excellent response to corticosteroid

Typical case (definite): Fulfill #1 and at least three criteria among #2–5.
Atypical case (probable): Fulfill #1 and one or two criteria among #2–5.

Note

1. Excellent response to corticosteroid therapy is helpful in establishing the diagnosis of AIH. However, in principle, it is desirable to perform a liver biopsy and diagnose according to its histological findings before corticosteroid treatment, irrespective of types of clinical course. When a liver biopsy is unavailable before treatment for some reasons, immediate treatment is recommended.
2. The revised diagnostic scoring system proposed by the International Autoimmune Hepatitis Group (IAIHG) may be helpful to diagnose as definite or probable AIH according to its scoring system as categorized in AIH.
3. Some patients have already progressed to cirrhosis at initial diagnosis.
4. Patients with acute hepatitis phase sometimes do not fulfill the criteria #2 and/or #3. In addition, some patients showed histological features of a predominance of centrizonal lobular necroinflammation with lymphoplasmacytic infiltration without significant changes in the portal and periportal areas.
5. Confirmation of diagnosis should be certainly followed by assessing the disease severity. Physicians should refer patients to a hepatologist immediately in case of severe condition or according to clinical course in case of moderate condition. The patients who fulfill only #1 but are suspected to progress to acute hepatic failure should also be referred to a hepatologist.
6. If the simplified diagnostic criteria are implemented as definite or probable AIH, corticosteroid treatment should be considered.
7. Corticosteroid treatment should be considered irrespective of the presence of antimitochondrial antibody (AMA) when the simplified diagnostic criteria are implemented as definite or probable AIH. Prevalence of AMA has been reported as ~10 % in patients with AIH.
8. Diagnostic scale proposed by a workshop on drug-induced liver injury (DILI) held during Digestive Disease Week, Japan, 2004, should be referred to for differential diagnosis of DILI.
9. This guide is not always applicable for AIH patients simultaneously affecting with other already known causes of liver injury.

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III. Severity

Clinical signs	Clinical laboratory tests	Imaging tests
① Hepatic encephalopathy	① AST/ALT of more than 200 U/L	① Hepatic atrophy
② Reduction or disappearance of hepatic dullness	② Bilirubin of more than 5 mg/dL ③ Prothrombin time of less than 60 %	② Heterogenous liver parenchyma pattern

Severe: Should fulfill at least one of the following three findings:
1. Clinical signs: ① or ②
2. Clinical laboratory tests: both ① and ③, or both ② and ③
3. Imaging tests: ① or ②

Moderate: Should fulfill these findings:
Clinical laboratory tests: one of the criteria (①, ② or ③) or both ① and ②, without clinical signs (neither ① nor ②), and imaging tests (neither ① nor ②)

Mild: None of the above criteria is observed.

Note

1. When patients are judged to have severe disease, they should be referred to a hepatologist immediately.
2. The scoring system for predicting mortality in patients with acute liver failure or late-onset liver failure, as established by the Intractable Hepatobiliary Diseases Study Group in Japan and the model for end-stage liver disease (MELD) score, should also be evaluated and considered in patients with severe disease.
3. When patients show prothrombin times <60 % or in cases where intense jaundice is present, referral to a hepatologist should be considered, even if the patients has moderate disease.

5.3.1 Age of Onset and Sex Difference

AIH predominantly occurs in women in Japan. According to the latest survey, the peak age of onset is in the 60s, and there are currently more elderly patients than in the past. While patients 60 years or older account for approximately 60 %, those 70 years or older account for approximately 30 %. Especially when patients 70 years or older with a hepatic disorder of unknown cause are diagnosed, it is important to take AIH into consideration. The male-to-female ratio is 1:6, showing an apparent female predominance. However, caution should be paid to the fact that there are more than a few male patients, as observed in overseas cases.

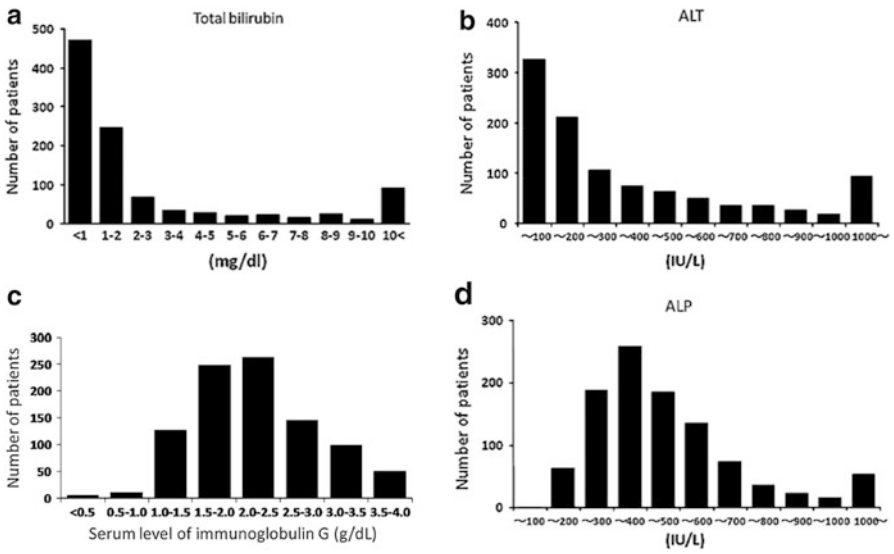

Fig. 5.1 Distribution of serum total bilirubin **a**) ($n = 1{,}037$), **b**) ALT ($n = 1{,}046$), **c**) ALP ($n = 1{,}035$), and **d**) IgG ($n = 1{,}056$) levels at diagnosis in patients with AIH. The upper limit of the normal range of ALP in all participating institutes was 343 ± 23 IU/Ll (median 348 IU/Ll)

5.3.2 Clinical Symptoms and Signs

The clinical picture of AIH ranges from an asymptomatic form to that presenting with acute liver failure. The pathological conditions of Japanese patients are relatively milder than those of European and American patients. Some asymptomatic patients (35 %) are accidentally pointed out to have a hepatic disorder and diagnosed as having AIH. As for the common symptoms, general malaise is observed in 50 % of patients, and jaundice and anorexia are observed in 30 %. Many patients present with signs rarely observed in common chronic viral hepatitis, such as overt jaundice, elevated sedimentation rate, and positivity for C-reactive protein. In patients who already have progression to hepatic cirrhosis at the time of diagnosis, findings of hepatic cirrhosis, spider angiomas, splenomegaly, and ascites may be observed. Moreover, complications with other autoimmune diseases are observed in approximately 30 % of the patients. It occurs more frequently in female patients. According to the latest survey, the common complications are chronic thyroiditis and Sjogren syndrome.

Figure 5.1 shows the distribution of levels of aspartate aminotransferase (AST), bilirubin, and alkaline phosphatase in Japanese patients. In Japan, many patients present with relatively mild hepatic disorders who have AST levels of 100 IU/L or lower. Moreover, although many patients have abnormal alkaline phosphatase levels, only a few patients have increased levels of more than twice the standard value. The previous survey showed that the IgG levels exceeded 2 g/dL in most

patients, whereas the latest survey showed a change in the proportion (i.e., 40 % or more) of patients with IgG levels of 2 g/dL or lower.

Regarding positive rates for serum autoimmune antibodies, ANA is positive in 89.1 % of the patients, ASMA is positive in 42.5 %, and ASMA is positive in 43.6 % of the patients negative for ANA. Although liver–kidney microsomal (LKM) antibody should be measured in patients negative for both antibodies, the incidence of type 2 AIH, in which patients are positive for LKM antibody alone, is extremely rare in Japan. In Japan, antimitochondrial antibody is positive in 9.5 % of the patients, and anti-pyruvate dehydrogenase antibody detected by ELISA is positive in 19.7 %. Moreover, hepatitis B surface antigen is positive in 1.3 %, and hepatitis C virus (HCV) antibody is positive in 5 %. Japanese patients positive for HLA-DR4 and HLA-DR2, which are predisposition genes for AIH in the Japanese population, account for 60 and 20 %, respectively.

5.4 Liver Histology

Liver tissue shows findings characteristic to AIH, such as interface hepatitis, panacinar inflammation, invasive cells predominantly composed of plasma cells, formation of liver cell rosettes, and emperipolesis (this is described in detail in Chap. 4). However, the diagnosis of AIH cannot be established with certainty using these findings alone, and the exclusion of other factors remains important. When the diagnosis is difficult to establish on the basis of blood biochemical findings, liver tissue findings are extremely useful for supporting the diagnosis. Moreover, liver tissue findings also provide important information for the assessment of the progression and activity of the liver lesions. Thus, assessment of liver tissue by liver biopsy is needed for the diagnosis of AIH. In addition, liver tissue findings also provide the most important information for determining therapeutic effects. In AIH, there are more than a few cases in which inflammatory findings are observed on histological examination despite the subsidence of a pathological state based on blood biochemical findings.

5.5 Patients for Whom the Diagnosis Is Difficult to Establish

5.5.1 Patients with Acute-Onset AIH

Regarding this pathological condition, the recommendations shown in Box 2 have been issued in Japan [8]. AIH used to be treated as chronic hepatitis. However, some patients have acute exacerbation of chronic hepatitis and those who have a pathological condition present with no histological picture of chronic hepatitis [9];

this fact has also been confirmed in Japan [10]. According to the latest nationwide survey, the incidence rate of such pathological conditions in all AIH patients is 10 % or higher, which is higher than the proportion of those who already have liver cirrhosis at the time of diagnosis. These conditions are more important in terms of pathology. The acute-onset type often lacks diagnostic features of AIH, high IgG levels, and positive findings of autoimmune antibodies; is difficult to diagnose; and consequently, has a poor prognosis [11]. However, the new diagnostic criteria allow AIH to be diagnosed on the basis of the responsiveness to CS if AIH is suspected after other already known factors are ruled out. Meanwhile, in patients with acute-onset AIH who present with no feature of AIH, centrizonal lobular necroinflammation has been reported as a characteristic finding [12] and that computed tomographic images of patients with a severe disease reveal geographic findings [13]. Because the new Japanese diagnostic criteria use severity assessment at the time of diagnosis, the application of the assessment is expected to contribute to more rapid treatment and improved prognosis.

5.5.2 The Diagnosis of the So-Called Overlap Syndrome and the So-Called Outlier

The clinical presence of pathological conditions in which AIH overlaps with primary biliary cirrhosis (PBC) or primary sclerosing cholangitis (PSC) has been known [14], and criteria for the diagnosis of AIH-PBC overlap syndrome have also been issued [15]. Washington reports that the so-called overlap syndrome can be diagnosed in approximately 10 % of patients with AIH or PBC [16]. However, under present circumstances, it is generally considered appropriate to diagnose and/or treat mainly based on major etiology instead of establishing the concept of overlap syndrome. The AASLD guidelines also recommend that the term overlap should not be used [17]. Moreover, the IAIHG also states that the IAIHG scoring system should not be used for the diagnosis of overlap syndrome [18]. Similarly, a study conducted in Japan indicates that the diagnosis should be established mainly based on major etiology [19]. To determine the major etiology, the diagnostic formula developed in Japan is useful [20]. Autoimmune cholangiopathy, which was first reported by Ben-Ari et al. [21], and autoimmune cholangitis [22] are currently called PBC. Although they appear similar to AIH, it is appropriate to consider that they are more severe in terms of findings of hepatitis. Thus, these terms should not be used.

AIH overlapping with PSC has also been reported [23]. Its incidence is higher in children [24], and differential diagnosis is important. There may be a greater need to consider AIH-PSC overlap in children as an independent pathological condition [25].

The coexistence of HCV infection and AIH has also been reported [26, 27].

There are also patients who cannot be diagnosed as having AIH by any diagnostic criteria or guidelines but in whom no etiology can be pointed out except for AIH. Especially in patients negative for autoimmune antibodies, the diagnosis is often difficult to establish, and Czaja et al. reported that such patients account for approximately 13 % [28]. Although it is proposed to call this pathological condition "cryptogenic chronic hepatitis," many cases are reported to be AIH [29]. Although responsiveness to CS therapy is useful for establishing the diagnosis in patients with such a condition, the diagnosis is difficult to establish based on only responsiveness to CS because hepatic disorders induced by non-AIH factors also respond to CS therapy. The AASLD guidelines show that relapse due to discontinuation of CS after amelioration of pathological conditions has been confirmed. However, continuation of CS therapy cannot be recommended because it is associated with a risk of acute exacerbation and progression to a severe disease. Moreover, AIH can spontaneously remit, depending on the clinical course. In addition to careful follow-up of patients, establishing the diagnosis by performing biopsy as needed is important.

5.5.3 Pediatric Patients

It has been apparent that diagnosis and/or treatment for pediatric patients differ from those for adult patients because of growth and/or development factors [30]. Because the IAIHG scoring system includes inappropriate factors for children in the development and/or growth stage, the IAIHG has also developed a separate scoring system for children. Moreover, biopsy findings in children also often differ from those in adults, and differentiation between AIH and PSC is also often difficult. Please refer to Chap. 9 for details.

5.5.4 IgG4-Associated AIH

Yada et al. [31] summarized this condition clearly as follows: A few studies have reported AIH cases with infiltrated IgG4-positive plasma cells in the liver, suggesting the involvement of IgG4 in the pathogenesis of AIH. This feature was called IgG4-associated AIH and only two studies have been published. However, the diagnostic criteria of IgG4-associated AIH have not been defined and the epidemiology and clinical features remain uncertain. The degree of IgG4-positive plasma cell infiltration in the liver was different in each article. The serum IgG4 level was not elevated in one study, whereas it was severely elevated in the other. Corticosteroid therapy normalized liver enzymes in both studies. Further studies are needed to define the epidemiological features or diagnostic criteria.

5.5.5 Type 2 AIH

Type 2 AIH, in which the LKM antibody is positive, is rare in Japan. Most of those positive for LKM antibody are positive for ANA and have type 1 AIH.

5.5.6 Hepatitis Virus

Because the HCV infection rate is high in middle-aged and elderly people in Japan, many patients have AIH accompanied by HCV infection [32]. Meanwhile, because HCV infection is also known to cause autoimmune reactions, such as cryoglobulinemia, it may be difficult to diagnose the infection [33, 34]. In principle, interferon used for the eradication of HCV infection is contraindicated for administration to patients with AIH due to the immunostimulatory effect of the drug. The diagnosis of AIH accompanied by HCV infection and the determination of indicated treatment are important.

5.5.7 De Novo AIH

The occurrence of AIH after organ transplantation, especially liver transplantation, has been reported, and such a case is called de novo AIH [35]. This also means that de novo AIH occurs in patients receiving immunosuppressants. Because of this condition and rejection, viral infection, use of other concomitant drugs, etc., the diagnosis and treatment of AIH are often difficult. In cases fulfilling the diagnostic criteria, clinical approaches with sufficient consideration of the presence of AIH are necessary.

Box 2. Proposal of Autoimmune Hepatitis Presenting with Acute Hepatitis, Severe Hepatitis, and Acute Liver Failure

Sometimes autoimmune hepatitis (AIH) may present in patients with no previous history of liver disease as acute hepatitis, severe hepatitis, or acute liver failure (fulminant hepatitis and late-onset liver failure (LOHF)). The disease is characterized by the presence of autoantibodies, such as antinuclear and anti-smooth muscle antibodies, and elevated serum bilirubin, transaminases, and immunoglobulin (IgG). However, atypical presentation may also occur. In such cases, it is necessary to exclude the possibilities of diagnosis of acute viral hepatitis and drug-induced liver injury. There are two types of

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AIH presentation, for which assessment of liver biopsy specimens is necessary for an accurate discrimination:

1. Acute exacerbation phase in which patients show clinical features of acute hepatitis with histological evidence of chronic hepatitis, such as the presence of fibrosis and moderate to severe inflammatory cell infiltrations in the portal tracts.
2. Acute hepatitis phase in which patients exhibit histological features of acute hepatitis, such as centrilobular necrosis, without or with minimal periportal hepatitis. These patients sometimes show lower serum levels of IgG and/or the absence or low titers of serum autoantibodies. Some patients may exhibit histological features of transition to chronicity. Patients with AIH usually respond well to corticosteroid treatment. For AIH patients in the acute hepatitis phase, however, diagnosis is sometimes difficult and the start of therapy is delayed. Patients progressing to acute liver failure (fulminant hepatitis and LOHF) become resistant to corticosteroid treatment, and their prognoses become poor. Consequently, liver transplantation is also considered as a therapeutic option in such patients.

Appendix

1. There are some cases with two types of presentation and a clear classification of these is difficult.
2. Some cases might be considered as non-A–E acute hepatitis.
3. Response to immunosuppressive therapy is sometimes better in young AIH patients with acute liver failure.
4. Further studies are necessary to clarify the relationship between the severity of hepatitis and the response to corticosteroid treatment.

5.6 Conclusion

The Japanese diagnostic guidelines based on the latest nationwide questionnaire survey conducted in Japan have been reviewed. Because no specific clinical indices have been established, the diagnosis of AIH depends on the exclusion of already known pathological conditions and guidelines indicating strategies to exclude them at present. The establishment of more specific clinical indices is an issue.

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Chapter 6
Acute Presentation of Autoimmune Hepatitis

Masanori Abe and Morikazu Onji

Abstract Autoimmune hepatitis (AIH) can present as acute hepatitis in its onset of symptoms and features, progressing to severe hepatitis and acute liver failure. In a recent national survey in Japan, 10.9 % of the patients with AIH were diagnosed with acute hepatitis. Some patients with acute presentation of AIH lack the typical features of AIH, such as increased serum immunoglobulin G levels and high autoantibody titers. For accurate diagnosis, it is essential to exclude the other possible causes that can lead to acute hepatitis, and liver biopsy should be performed early if appropriate. Centrilobular necrosis without interface hepatitis or fibrosis is considered to be the histological characteristic in the acute hepatitis phase of AIH. Interface hepatitis is a hallmark characteristic of AIH; therefore, its absence in acute hepatitis phase can lead to missed diagnosis of AIH. Most patients with an acute presentation of AIH respond well to corticosteroid therapy; however, the prognosis of AIH patients worsens with progression to acute liver failure. Liver transplantation is a possible treatment option for patients with acute liver failure who do not respond to corticosteroid therapy.

Keywords Acute hepatitis • Acute liver failure • Autoimmune hepatitis

6.1 Introduction

Autoimmune hepatitis (AIH) is a persistent inflammatory liver disorder from unknown causes that is histologically characterized by lobular hepatitis, interface hepatitis, and mononuclear cell infiltration predominantly consisting of plasma

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cells; the serological characteristics of AIH include the presence of autoantibodies, hypergammaglobulinemia, and elevated aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels [1, 2]. As the diagnostic techniques for detecting hepatitis viruses have been improved, the clinical entry criteria of AIH have been more clearly established. The diagnostic criteria of AIH, based on clinicopathological features, were proposed by the International AIH Group (IAIHG) [3, 4]; these criteria have been widely accepted and used as useful diagnostic tools in clinical practice. Recently, a diagnostic guide for AIH was proposed by the Intractable Hepatobiliary Disease Study Group in Japan [5].

Most patients with AIH present with chronic hepatitis with or without specific symptoms or are observed to have liver dysfunction during routine medical examination. However, since Lefkowitch et al. first reported a case in 1984 [6], the numbers of patients with acute presentation of AIH have increased in Japan [7–13]. The acute presentation of AIH comprises clinical features of acute hepatitis with histological evidence of chronic hepatitis (acute exacerbation) or those of acute hepatitis (acute hepatitis phase); in these patients, histopathological assessment of the liver is necessary for accurate discrimination and diagnosis [14–16] (Box 1). In addition, some patients with AIH progress to acute liver failure, including fulminant hepatitis or late-onset liver failure (LOHF) [17–21].

The clinical features of Japanese AIH differ from those of Caucasian patients. In this chapter, we have mainly focused on the clinical features of Japanese AIH patients with acute presentation.

Box 1. Proposal of Autoimmune Hepatitis Presenting with Acute Hepatitis, Severe Hepatitis, and Acute Liver Failure [14]

Sometimes autoimmune hepatitis (AIH) may present in patients with no previous history of liver disease as acute hepatitis, severe hepatitis, or acute liver failure (fulminant hepatitis and late-onset liver failure [LOHF]). The disease is characterized by the presence of autoantibodies, such as antinuclear and anti-smooth muscle antibodies, and elevated serum bilirubin, transaminases, and immunoglobulin (Ig)G. However, atypical presentation may also occur. In such cases, it is necessary to exclude the possibilities of diagnosis of acute viral hepatitis and drug-induced liver injury.

There are two types of AIH presentation, for which assessment of liver biopsy specimens is necessary for an accurate discrimination:

(1) Acute exacerbation phase in which patients show clinical features of acute hepatitis with histological evidence of chronic hepatitis, such as the presence of fibrosis and moderate to severe inflammatory cell infiltrations in the portal tracts.
(2) Acute hepatitis phase in which patients exhibit histological features of acute hepatitis, such as centrilobular necrosis, without or with minimal

(continued)

(continued)

periportal hepatitis. These patients sometimes show lower serum levels of IgG and/or the absence or low titers of serum autoantibodies. Some patients may exhibit histological features of transition to chronicity.

Patients with AIH usually respond well to corticosteroid treatment. For AIH patients in the acute hepatitis phase, however, diagnosis is sometimes difficult and the start of therapy is delayed. Patients progressing to acute liver failure (fulminant hepatitis and LOHF) become resistant to corticosteroid treatment, and their prognoses become poor. Consequently, liver transplantation is also considered as a therapeutic option in such patients.

Appendix

1. There are some cases with two types of presentation and a clear classification of these is difficult.
2. Some cases might be considered as non-A–E acute hepatitis.
3. Response to immunosuppressive therapy is sometimes better in young AIH patients with acute liver failure.
4. Further studies are necessary to clarify the relationship between the severity of hepatitis and the response to corticosteroid treatment.

6.2 Prevalence of the Acute Presentation of AIH

Most epidemiology data concerning the acute presentation of AIH in Japan is obtained from single-center studies (mainly tertiary referral hospitals) [8–12]. The reported prevalence has varied widely probably due to selection bias, rarity of the disease, and lack of uniformity in the diagnostic criteria used to identify cases. In a Japanese nationwide survey study conducted in 1991, 5.6 % of patients with AIH were found to have a feature of acute hepatitis upon histological examination [7]. The most recent Japanese survey was performed in 2009, which enrolled 1,056 patients with AIH from 153 hospitals and clinics throughout Japan [13]. Histological assessment results were available for 871 patients, and 95 patients (10.9 %) were diagnosed with acute hepatitis.

Few descriptive epidemiology studies are available concerning the prevalence of AIH in Japanese patients presenting with acute hepatitis. In 2006, Fukumoto et al. [22] surveyed the etiology of acute hepatitis in 1,815 patients from 15 hospitals in the Chugoku area; the prevalence of AIH was 1.3 %. In 2011, a nationwide survey of acute hepatitis was conducted in Japan [23]. The etiology of 2,547 patients with acute hepatitis from 103 hospitals throughout Japan was analyzed. The most prevalent cause was hepatitis B virus (31.8 %), followed by AIH (14.0 %). These results cannot be directly compared since the criteria used in these studies were different; however, these data suggest that the number of patients with acute presentation of AIH has increased recently.

The definition, classifications, and diagnostic criteria for acute liver failure differ between Japan and European countries and the United States. In 2011, the Intractable Hepatobiliary Disease Study Group in Japan established novel diagnostic criteria for acute liver failure, including the disease entity of fulminant hepatitis [24]. A nationwide survey of patients with acute liver failure in 2010 using this criteria showed that AIH was found in 16 of 220 patients (7.2 %) with acute liver failure and LOHF, including 9 of 96 patients (9.3 %) with acute liver failure without hepatic coma, 4 of 54 patients (7.4 %) with the subacute type of fulminant hepatitis, and 3 of 9 patients (33.3 %) with LOHF [25]. Very recently, the classification of the etiologies of acute liver failure was proposed by the same group [26]. In this classification, patients satisfying the diagnostic guide for AIH [5], or those positive for antinuclear antibody (ANA) or serum immunoglobulin G (IgG) concentrations 1.1 times the upper limit of normal range or greater, are diagnosed with acute liver failure due to AIH. Further studies using this classification are considered necessary.

6.3 Pathogenesis of the Acute Presentation of AIH

The causes of the acute presentation of AIH—either as acute hepatitis or as acute exacerbation—are unknown. As in other autoimmune diseases, it is believed that AIH develops in individuals with genetic predisposition under the influence of environmental factors. Most genetic data for AIH is derived from studies of HLA genes. Several studies indicate that HLA may influence the clinical phenotype of AIH [27]. HLA-DR4 is the major susceptibility factor for AIH in Japan [28, 29]; however, a difference in HLA for the acute presentation of AIH has not been indicated. Genetic polymorphisms of non-HLA genes, such as *cytotoxic T lymphocyte antigen 4* and *tumor necrosis factor alpha*, may also influence the clinical phenotypes of AIH [30]. To identify the genes influencing the susceptibility and clinical profile of AIH, genome-wide association studies are underway in a Japanese cohort.

It is very difficult to obtain information regarding the immunological reactions responsible for the initiation of AIH. The use of animal models might improve our understanding of the pathophysiology of this disease. Several models of experimental AIH have been reported by Japanese researchers (described in detail in Chap. 2); most of these models show acute hepatitis rather than chronic hepatitis. Recently, Kido et al. [31] reported a mouse model of spontaneous acute presentation of AIH model by inducing concurrent loss of $FoxP3^{+}$ regulatory T cells and PD-1-mediated signaling. $PD\text{-}1^{-/-}$ mice develop AIH characterized by T cell infiltration and increased ANA titers, indicating a role of autoreactive and regulatory T cells in the pathogenesis of acute presentation of AIH.

Defects in liver regeneration have been implicated as a factor contributing to the acute presentation of AIH. Fujiwara et al. [32] reported the immunohistochemical evaluation of liver tissue with cytokeratin 7 in patients with acute presentation of

AIH and demonstrated the presence of intermediate hepatocytes and intralobular progenitor cells in patients with severe acute presentation of AIH. These findings were less common in patients with non-severe presentation, indicating that impairment in the liver's regenerative response may contribute to the development or progression to acute liver failure.

6.4 Diagnostic Features of the Acute Presentation of AIH

Acute presentations of AIH are recognized in both adults and children. Fujisawa et al. [33] reported that 5 out of 12 Japanese children with AIH showed acute presentation. The clinical features of AIH in Japanese children are described in detail in Chap. 9; therefore, we have mainly focused on the acute presentation of AIH in Japanese adults in this chapter.

Most reports have demonstrated that the acute presentation of AIH typically affects middle-aged women, and their profiles are indistinguishable from those with chronic presentation in terms of age and gender [8–11]. However, Miyake et al. [12] showed that the age at diagnosis of acute hepatitis phase was younger than that of chronic hepatitis.

6.4.1 Laboratory Features

Blood biochemistry tests have shown that the acute presentation of AIH is associated with higher serum levels of AST, ALT, and total bilirubin and with lower prothrombin time (PT) activity as compared with chronic presentation [8–12]. In contrast, serum levels of γ-globulin or IgG have been reported to be lower in the acute presentation of AIH [8, 9, 11, 12]. In addition, some patients with acute presentation of AIH showed the absence or low titers of serum autoantibodies, particularly ANA. There are several differences in laboratory data among studies concerning the acute presentation of AIH, with many possible reasons for these discrepancies. First, the time points at which laboratory data were obtained may be different for each patient since all the studies were conducted retrospectively. The duration between the initial onset of symptoms and the time of diagnosis of AIH was variable, and liver injury was already in progress at the time of admission in some patients. In fact, several groups have reported IgG levels to be higher in patients with severe hepatitis or acute liver failure than those in non-severe patients [18, 19]. Second, some studies have analyzed the "acute hepatitis phase" (histologically proven acute hepatitis) as acute presentation [7, 8, 11, 12], while others have included both "acute hepatitis phase" and "acute exacerbation phase" in the definition [9, 10, 12]. Further studies with larger cohort using a well-defined classification may be necessary to clarify the clinical features of the acute presentation of AIH.

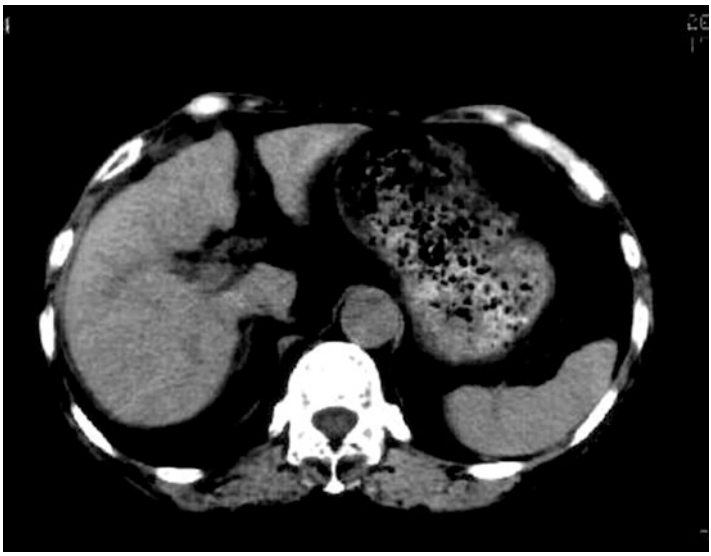

Fig. 6.1 Computed tomography image of a patient with fulminant hepatitis resulting from AIH. In addition to the liver atrophy (estimated liver volume, 440 g), an area of heterogeneous hypoattenuation was present

6.4.2 Imaging Study Findings

As described later, histological examination is useful for diagnosing patients with acute presentation of AIH. However, it is sometimes difficult to perform liver biopsy in such patients due to the presence of complicated coagulopathy and/or ascites. Yasui et al. [34] reported the usefulness of computed tomography (CT) in the diagnosis of acute liver failure due to AIH (Fig. 6.1). They showed that the heterogeneous area of hypoattenuation in the liver, which may reflect massive necrosis and preserved parenchyma, was present in 65 % of the patients with acute liver failure due to AIH.

6.4.3 Histological Features

Histological examination of liver tissue is essential in order to confirm the diagnosis of AIH and evaluate the severity of the disease. The typical histological pattern of AIH comprises interface hepatitis with portal and periportal plasma cells, hepatocyte rosettes, and in severe cases bridging necrosis or acinar collapse [1, 2]. In addition, histological evaluation is necessary to discriminate between acute hepatitis phase and acute exacerbation in patients with acute presentation of AIH [14–16].

In acute presentation, centrilobular necrosis (zone 3 necrosis), including submassive and massive necrosis—rarely seen in patients with chronic

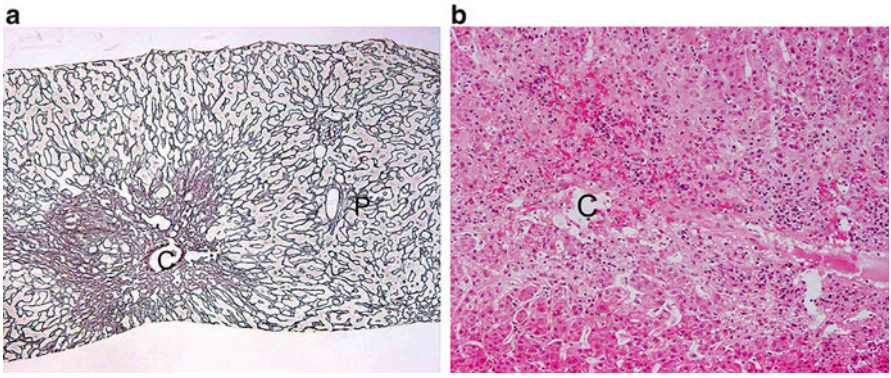

Fig. 6.2 Liver histology of a patient in the acute hepatitis phase of AIH. Hepatocellular zonal necrosis was present in the centrilobular area (C). Portal tracts (P) are preserved without evident inflammatory changes (**a**, reticulin staining; **b**, H&E staining)

presentation—has been observed frequently (Fig. 6.2) [8–12, 18, 19, 35–38], although it is a nonspecific histological finding also observed in other liver diseases, such as acute viral hepatitis or drug-induced liver injury. In particular, centrilobular necrosis with minimal to mild portal inflammation and without interface hepatitis or fibrosis is considered to be the characteristic histological feature of the acute hepatitis phase of AIH [14–16]. According to the studies that performed follow-up biopsy at the time of recurrence of AIH, some patients progressed to the typical histological features of AIH, indicating that centrilobular necrosis may reflect early lesion preceding portal involvement; in contrast, other patients showed recurrent episodes of centrilobular necrosis. These data suggest that centrilobular necrosis with acute presentation of AIH may not be a single disease entity [36], and further studies are necessary in this regard. Further, plasma cell enrichment has also been demonstrated to be characteristic of the acute presentation of AIH [18, 39].

The Acute Liver Failure Study Group in the United States analyzed the histological characteristics of acute liver failure due to AIH [39]. Massive hepatic necrosis with centrilobular hemorrhagic necrosis or classical interface hepatitis was frequently observed in patients with AIH. In addition, they also proposed that the presence of central venulitis, plasma cell enrichment, and lymphoid aggregates represent histological features of acute liver failure due to AIH.

6.4.4 Diagnostic Scoring System

The revised scoring system proposed by IAIHG [4] has been widely accepted and incorporated into clinical practice. However, the acute presentation of AIH often lacks the typical features of AIH, such as high immunoglobulin levels and autoantibody titers, as described above. These features sometimes make the diagnosis of the acute presentation of AIH difficult according to this diagnostic scoring system [8, 9, 11, 12, 14–16].

In 2008, the simplified diagnostic criteria for the diagnosis of AIH were proposed by IAIHG [40]. Miyake et al. [41] reported that 77 % of patients with acute presentation and 50 % of those presenting with the acute hepatitis phase of AIH fulfilled the diagnostic requirements of the simplified criteria. In addition, Fujiwara et al. [42] reported that only 28 % of patients presenting with the acute hepatitis phase met the diagnostic criteria of the simplified system, although the frequency was 91 % for the revised scoring system. The superiority of the revised system for the diagnosis of the acute presentation of AIH probably reflects its comprehensive nature and the frequency at which serum immunoglobulin levels and autoantibody titers are low in such patients. However, it is important to make the diagnosis based on the scoring systems as well as using the comprehensive assessments.

6.5 Treatment of the Acute Presentation of AIH

The severity of the disease should be judged based on the diagnostic guide for AIH in Japan (Table 6.1) [5] when the diagnosis is made. According to the severity, the referral to an expert hepatologist should be also considered.

In Japan, the majority of the patients with acute presentation of AIH are treated with corticosteroid (CS) therapy as the initial medical treatment. In the most cases, the medication can be given as an oral dose; however, in severe cases, intravenous therapy, including pulse steroid treatment, may be necessary. In general, patients with acute presentation as well as chronic presentation respond well to CS therapy. However, patients with acute presentation tend to require a higher dose of initial CS than those with chronic presentation. In the Japanese treatment guide for AIH published in 2013 [5], it is recommended that predonisolone (PSL) should be administered at an initial daily dose of $\geq$0.6 mg/kg. However, a higher dose ($\geq$0.8 mg/kg) of PSL is necessary for more severe cases of AIH [43]. Some patients may require pulse steroid treatment for the rapid improvement of intrahepatic inflammation.

However, some reports have shown that the acute presentation of AIH is more refractory to CS therapy than chronic presentation. In our experience [18], patients with acute presentation of AIH without severe jaundice (total bilirubin $<$10 mg/dL) exhibited a very good response to CS treatment, whereas more than 50 % of the patients with severe jaundice did not respond to CS. However, our AIH patients without severe jaundice showed lower ANA titers, making the diagnosis of AIH considerably difficult. These findings indicate that the response to CS therapy may reflect the promptness of diagnosis and treatment.

The efficacy of steroid pulse treatment in AIH has been uncertain. Recently, Yamamoto et al. [21] reported that pulse steroid treatment showed favorable outcomes in AIH patients with PT activity of $>$40 % or PT-INR $<$1.5, but poorer prognosis in those with PT activity of $\leq$40 % or PT-INR $\geq$1.5. A prospective validation study is thus necessary to prove the efficacy of steroid pulse treatment for AIH.

Table 6.1 Severity of autoimmune hepatitis [5]

Clinical signs	Clinical laboratory tests	Imaging tests
① Hepatic encephalopathy ② Reduction or disappearance of hepatic dullness	① AST/ALT > 200 U/L ② Bilirubin > 5 mg/dL ③ Prothrombin time < 60 %	① Hepatic atrophy ② Heterogenous liver parenchyma pattern
Severe: should fulfill at least one of these three findings: 1. Clinical signs: ① or ② 2. Clinical laboratory tests: both ① and ③ or both ② and ③ 3. Imaging tests: ① or ②		
Moderate: should fulfill these findings: Clinical laboratory tests: one of the criteria (①, ②, or ③) or both ① and ②, without clinical signs (neither ① nor ②), and imaging tests (neither ① nor ②)		
Mild: none of the above criteria are observed		

Notes:

1. When patients are judged in to have severe disease, they should be referred to a hepatologist immediately
2. The scoring system for predicting mortality in patients with acute liver failure or late-onset liver failure, as established by the Intractable Hepatobiliary Diseases Study Group in Japan and the model for end-stage liver disease (MELD) score, should also be evaluated and considered in patients with severe disease
3. When patients show prothrombin time of <60 %, or in cases where intense jaundice is present, referral to a hepatologist should be considered, even if the patient has moderate disease

6.6 Treatment and Outcomes of Acute Liver Failure Due to AIH

In a Japanese nationwide survey involving patients with fulminant hepatitis and LOHF between 2004 and 2009 [44], the survival rate without liver transplantation was 32.4 % in AIH patients. Despite new therapeutic approaches and intensive care, the prognosis of such patients remains poor without liver transplantation.

Although there are certain controversies regarding this point [45, 46], CS therapy is considered the first treatment choice for patients with acute liver failure due to AIH [17–21, 47, 48]. However, not all patients respond to CS therapy. Protracted therapy with CS can be complicated with the risk of serious infections with a subsequent inability to undergo liver transplantation. Therefore, the decision whether to continue CS therapy or abandon it for liver transplantation is very important to a successful outcome. Miyake et al. [17] showed that serum bilirubin levels are an important prognostic factor, with worsening levels observed during days 8–15 after the diagnosis in non-survivors. Yasui et al. [19] showed that lower PT activity at the start of CS treatment and insufficient improvement in PT activity during the first 2 weeks after the initiation of CS treatment was associated with poor outcomes. These findings indicate that the treatment response to CS can be determined at 2 weeks after initiating treatment; further, alternative treatments should be considered in patients not showing improvement. Close monitoring for the

occurrence of infectious diseases, such as viral and/or fungal infections, is also required during CS therapy.

Liver transplantation is a possible treatment choice for acute liver failure due to AIH. In Japan, the indication for liver transplantation has been determined thus far based on the guideline proposed by the Intractable Hepatobiliary Disease Study Group of Japan in 1996 [49], and new guidelines have been recently proposed by the same group [50]. The survival of patients with acute liver failure due to AIH following transplantation was 68.8 % at 5 years in Japan; this outcome was similar to that for patients undergoing liver transplantation for acute liver failure due to other etiologies [51].

6.7 Summary

AIH can present with symptoms and features of acute hepatitis and develop into severe hepatitis and acute liver failure. Patients with acute presentation of AIH do not always show increased serum IgG levels and/or autoantibody positivity; the lack of such markers makes the diagnosis of AIH difficult in these cases. For accurate diagnosis, it is essential to exclude other possible causes of acute hepatitis, and early liver biopsy should be performed if appropriate. Most patients with acute presentation of AIH respond well to CS therapy; however, when the disease progresses to acute liver failure, the response of the patient to CS treatment as well as prognosis is poor. Although the acute presentation of AIH is associated with several unsolved problems, it is clinically important that AIH be considered in the differential diagnoses for patients with unexplained acute hepatitis or acute liver failure. Early diagnosis and treatment may improve the prognosis of these patients.

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Chapter 7
Treatment of Autoimmune Hepatitis

Yasuhiro Miyake

Abstract Japanese AIH patients respond well to immunosuppressive treatment; however, failure to achieve the persistent normalization of serum ALT levels and repeated relapses are risk factors for poor outcomes (liver failure, or hepatocellular carcinoma, liver transplantation, death). Patients showing abnormalities of serum ALT levels (>30 IU/L), which indicate the presence of intrahepatic inflammation, should be treated with immunosuppressive treatment, and the persistent normalization of serum transaminase levels (ALT ≤30 IU/L) should be achieved. A first-line treatment is PSL monotherapy. PSL treatment with the initial dose ≥0.6 mg/kg/day is effective to promptly achieve the normalization of serum transaminase levels. In patients showing serum ALT levels more than tenfold the upper normal limit at starting treatment, PSL treatment with the initial dose ≥0.8 mg/kg/day is appropriate. The initial dose is generally maintained for 2–4 weeks. Thereafter, PSL dose is carefully tapered down to the maintenance dose (5–10 mg/day). To avoid early relapse, PSL dose should be maintained at 0.2 mg/kg/day or more until the normalization of serum transaminase levels. For patients who show treatment failure or incomplete response to conventional PSL therapy, or who relapse during PSL maintenance therapy, the administration of AZP at 1–2 mg/kg/day is effective. Termination of immunosuppressive treatment should be considered in patients achieving the persistent normalization of serum transaminase and IgG levels during at least 2 years. But, relapse after drug withdrawal is usual.

Keywords Autoimmune hepatitis • Azathioprine • Budesonide • Prednisolone • Ursodeoxycholic acid

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7.1 Treatment Indication

The guidelines by the American Association for the Study of Liver Diseases have recommended that immunosuppressive treatment should be introduced for patients showing serum AST or ALT levels greater than tenfold the upper normal limit, at least fivefold the upper normal limit in conjunction with a serum γ-globulin level at least twice the upper normal limit, and/or histological features of bridging necrosis or multilobular necrosis [1]. But, treatment indications for patients who show mild changes of laboratory data and histological features without any symptoms have not been established.

Even in AIH with mild activity, spontaneous remission is rare [2]. A 10-year survival is lower in untreated patients with mild activity than in overall treated patients including those with severe disease (67 versus 98 %) [2]. Even AIH with mild activity can progress to liver failure if the appropriate treatment is not performed.

Next, initial response to immunosuppressive treatment is generally good, but the long-term prognosis is poor in AIH patients treated with immunosuppressant than in the general population [3]. Failure to achieve the persistent normalization of serum ALT levels and repeated relapses are risk factors for liver-related death in AIH patients treated with immunosuppressant [3–5]. Even if serum ALT levels fluctuate under twice the upper normal limit, failure to achieve the persistent normalization of serum ALT levels leads to the poor outcomes.

Thirdly, although intrahepatic inflammation promotes liver fibrotic change, sustained suppression of inflammatory activity by immunosuppressive treatment results in the decreased liver fibrosis [6]. Even cirrhosis is reversible in patients achieving the sustained remission [6, 7].

Thus, patients showing abnormalities of serum ALT levels (>30 IU/L), which indicate the presence of intrahepatic inflammation, should be treated with immunosuppressant. Liver biopsy examination prior to starting immunosuppressive treatment is recommended in order to guide the treatment decision.

7.2 Treatment Strategy

The normalization of serum transaminase levels (ALT ≤30 IU/L) should be achieved in order to improve the prognosis of AIH patients. Worldwide, PSL monotherapy and the combination therapy of PSL and AZP are accepted as initial treatment for AIH. A systemic review has indicated that remission rate and mortality rate are comparable between these two therapies [8]. In a Caucasian study, 76 % of AIH patients without cirrhosis at presentation and 78 % of those with cirrhosis achieved remission by PSL monotherapy or the combination therapy of PSL and AZP [9]. On the other hand, in Japan, 75 % of AIH patients are treated with PSL, and the normalization of serum transaminase levels is achieved in 94 % of

those treated with PSL [10]. Response to immunosuppressive treatment, especially to PSL monotherapy, in Japanese AIH patients seems to be better than Caucasian patients. Thus, a first-line treatment for Japanese AIH patients is PSL monotherapy. As initial treatment, AZP monotherapy is not recommended because a mortality rate is higher in patients treated with AZP monotherapy than in patients treated with PSL monotherapy or the combination therapy of PSL and AZP [8].

After achieving the normalization of serum transaminase levels, PSL dose is tapered gradually. But, relapse during the taper of PSL dose is usual. In patients who respond well to initial PSL treatment, the re-treatment with PSL or the increase in PSL dose is effective after relapse [11]. On the other hand, prolonged PSL treatment increases the probability of steroid-specific side effects. In patients intolerant of prolonged PSL treatment due to steroid-specific side effects or concurrent diseases, the addition of AZP or the maintenance therapy with AZP alone is effective. As maintenance treatment, AZP monotherapy or the combination therapy of PSL and AZP has been reported to be superior to PSL monotherapy to maintain remission [8]. In the disease with mild activity (serum transaminase levels less than twice the upper normal limit), the addition of UDCA to PSL treatment may be effective to maintain remission.

As prognostic factors for predicting treatment response, the importance of HLA-DR status is accepted in Caucasian AIH patients. In Caucasians, DR3 and DR4 are associated with the disease susceptibility [12, 13]. Patients with DR3 have a higher frequency of treatment failure, relapse after drug withdrawal, and requirement for liver transplantation than others. In contrast, patients with DR4 better respond to corticosteroid treatment than those with DR3. On the other hand, in Japanese AIH patients, few have DR3 [14]. In addition, response to PSL treatment is better in Japanese patients. So, HLA-DR status may be useless for predicting treatment response in Japanese patients. Recently, the presence of anti-programmed cell death (PD)-1 antibody in sera has been shown to predict the response to PSL treatment in Japanese patients [15]. PD-1 is expressed on activated T and B cells and has inhibitory properties, and its dysfunction activates autoreactive T cells and results in the development of autoimmune diseases [16]. Patients positive for serum anti-PD-1 antibody have the severer disease and show the later normalization of serum transaminase levels and more frequently relapse during the taper of corticosteroid. Patients negative for serum anti-PD-1 antibody better respond to corticosteroid treatment. The multicenter validation study carried out by the Intractable Hepatobiliary Disease Study Group of Japan, which is supported by the Ministry of Health, Welfare and Labor of Japan, has confirmed these findings. Measurement of serum levels of anti-PD-1 antibody is useful for predicting treatment response as a new biomarker for AIH.

7.2.1 Prednisolone

Recently, the Intractable Hepatobiliary Disease Study Group of Japan has revealed that PSL treatment with the initial dose $\geq$0.6 mg/kg/day is effective to promptly achieve the normalization of serum transaminase levels in Japanese AIH patients [17].

In patients showing serum ALT levels more than tenfold the upper normal limit at starting treatment, PSL treatment with the initial dose ≥0.8 mg/kg/day is appropriate. Insufficient initial dose of PSL is a risk factor for the disease progression [18]. The initial dose is generally maintained for 2–4 weeks. Thereafter, PSL dose is tapered down to the maintenance dose. Reduction should be done by 2.5–5 mg/1–2 week until 20 mg/day and thereafter 2.5 mg/2–4 week until the maintenance dose. In order to avoid early relapse, PSL dose should be maintained at 0.2 mg/kg/day or more until achieving the normalization of serum transaminase levels [17]. The maintenance dose is usually 5–10 mg/day [10], and this maintenance treatment should be continued during 2 years at least.

In patients showing relapse during the taper of PSL dose or the maintenance treatment, the increase of PSL dose to 20 mg/day will be generally effective. After the renormalization of serum transaminase levels, PSL dose should be tapered carefully.

As major side effects for 6-month PSL treatment, weight increase (19.0 %), headache (7.6 %), mood alterations (7.6 %), muscular weakness (7.6 %), hypertension (6.7 %), and insomnia (4.8 %) have been reported [19]. Cosmetic side effects, such as facial rounding, weight gain, and dorsal hump formation and somatic changes, including emotional instability, glucose intolerance, or cataract formation, are present in 80 % of patients after 2-year PSL treatment [20].

Osteoporosis is one of the frequent adverse effects of corticosteroid. The Japanese Society for Bone and Mineral Research has developed the guidelines on the management and treatment for corticosteroid-induced osteoporosis [21]. The criteria for starting treatment for corticosteroid-induced osteoporosis are patients with prior fragility fracture and with new fractures during corticosteroid treatment, patients with less bone mineral density than 80 % of young adult mean, and patients using a dose of 5 mg/day or higher (mean daily dose) as prednisolone equivalent. Bisphosphonates are recommended as the first-line drugs and active vitamin D_3 and vitamin K_2 are recommended as the second-line drugs.

7.2.2 Pulse Steroid Treatment

Methylprednisolone pulse therapy (125–1,000 mg/day, intravenous, 3 days) is sometimes performed for the purpose of immediately improving intrahepatic inflammation in patients with severe disease; however, the efficacy has been uncertain. In future, the efficacy of pulse steroid treatment for AIH with severe liver disease should be evaluated. On the other hand, pulse steroid treatment for acute liver failure due to AIH showing prothrombin activity of 40 % or lower and/or PT-INR of 1.5 or higher increases a risk of death from infectious diseases [22]. Attention to the development of infectious diseases, especially fungal infection and pneumocystosis, should be paid when pulse steroid treatment is performed. Serum procalcitonin levels will be elevated in bacterial infection. In addition, measurement of serum beta-D-glucan levels will be useful for screening fungal infection and pneumocystosis.

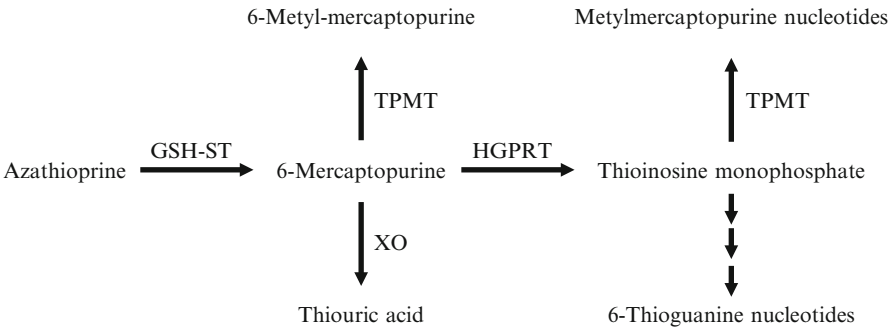

Fig. 7.1 Metabolism of azathioprine. *GSH-ST* glutathione-S-transferase, *TPMT* thiopurine methyltransferase, *XO* xanthine oxidase, *HGPRT* hypoxanthine-guanine phosphoribosyltransferase

7.2.3 Azathioprine

AZP is a purine analogue that acts as an antagonist to the endogenous purines that are essential components of DNA, RNA, and some coenzymes. As shown in Fig. 7.1, AZP is converted to active metabolites, 6-thioguanine nucleotides (6-TGN). 6-TGN is incorporated into cellular nucleic acids, resulting in the inhibition of nucleotide and protein synthesis and ultimately in the inhibition of lymphocyte proliferation [23]. On the other hand, measurements of AZP metabolite concentrations such as 6-TGN and methylmercaptopurine nucleotides (MeMPN) in red blood cells (RBCs) are useful for the prediction of AZP efficacy and adverse effects. Higher concentrations of RBC 6-TGN and lower concentrations of RBC MeMPN are associated with good response to AZP, while higher levels of RBC MeMPN are associated with the development of cholestatic hepatitis [24, 25]. In addition, thiopurine S-methyltransferase (TPMT) activities are lower in patients intolerant of AZP [26]. Single nucleotide polymorphisms in the TPMT gene have been shown to affect TMPT activities and AZP tolerance [26]. In Japanese population, 0.2–0.8 % have heterozygosity for the low-activity allele [27, 28].

In Japan, AZP at 1–2 mg/kg/day is administered for patients who show treatment failure or incomplete response to conventional PSL therapy or who relapse during PSL maintenance therapy. Approximately 90 % of patients with the refractory disease respond to the combination of PSL and AZP [29]. In addition, after remission, AZP monotherapy is useful for the long-term maintenance of remission. Eighty-three percent of patients in complete remission with the combination of PSL at 5–15 mg/day and AZP at 1 mg/kg/day have been reported to remain in remission while receiving AZP alone for a median of 67 months [30].

The frequency of AZP-related side effects in AIH patients is 10 %, and the most frequent side effects are hematological [20]. Mild lymphopenia is shown in 57 % of patients administered with AZP at 2 mg/kg/day [30], but severe myelosuppression

that justifies premature discontinuation of medication or dose reduction occurs in ≤6 % [20]. Gastrointestinal intolerance (abdominal pain, nausea/vomiting, and diarrhea) and hepatotoxicity are shown in 10 and 3 %, respectively [25]. Serious complications, such as pancreatitis, venoocclusive disease, extrahepatic malignancies, intestinal villous atrophy with malabsorption, and nodular regenerative hyperplasia, are possible.

Cirrhosis is a risk factor for AZP-related side effects, and 39 % of cirrhosis patients have been reported to exhibit the toxicity to AZP [31]. TMPT enzyme activity is shown lower in patients showing advanced fibrosis.

7.2.4 *Ursodeoxycholic Acid*

Efficacy of UDCA for AIH is dose-dependent, and UDCA at 600 mg/day is generally administered for AIH patients. UDCA has some degree of immunomodulating effects. UDCA suppresses the secretion of interleukin-2, interleukin-4, and interferon-γ from activated T lymphocytes and immunoglobulin production from B lymphocytes [32]. Of AIH patients treated with UDCA monotherapy, 64 % were reported to achieve the normalization of serum ALT levels within 12 months after starting treatment [33]. Prognosis of patients achieving the persistent normalization of serum ALT levels by UDCA monotherapy will be good. But, until the normalization of serum ALT levels, longer periods are required in patients treated with UDCA monotherapy than in those treated with PSL. In addition, regression of liver fibrosis is rare in patients treated with UDCA monotherapy although in patients achieving the normalization of serum ALT levels, histologically inflammatory activity improves [34]. Thus, UDCA monotherapy for patients with high-grade inflammatory activity, poor residual capacity of liver function, or cirrhosis is not recommended because they may reach liver failure before the achievement of remission.

On the other hand, additional use of UDCA to PSL treatment may be effective. Additional use of UDCA in patients showing repeated relapse or incomplete response during PSL treatment decreases serum transaminase levels [35]. Furthermore, additional use of UDCA during the taper of corticosteroid is effective for the prevention of early relapse [33].

UDCA is generally well tolerated. There are few serious adverse effects. Diarrhea has been reported at an incidence of 2–9 % [36].

7.2.5 *Budesonide*

Budesonide is a second-generation corticosteroid showing 90 % first-pass metabolism in the liver. In untreated AIH patients, 3-month oral budesonide monotherapy at 9 mg/day improves serum transaminase levels and is well tolerated in 83 % [37]. In addition, the combination therapy of budesonide at 9 mg/day and AZP at

1–2 mg/kg/day for 6 months has the advantage of more frequently achieving complete biochemical remission (47 % versus 18 %) and inducing few steroid-specific side effects such as moon face and acne (26 versus 52 %) compared with the combination therapy of PSL at 40 mg/day, tapered to 10 mg/day, and AZP at 1–2 mg/kg/day [19]. So, budesonide is promising for the treatment of AIH and expected in Japan.

On the other hand, in patients showing advanced fibrosis or cirrhosis, efficacy of budesonide is not always sufficient, and a frequency of steroid-specific side effects is higher [38]. These are due to increased serum levels of budesonide by reduced hepatic metabolism and portosystemic shunting [38, 39]. The efficacy and safety in cirrhotic patients need to be confirmed in Japanese patients.

7.3 Definition of Treatment Response

Remission. Improvement of symptoms such as fatigue, arthralgia and anorexia, normalization of serum AST or ALT, bilirubin and immunoglobulin levels, and disappearance of intrahepatic inflammation [1, 40]. Improvement of serum AST or ALT levels below twice the upper normal limit, which was suggested as remission in the previous report [40], should not be accepted as remission.

Treatment Failure. Worsening clinical, laboratory, and histological features despite compliance with conventional treatment schedule [1].

Incomplete Response. Insufficient improvement of the clinical, laboratory, and histological findings that does not satisfy criteria for remission and treatment failure [1, 20].

Drug Toxicity. Development of a side effect due to treatment [1, 20].

Relapse. Increase in serum AST or ALT levels of greater than twice the upper normal limit or reappearance of intrahepatic inflammation after induction of remission [1, 40].

7.4 Monitoring

Conventional laboratory tests including serum transaminase and IgG levels should be checked once or twice per week for the first 4 weeks after starting treatment. After remission, these are checked at least once per 1–3 months. During the clinical course of AIH, elevations of serum transaminase levels frequently happen. These are not always due to relapse or flare of AIH and may be due to drug-induced liver injury, fatty liver diseases, overlaps with PBC or PSC, autoimmune thyroiditis, etc. If relapse or flare of AIH, serum IgG levels will be increased before or together with elevations of serum transaminase levels. So, measurement of not only serum transaminase levels but also serum IgG levels is important.

A recent nationwide survey of AIH in Japan indicates that HCC develops in approximately 5 % of AIH patients [41]. Especially, the presence of cirrhosis is an important risk factor for the development of HCC. Thus, in AIH patients, ultrasonography, computed tomography, or magnetic resonance imaging should be performed at least every 6–12 months.

Some cirrhotic patients have a variceal bleeding. Variceal bleeding often leads to the fatal outcomes. In cirrhotic patients, esophagogastroduodenoscopy for screening of gastroesophageal varices should be performed every 1–2 years. If necessary, preventive therapy including endoscopic treatment, balloon-occluded retrograde transvenous obliteration, and splenectomy should be considered.

7.5 Treatment Endpoints

Approximately 90 % of AIH patients receiving immunosuppressive treatment achieve the normalization of serum ALT levels within 12 months after starting treatment [3]. However, histological improvement lags behind clinical and laboratory improvement by 3–8 months [1]. So, only a portion of patients may achieve remission within 12 months after starting treatment.

Next, even in patients persistently showing remission for at least 2 years, the incidence of relapse or loss of remission is 59 % at 1 year, 73 % at 2 years, and 81 % at 3 years after drug withdrawal [42]. Relapse after drug withdrawal is usual. On the other hand, abnormalities of serum transaminase and IgG levels at drug withdrawal are risk factors for relapse [43]. At drug withdrawal, patients who sustain their remission after drug withdrawal show normal laboratory data (serum transaminase, γ-globulin, and IgG levels) more frequently than patients who relapse (88 versus 46 %). In addition, histological portal plasma cell infiltration prior to drug withdrawal is associated with relapse after drug withdrawal [44].

Thus, termination of immunosuppressive treatment should be considered in patients achieving the persistent normalization of serum transaminase and IgG levels during at least 2 years. Liver biopsy examination prior to the termination of immunosuppressive treatment is recommended to ensure the disappearance of intrahepatic inflammation.

7.6 Specific Clinical Condition

7.6.1 Acute Liver Failure

Of Japanese AIH patients, approximately 30 % patients show acute presentation [45]. Generally, patients with acute presentation respond well to PSL treatment, and their prognoses are good [22]. Survival of patients showing both

prothrombin activity >40 % and PT-INR <1.5 at presentation is higher than 90 %. However, approximately 50 % of patients showing prothrombin activity of 40 % or lower and/or PT-INR of 1.5 or higher (acute liver failure) at presentation reach fatal outcomes. Patients showing acute liver failure have been reported to respond poorly to PSL treatment [46]. PSL treatment decreases the serum transaminase levels; however, liver failure condition is not improved in spite of intensive immunosuppressive therapy. If anything, PSL treatment may increase a risk of death from infectious diseases [47]. In patients showing acute liver failure, the determination of therapeutic strategy that takes account of liver transplantation is important.

On the other hand, in AIH patients showing acute liver failure, higher serum IgG levels are associated with poor response to PSL treatment [46]. Serum IgG of these patients may contain some autoantibodies associated with the pathogenesis of AIH. Recently, some autoantibodies have been reported to be associated with the severity and corticosteroid-resistance of AIH [15]. Thus, in order to remove the pathogenic immunoglobulins, plasma exchange may be effective [48, 49]. Further investigation is required.

7.6.2 Pregnancy

In Japan, approximately 10 % of AIH patients are women under the age of 30 years [10]. Information about the relationship of the disease and pregnancy is important.

In earlier studies, pregnancy in AIH women was shown to be associated with a high rate of fetal and maternal complications. But, recent studies indicate that pregnancies can be well tolerated by AIH patients and that successful completion of pregnancy is based on the well-controlled disease [50–52].

In AIH women, pre-conceptional counseling is important in order to reduce fetal exposure to potentially teratogenic drugs. In Japan, AZP is administered to limited number of AIH patients [10]. Limited reports have indicated that the administration of AZP at conception does not seem to be associated with miscarriage, fetal death, and abnormalities in AIH [50–53]. But, in patients with inflammatory bowel disease (IBD), infants who are exposed to AZP in early pregnancy are reported to be at a moderately increased risk of congenital malformations, specifically ventricular and atrial septal defects [54]. Furthermore, thiopurine exposure in IBD women is associated with preterm birth [55]. In Japan, pregnancy has been a contraindication for the administration of AZP. Thus, pregnant patients should be treated with corticosteroid.

Flare and relapse of AIH during pregnancy and postpartum are well known although the disease activity tends to improve in many pregnant patients. Approximately 10–20 % of pregnant patients have a flare in the disease activity, and approximately 10–50 % of patients experience flares after delivery [50–52]. Serious maternal adverse events (death or liver transplantation) have been reported to occur

in approximately 10 % of pregnancies [50–52]. Disease control before conception and cirrhosis at conception seem to be associated with poor outcomes while pregnant [50]. In order to avoid maternal poor outcomes, sufficient disease control before conception is necessary.

7.6.3 Elderly Patients

Elderly patients respond well to PSL monotherapy and the combination therapy of PSL and AZP. Frequencies of remission, relapse, sustained remission after drug withdrawal, death from hepatic failure, and need for liver transplantation are similar between elderly patients and younger patients [56, 57]. However, postmenopausal women have a higher cumulative frequency of complications (77 % vs. 48 %) than premenopausal counterparts [58]. Especially, vertebral compression occurred more frequently (23 % versus 7 %). In postmenopausal women, the prevention of corticosteroid-related adverse events is needed.

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Chapter 8
Management Strategies for Autoimmune Hepatitis Treatment Nonresponders

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Abstract The predominant type of autoimmune hepatitis (AIH) in Japanese patients is type 1 AIH, which responds to steroid treatment, and the prognosis is mostly favorable. However, some treatment-resistant AIH cases progress to a more severe or fulminant state or cannot achieve the normalization of liver function. The following conditions are reported in patients with steroid-resistant AIH: (1) treatment failure or an incomplete response, (2) repeated relapse, and (3) difficulty in continuing steroid treatments. The American Association for the Study of Liver Diseases guidelines recommend steroid monotherapy or steroid/azathioprine (AZA) combination therapy for patients with AIH. But AZA is not approved by the national health insurance scheme in Japan. In this survey, treatments with AZA may be beneficial for AIH patients with recurrence following steroid treatments, complications, and adverse effects. Therefore, an investigation of the pathological conditions associated with this disease, such as severity, complications, and age, as well as the use of immunosuppressant, AZA therapy, and other treatments, is warranted for treatment-resistant cases. The applicability of liver transplantation should also be determined in cases of steroid- and various immunosuppressant-resistant severe and fulminant hepatitis and liver failure by accurately judging the pathological conditions of AIH patients and timing of treatments.

Keywords Immunosuppressant treatment • Liver transplantation • Nonresponder • Relapse • Treatment failure

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8.1 Introduction

Autoimmune hepatitis (AIH) is an organ-specific autoimmune disease mainly involving hepatocytes, and the autoimmune mechanism responsible is considered to play an important role in the development and persistence of liver disorders. Steroid treatments are known to be effective for AIH and have been included in the scoring system established as diagnostic criteria by the International Autoimmune Hepatitis Group (IAIHG) [1]. Approximately 60–70 % of AIH patients in Japan were shown to be human leucocyte antigen (HLA) DR4-positive [2–4], with no patient being treatment-resistant HLA DR3-positive, which is in contrast to patients in Western countries. Furthermore, liver kidney microsome-1 antibody-positive patients are rare, i.e., type 1 AIH is the most common in Japanese patients, steroid treatments and immunosuppressants are effective, and the prognosis is mostly favorable [4, 5]. However, some treatment-resistant AIH cases, such as those that progress to a serious or fulminant state, cannot achieve remission and relapse with reductions in the dose of the drugs administered. Treatment-resistant AIH and treatment approaches have been described in this review, including new information obtained in Japan and other countries.

8.2 Treatment Nonresponders

The treatment goals for patients with AIH are to normalize alanine aminotransferase (ALT) and immunoglobulin G (IgG) levels, improve histological inflammation and fibrosis, and achieve a sustained remission status. A previous study reported that achieving a good response to immunosuppressive therapies at the treatment onset was important [6], and the poor control of transaminase levels and repeated relapse are risk factors for progression to liver cirrhosis and hepatocellular carcinoma (HCC) [4, 5, 7]. Although the guidelines established by the American Association for the Study of Liver Diseases (AASLD) [8] recommend steroid/azathioprine (AZA) combination therapy, as well as steroid therapy, this is not employed in Japan because AZA is not approved by the national health insurance scheme. Thus, the term “treatment nonresponders” refers to steroid resistance.

Steroids exhibit anti-inflammatory and anti-immune effects by inhibiting various stimulation-induced activations of T and B lymphocytes then decreasing cytokine production and their actions. Regarding the mechanisms responsible, the steroid-induced inhibition of the transcription activities of nuclear factor (NF)-κB [9], which is a nuclear transcription factor that is activated by antigens and cytokines, and activator protein-1 [10], which is a nuclear transcription factor that induces protease and various cytokines, through binding to the glucocorticoid receptor (GR), and glucocorticoid-induced apoptosis [11] has been reported and has been implicated in glucocorticoid resistance. NF-κB has also been shown to

induce the expression of the β-isoform of GR (GRβ) [12]. The increased expression of GRβ has been reported in glucocorticoid-resistant asthma [13], rheumatoid arthritis [14], and ulcerative colitis [15]. Previous studies on AIH also confirmed that hepatitis was active, the steroid dose was high, and the relapse rate was high in patients overexpressing GRβ, and human GRβ expression levels reflected steroid resistance, serving as an index of the progression to a more serious state [16]. Other factors involved in resistance to steroids include heat shock protein 70/90 [17–19] and P-glycoprotein [20, 21]; however, no reliable predictive marker has been identified to date.

The following three conditions are typically present in patients with steroid-resistant AIH: (1) the aggravation of transaminase levels in spite of the administration of steroids at a high dose and/or for a sufficient period (treatment failure), or the inability to normalize these levels (incomplete response) [22], (2) repeated relapse when the steroid dose is reduced or withdrawn, and (3) difficulty in continuing a steroid treatment due to adverse effects and complications.

8.2.1 States in Which Transaminase Levels Cannot Be Normalized by Steroids Administered at a High Dose and/or for a Sufficient Period (Treatment Failure, Incomplete Response)

8.2.1.1 Severe Hepatitis, Fulminant Hepatitis, and Late-Onset Hepatic Failure

Some patients with AIH develop acute hepatitis and may then progress to a more serious state, leading to fulminant hepatitis (FH) or late-onset hepatic failure (LOHF). Previous studies reported that the IgG level and antinuclear antibody titer were low in many cases of acute-onset AIH [23–27], but were high in cases progressing to a more serious state [28]. A recent nationwide survey in Japan confirmed that the prognosis of acute-onset AIH was poor when serum bilirubin levels were high and prothrombin time (PT) activity levels were low or PT-international normalized ratio (PT-INR) were high [29]. The Intractable Hepato-Biliary Diseases Study Group of the Ministry of Health, Labour and Welfare presented autoimmune hepatitis severity judgment criteria in its meeting report [30] (Table 8.1). Critical care including the administration of very large doses of steroids at a medical institution with a hepatologist was recommended when the patient's condition was judged to be severe based on these criteria. However, recovery is still difficult to achieve in many severe cases, even in those treated with very large doses of steroids. In the 2004–2009 nationwide survey conducted in Japan, 8.3 and 32.1 % of FH and LOHF cases, respectively, were attributed to AIH, and survival rates were 32.4 and 12.5 %, respectively, which indicated a very poor prognosis [31]. In the 1998–2003 survey, 6.2 and

Table 8.1 Severity assessment of autoimmune hepatitis

Clinical signs	Laboratory test findings	Imaging findings
(1) Hepatic encephalopathy	(1) AST, ALT > 200 IU/l	(1) Liver size reduction
(2) Reduction or loss of hepatic dullness	(2) T. bilirubin > 5 mg/dL (3) PT < 60 %	(2) Liver parenchymal heterogeneity

Severe:

One of the following three items was observed:

1. Clinical signs (1) or (2)
2. Laboratory test findings (1) + (3) or (2) + (3)
3. Imaging findings (1) or (2)

Moderate:

Clinical signs (1) and (2), laboratory test findings (3), and imaging findings (1) and (2) were absent, but clinical signs (1) or (2) was observed

Mild:

None of clinical signs (1) or (2), laboratory test findings (1), (2), or (3), and imaging findings (1) or (2) were observed

14.1 % of FH and LOHF cases, respectively, were attributed to AIH [32]. These findings revealed an increase in these rates in recent years. AIH is not a rare cause of FH and LOHF. The prognosis of patients with AIH-induced acute liver failure is very poor, for which an early diagnosis and the administration of very large doses of steroids and immunosuppressants are necessary from the early phase while evaluating responses. Furthermore, the applicability of liver transplantation needs to be determined by accurately judging the pathological conditions of these patients.

8.2.1.2 AIH + PBC and AIH + PSC Overlap Syndromes

The frequency of the concomitant clinical features of primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC) in AIH, i.e., overlap syndrome, is not high. The incidence of overlap syndrome was reported to be between 8.4 and 12 % in autoimmune liver disease or PBC [33–37] and 1.9–4.2 % in AIH [3, 7, 38]. Diagnostic criteria currently remain unclear, and the International Autoimmune Hepatitis Group (IAIHG) stated in its 2011 report that there was no independent concept of overlap syndrome and described conditions in which other autoimmune liver diseases were present as accessory clinical features in addition to the main clinical features of AIH or PBC [39]. Pathological conditions with the coexistence of bile duct lesions associated with PBC and PSC in AIH that were diagnosed using both simplified criteria [40] and Paris criteria [33] have frequently been reported recently. These studies also demonstrated that steroid treatment was useful for overlap syndrome, and although combinations with ursodeoxycholic acid and AZA were performed, fewer patients achieved a complete response than those with AIH, the frequencies of nonresponders, liver-related death, and liver transplantation were high, and the outcomes were poor [37, 38, 41–44]. Therefore, when

steroid treatments for AIH fail, complications associated with bile duct diseases, such as PBC and PSC, need to be reinvestigated using immunological and pathological examinations and imaging, such as magnetic resonance imaging (MRI) and endoscopy.

8.2.1.3 Others

The presence of diseases manifesting biochemical features similar to those of AIH, for example, hepatitis C virus infection, Wilson's disease, metabolic diseases, such as nonalcoholic fatty liver disease, and diseases causing immunological abnormalities, such as collagen disease, also needs to be reexamined. Therefore, the reconsideration of diagnoses in all compliant AIH patients with treatment failure is required by reevaluating the findings of histology and autoimmune serology. In addition, an investigation of genetic or metabolic diseases in the liver and endoscopic or magnetic resonance cholangiography is also mandatory in this setting.

8.2.2 Repeated Relapse Associated with Steroid Dose Reductions and Withdrawal

The AASLD guidelines define "relapse" as the re-elevation of disease activity after remission or treatment completion with a threefold or higher aspartate aminotransferase (AST) level than the upper limit of the normal level or a 2 g/dL or higher γ-globulin level [22]. Laboratory changes in these levels are invariably associated with the reappearance of interface hepatitis in liver tissues and preclude the need for a liver biopsy examination to document relapse. The rate of relapse of AIH with steroid withdrawal or dose reductions was reported to be as high as 30–70 % [45–47], which is a characteristic feature of AIH. Advanced liver tissue fibrosis at the time of diagnosis, a high γ-globulin or IgG level, a score on the international AIH scoring system $\geq$17 points at the time of diagnosis, HLA DRB1*03 positivity, complications associated with autoimmune disease, onset at a young age, high ALT and IgG levels at the time of withdrawal, and portal plasma cell infiltration at the time of withdrawal have been reported as risk factors for relapse [46–49].

The relapse of AIH due to steroid withdrawal or dose reductions can be improved in many cases by re-initiating the steroid treatment or elevating the doses administered; however, the control of AIH by low doses of steroids becomes difficult with repeated relapse. The desired maintenance dose of steroids is 5–10 mg/day or lower because treatments requiring 20 mg/day or higher has been shown to increase the risk of adverse effects, complications, and concomitant diseases. This dose has been established as the limit of steroid treatments, i.e., steroid resistance, and the reinvestigation of treatment policies is necessary.

8.2.3 States in Which the Continuation of Steroid Treatments Is Difficult due to Adverse Effects and Complications

Steroid treatments for AIH need to be administered for years; therefore, various adverse effects associated with the administration of steroids become problematic. Infections (particularly fungal infections), intestinal disorders (ulcer, hemorrhage, and perforation), the development and exacerbation of diabetes, osteoporosis, pathological fractures and bone necrosis, muscle weakness, mental disorders, heart failure, and vasculitis may threaten the lives of these patients. Preventive drug administration is recommended for these adverse effects. However, problems related to adverse effects have become more serious because the AIH onset age has recently advanced in Japan [3], and steroid dose reductions and withdrawal are inevitable in many cases, which interrupts the normalization of liver function.

8.3 Countermeasures for Treatment Nonresponders

The prognosis of patients is known to be poor when the response to the initial steroid treatment is weak [6]. The insufficient control of transaminase levels and frequent relapse increase the risk of progression to liver cirrhosis and HCC. Thus, the maintenance of normal transaminase levels is crucial in these patients [4, 5, 7]. A combination with or switch to AZA and other immunosuppressant needs to be investigated when the normalization of ALT and IgG levels, improvements in histological inflammation and fibrosis, and sustained remission are not achieved by 6 months following the initiation of steroid treatments and dose adjustments. Liver transplantation should be considered from the early phase in cases that progress to acute liver failure as well as fulminant and more serious states, and an early diagnosis and the initiation of steroids and immunosuppressants are necessary. Responses should be evaluated during these treatments in order to prepare for liver transplantation prior to the development of complications in poor responders.

8.3.1 Azathioprine (AZA)

AZA exhibits its pharmacological effects after being converted to 6-thioguanine nucleotides (6-TGN), which involves several metabolic enzymes in the body (Fig. 8.1). When the activity of one of the metabolic enzymes, thiopurine methyltransferase (TPMT), is absent or low, 6-TGN levels increase, with which the effects of AZA and adverse effects increase. The frequency of the TPMT gene polymorphism in Japanese AIH patients was shown to be 10.2 %, which was higher than that (2–3 %) in healthy subjects [50]; however, it is difficult to predict the development of adverse effects from gene polymorphisms alone. Monitoring drug

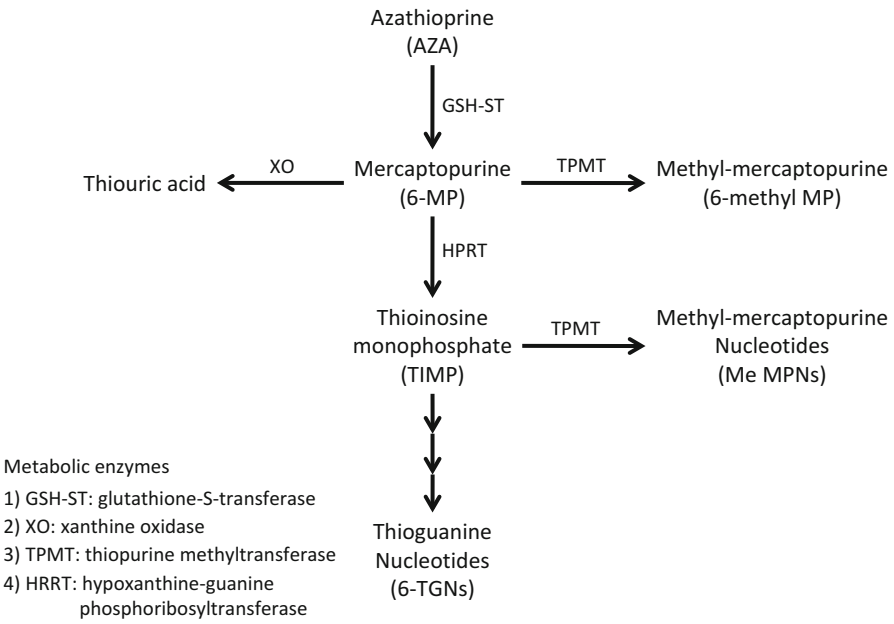

Fig. 8.1 Metabolic pathway of azathioprine

levels by measuring TPMT enzyme activity and 6-TGN levels may be useful for evaluating the therapeutic effects of drugs. The main adverse effects that have been reported include cytopenia, bone marrow suppression, digestive symptoms, such as nausea and vomiting, and liver disorders.

The AASLD guidelines recommend prednisolone (PSL) 60 mg/day monotherapy or PSL 30 mg/day + AZA 50 mg/day combination therapy for patients with AIH. In the 2010 systematic review of randomized controlled trials, the efficacies of PSL monotherapy and PSL/AZA combination therapy for induction treatment were found to be equivalent in naïve and relapsing AIH patients. Furthermore, PSL/AZA combination therapy and AZA monotherapy were reported to be superior to PSL monotherapy for maintenance therapy [51]. AZA is not approved by the national health insurance scheme in Japan, and few studies have examined a large number of cases. Okayama University reported that AZA/low-dose PSL combination therapy achieved remission in 12 out of 13 patients with intractable AIH [52], while all seven recurrent AIH patients treated with AZA/PSL combination therapy achieved remission at Fukushima University [46]. In Kurume University, 45 patients were treated with AZA (Fig. 8.2); AZA was used because steroids were ineffective in 8 patients (17.8 %) and for relapse in 30 patients (66.7 %). Treatment with steroid/AZA combination therapy was initiated in 7 patients (15.6 %) with complications, such as femoral head necrosis, diabetes, and mental disorders. Regarding the effects of the treatment in 38 cases, excluding early discontinuation cases, liver function was normalized in 28 (73.7 %). The

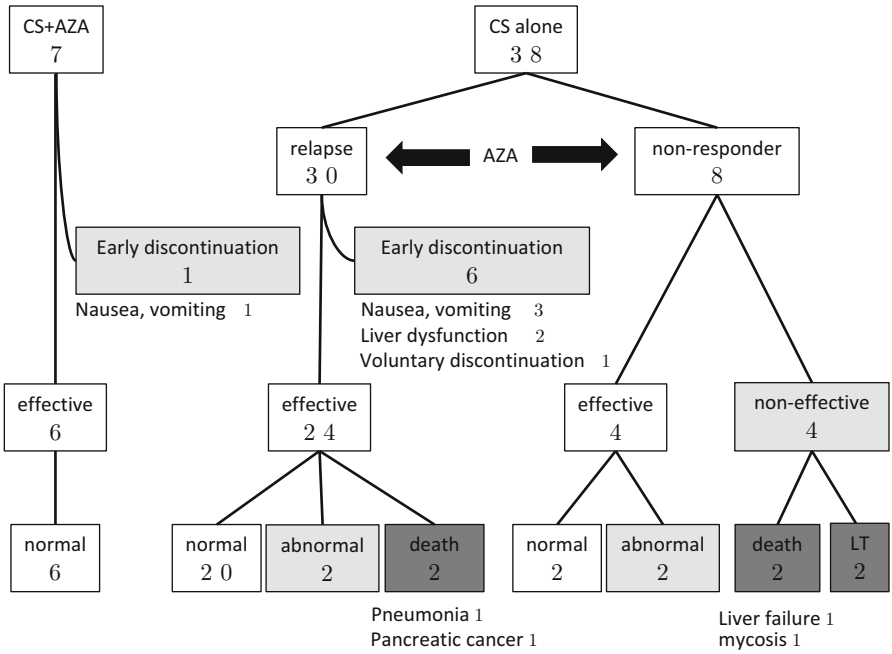

Fig. 8.2 Courses and outcomes of azathioprine-treated cases. *AZA* azathioprine, *CS* corticosteroid, *LT* liver transplantation. Figures represent the number of cases. The external boxes represent reasons for early discontinuation and causes of death

disease did not recur in any patient in whom treatment was initiated with steroid/AZA combination therapy. In the eight cases in which steroids were ineffective as a previous treatment, liver function was normalized in two, and AZA was also ineffective in four. Two patients died (due to infection and liver failure, respectively), and the other two underwent liver transplantation.

Based on the above findings, treatments with AZA may be beneficial for AIH patients with recurrence following steroid treatments, complications, and adverse effects. However, this treatment was markedly effective for some nonsteroid responders, but ineffective for others. The further accumulation of relevant cases is needed.

8.3.2 Other Immunosuppressants

Very few steroid- and AZA-ineffective cases have been reported in Japan, and these have only been described in case reports. Other therapeutic drugs include cyclosporine A (CyA), tacrolimus, mycophenolate mofetil, and budesonide.

CyA exhibits a potent immunosuppressive effect mainly by inhibiting the production of cytokines by T cells, such as interleukin-2. Regarding the mechanism

responsible, CyA may inhibit the activation of calcineurin, which is an important molecule in signal transmission for the activation of T cells, by forming a complex with the intracellular binding protein, cyclophilin. This subsequently leads to the dephosphorylation-induced inhibition of the nuclear translocation of the transcription factor, nuclear factor of activated T-cell (NFAT), thereby inhibiting the production of cytokines, such as interleukin-2 [53, 54]. CyA is considered to be the main therapeutic drug for steroid-resistant AIH [55–57], and histological improvements have been reported. The main adverse effects associated with CyA include renal dysfunction, hypertension, and neurotoxicity. Improvements have been reported in intractable cases including acute severe AIH in Japan following the initiation of CyA treatments [58–61].

Tacrolimus is another potent calcineurin inhibitor [62] that is used for post-transplantation treatments in Japan; however, its application to steroid-resistant AIH has not been reported.

Mycophenolate mofetil is a selective and reversible inhibitor of inosine monophosphate dehydrogenase in activated lymphocytes. This is a rate-limiting enzyme in purine biosynthesis and exerts selective antiproliferative effects on T and B lymphocytes by inhibiting the synthesis of deoxyribonucleic acid (DNA) [53]. Mycophenolate mofetil has been used to treat inflammatory bowel disease and rheumatoid arthritis and also as a transplantation medicine.

Budesonide is a next-generation glucocorticoid that has >90 % first-pass hepatic clearance and metabolites devoid of glucocorticoid activity [64]. Therefore, budesonide efficiently exhibits pharmacological effects by directly acting on the cause of the disease, such as lymphocytes in the liver, thereby inducing less adverse effects. In randomized controlled trials performed in Europe on budesonide + AZA and steroid + AZA for the treatment of AIH without liver cirrhosis, the effects of budesonide were higher than those of steroid while inducing less adverse effects [65]. Budesonide is mainly used in Japan as an inhalant for bronchial asthma and has not yet been applied to the treatment of AIH.

8.3.3 Liver Transplantation

Liver transplantation is necessary in AIH cases not responding to steroids and immunosuppressants under the following conditions: (1) when the disease acutely developed and progressed to acute liver failure, fulminant hepatitis, or severe hepatitis and (2) when the disease acutely or slowly progressed to decompensated liver cirrhosis, HCC, or chronic liver failure. An acute liver failure scoring system has been published (Table 8.2), in which the prognosis is considered to be poor when the score is five or higher [66]. The indication and timing of liver transplantation for acute liver failure and fulminant hepatitis should be judged using this system. Judgments should also be made for chronic liver failure following the indication of transplantation for liver cirrhosis.

Table 8.2 Scoring system to predict the mortality of patients with fulminant hepatitis and LOHF established by the Intractable Hepatobiliary Diseases Study Group in Japan in 2010, from reference [63]

Score	0	1	2
O-C (days)	≤5	6–10	11≤
PT (%)	20<	5<, ≤20	≤5
TB (mg/dL)	<10	10≤, <15	15≤
D/T ratio	0.7≤	0.5≤, <0.7	<0.5
PLT (10^4/μL)	10<	5<, ≤10	≤5
Liver atrophy	Absent	Present	

O-C the interval between hepatitis onset and development of hepatic encephalopathy, *PT* prothrombin time, *TB* total bilirubin, *D/T ratio* ratio of direct to total bilirubin concentration, *PLT* platelet

In the 2011 report on Liver Transplantation in Japan-Registry by the Japanese Liver Transplantation Society [67], the total number of transplantations performed in 2010 at 65 institutions cooperating for the survey was 6,195, and the number of living-donor liver transplantations was 6,097. Ninety-one AIH patients (1.59 %) underwent living-donor liver transplantation, 24 of whom had acute liver failure. In other countries, AIH cases account for 1–3 % of adult cases of liver transplantation, which is similar to that reported in Japan. The cumulative post-transplantation survival rate of AIH patients was relatively favorable, and the 1-, 5-, and 10-year survival rates were 77.6, 75.9, and 75.9 %, respectively. The 1- and 10-year survival rates of AIH patients with acute liver failure were 66.7 and 66.7 %, respectively, which were similar to those of patients with acute liver failure associated with other causative diseases. Only a few studies on liver transplantation in Japan have included a large number of AIH patients. Yamashiki et al. [68] reported a total of 16 cases of AIH and AIH + PBC, in which the age at the time of transplantation was 48 years, which was considered to be relatively young, and 4 patients (25 %) had concomitant PBC. The disease did not recur following liver transplantation, which may have been due to the continuation of treatment with calcineurin inhibitors and maintenance corticosteroids; however, the incidence of relapse after liver transplantation was reported to be approximately 20 % in other countries [69, 70]. Although differences in the immunosuppressant administration method used and between races were considered, further investigations using a larger number of cases are needed.

8.4 Summary

Steroid treatments are generally considered to be effective for AIH; however, many intractable cases require individual approaches, for which a wealth of knowledge and precise judgments are required. The identification of pathological conditions including severity, complications, and age and an investigation of the use of AZA

and other immunosuppressants for relapse cases and treatment resistance due to adverse effects are needed. The applicability of liver transplantation should be determined in cases with chronic liver failure resistant to not only steroids but also various immunosuppressants, such as fulminant hepatitis, acute liver failure, and decompensate liver cirrhosis, by accurately judging the pathological conditions of patients and timing with reference to the corresponding liver transplantation guidelines.

Abbreviations

6-TGN	6-thioguanine nucleotide
AASLD	American Association for the Study of Liver Diseases
AIH	Autoimmune hepatitis
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
AZA	Azathioprine
CyA	Cyclosporine A
FH	Fulminant hepatitis
GR	Glucocorticoid receptor
HCC	Hepatocellular carcinoma
HLA	Human leucocyte antigen
IgG	Immunoglobulin G
IAIHG	International Autoimmune Hepatitis Group
LOHF	Late-onset hepatic failure
MRI	Magnetic resonance imaging
NF-κB	Nuclear factor-κB
PBC	Primary biliary cirrhosis
PSC	Primary sclerosing cholangitis
PSL	Prednisolone
PT	Prothrombin time
PT-INR	PT-international normalized ration
TPMT	Thiopurine methyltransferase

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Chapter 9
Pediatric Autoimmune Hepatitis

Tomoo Fujisawa

Abstract Autoimmune hepatitis (AIH) and primary sclerosing cholangitis (PSC) are immune-mediated liver diseases in children that sometimes overlap, a condition which is usually called autoimmune sclerosing cholangitis (ASC). Pediatric ASC and PSC are often complicated with inflammatory bowel disease.

AIH can occur at any age of life. AIH has been diagnosed as early as 6 months of age. There have been no reports on the frequency of pediatric AIH in Japan, but studies from overseas have reported a prevalence of pediatric AIH and PSC or ASC of approximately 3 and 1.5 people per 100,000 respectively; the prevalence is considered to be similar in Japan.

The diagnosis of ASC is similar to that of the overlap of AIH and PSC in adults, but acute-onset AIH is more frequent and can be difficult to distinguish from ASC, making endoscopic retrograde cholangiopancreatography (ERCP) and other forms of cholangiography necessary.

Keywords Autoimmune hepatitis • Autoimmune sclerosing cholangitis • Overlap • Primary sclerosing cholangitis

9.1 Introduction

Immune-mediated liver diseases (IMLDs) that occur during childhood include autoimmune hepatitis (AIH) and primary sclerosing cholangitis (PSC), the overlap condition of which is called autoimmune sclerosing cholangitis (ASC). Among the

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autoimmune diseases that occur during childhood, there have been no reports on primary biliary cirrhosis (PBC) in children. Liver disorders can accompany autoimmune pancreatitis in adults, but the same disease has not been reported in children.

9.2 AIH

Pediatric AIH can occur at any age in boys and girls [1–3]. Most overseas case reports (75 %) focus on girls [1]. AIH is characterized by specific autoantibody positivity, and with inappropriate treatment, AIH can progress rapidly to cirrhosis. However, corticosteroids (CS) can elicit a complete response and usually suppress lesion progression.

9.2.1 Onset Mechanism [2]

Onset is believed to occur when T cells are activated by some factors that include genetic factors, infection such as Epstein–Barr virus, drugs such as Minomycin, age, and endocrine changes. This activation induces an autoimmune reaction that causes the immune system to attack its own hepatocytes. With regard to genetic factors, human leukocyte antigens (HLA) DR3 and HLA DR4 are known to be related to AIH, and HLA DR3 in particular is significantly correlated with AIH in adults in Europe and America. The revised AIH scoring system (AIH score) of the International Autoimmune Hepatitis Group (IAIHG) gives one point each for DR3 and DR4 [4]. Of the pediatric AIH cases we encountered, 37.5 % (three of eight cases) were HLA DR4, which is relatively common in Japanese patients with AIH. In fact, there are differences between European–American and Japanese patients in AIH pathogenesis and responses to treatment [3].

9.2.2 Clinical Manifestations

The age of onset exhibits two peaks, prior to the age of 30 years and in the sixth decade of life [1]. According to European and US studies, many patients experience onset before the age of 18 years, most frequently during prepubescence. Pediatric AIH is often diagnosed as acute hepatitis based on symptoms such as jaundice or fatigue as well as incidentally from abnormal serum levels of transaminase. While AIH does not exhibit specific symptoms, it is diagnosed based on symptoms such as thrombocytopenia, joint pain, fever, and skin rash. Children with acute hepatitis may include acute exacerbations of latent chronic hepatitis for some reason or those that start as acute hepatitis. Without a liver biopsy, it is difficult to distinguish between these two conditions [2, 3].

9.2.3 Diagnosis

The diagnosis of AIH in children is usually made using the revised IAIHG diagnostic criteria and AIH score, which were reported in 1999 [1, 4]. The AIH score was originally developed to stratify patient characteristics for the purpose of research, so high scores can sometimes be obtained in diseases other than AIH such as PSC, collagen disease, Wilson's disease, and malignant diseases, such as leukemia. It should be noted that the diagnosis of AIH is ultimately established by differentiation and exclusion, but as long as this is taken into consideration, the scoring system is considered sufficiently useful for diagnosing pediatric AIH [2]. However, since children do not drink alcohol, the female sex merits four points. In addition, children may receive points because they have physiologically high alkaline phosphatase (ALP) levels, and their serum immunoglobulin G (IgG) levels fluctuate around the upper limit of the normal range with aging. Thus, this system may be unsuitable for use in some situations. A scoring system better suited to pediatric AIH diagnosis is needed, which is being considered by an international working group on pediatric hepatitis [5].

Since the immune system targets hepatocytes in AIH, the increase in transaminase levels is more marked than the rise in biliary system enzymes such as ALP, γ-glutamyl transpeptidase (γ-GTP), and leucine aminopeptidase. Moreover, while autoantibody detection is most important in AIH, the autoantibodies observed include antinuclear antibodies (ANA), anti-smooth muscle antibodies (SMA), anti-liver kidney microsome antibody type 1 (LKM-1), anti-soluble liver/liver–pancreas antibodies, anti-liver cytosol antibody type 1, and anti-asialoglycoprotein receptor antibodies [3]. AIH is divided into type 1, which is associated with ANA and/or SMA positivity, and type 2, which is associated with LKM-1 positivity alone. Type 2 AIH is very rare in Japan; of the patients encountered, 75 % were positive for ANA and 25 % were positive for SMA, but none of the patients were positive for LKM-1. It is reported that 4 % of adult patients with AIH in the United States are LKM-1 positive, but the rate is considerably higher in Western Europe (20 %), with regional differences seen in the rates of positivity for other antibodies [6]. Type 2 AIH is generally more common in young patients and is more severe than type 1 AIH.

Liver tissue is of most importance in AIH diagnosis. We defined severe necrosis and inflammation in the centrilobular region but with almost no fibrosis as the acute hepatitis type and compared it with the conventional chronic hepatitis type. The acute hepatitis type was associated with significantly higher total bilirubin and ALT levels and significantly lower autoantibody positive ratios and serum IgG values [7]. In the case acute hepatitis of unknown cause accompanying jaundice, AIH cannot be ruled out even if the patients are negative for autoantibodies and serum IgG values are low. AIH is also characterized by interface hepatitis (periportal necrosis and inflammation with destruction of the limiting plate), rosette-forming hepatocytes, and infiltration of lymphocyte plasma cells, all of which are given points in the AIH scores. In addition to interface hepatitis, children exhibit high rates of centrilobular necrosis and inflammation, giant cell transformation of hepatocytes,

Table 9.1 Pediatric autoimmune hepatitis diagnostic criteria

Abnormal transaminase values
Autoantibody positivity
ANA- and/or SMA-positive type 1 AIH
Only LKM-1-positive type 2 AIH
High serum IgG level determined from age-based criteria
Liver biopsy findings of interface hepatitis and lobular hepatocyte collapse
Confirm absence of viral hepatitis and exclude hepatitis A–E
Confirm absence of liver dysfunction accompanying other viral infections, particularly EBV or CMV
Confirm absence of metabolic disorder, particularly Wilson's disease
Normal biliary tract findings (by MRCP or ERCP)

AIH autoimmune hepatitis, *ANA* antinuclear antibodies, *SMA* smooth muscle antibody, *LKM-1* anti-liver kidney microsome antibody type 1, *IgG* immunoglobulin G, *EBV* Epstein–Barr virus, *CMV* cytomegalovirus, *MRCP* magnetic resonance cholangiography, *ERCP* endoscopic retrograde cholangiopancreatography

and eosinophil infiltration [7]. However, points are subtracted from the AIH score for bile duct lesions. Point reductions are made for onionskin fibrosis, which is characterized in PSC, and nonsuppurative destructive cholangitis accompanying granuloma, which resembles PBC. Bile duct lesions are observed in >90 % of pediatric AIH cases, most of which exhibit bile duct degeneration accompanied by the periportal infiltration of inflammatory cells. However, some cases of suspected PSC also exhibit onionskin fibrosis around the bile duct [7]. Whether this phenomenon is characteristic of pediatric AIH needs to be investigated. Table 9.1 shows the diagnostic criteria for pediatric AIH.

9.2.4 Complications

Adult AIH, but not pediatric AIH, is often complicated by autoimmune disease. Complicating autoimmune diseases include chronic thyroiditis, Behcet disease, type 1 diabetes, idiopathic thrombocytopenia, systemic lupus erythematosus (SLE), and articular rheumatism. Studies from Europe and the United States have mentioned complication of AIH with inflammatory bowel disease (IBD) [8]; however, we believe that complicating IBD is rare and should be considered an indication of PSC or ASC.

9.3 PSC

Diagnostic criteria from the Mayo Clinic are generally used when diagnosing pediatric PSC, and cholangiography findings are the most important consideration. Since children have physiologically high ALP levels due to the effects of

age-related bone metabolism, γ-GTP is used as a substitute. Magnetic resonance cholangiopancreatography (MRCP) is sometimes used instead of cholangiography, but since identification of minute lesions of the intrahepatic bile duct in the initial stage of the disease is difficult, endoscopic retrograde cholangiopancreatography (ERCP) should be preferably used when PSC is suspected [9].

Childhood-onset PSC initially involves the presence of high autoantibody levels, and some patients have high IgG values. It is not uncommon for cases to be scored "definite AIH" on the aforementioned AIH score. Sclerosing cholangitis with a strong autoimmune phenomenon is called ASC, mainly by a group at King's College, theUnited Kingdom [5, 10]. In the United Kingdom, ASC is said to occur at the same frequency as pediatric type 1 AIH. At King's College, all children who are exhibiting liver dysfunction, autoantibody positivity, high serum IgG levels, and interface hepatitis in the liver tissue are recommended to undergo routine ERCP or other forms of direct cholangiography. Mild forms of PSC findings are observed in approximately 50 % of cases on cholangiography [10]. Abnormal cholangiography findings are naturally found in ASC, but it is also important to consider important points such as the absence of acute onset, frequent IBD complications, high perinuclear antineutrophil cytoplasmic antibody ratios, and a high incidence of the HLA DR13 type. Steroids have also been shown to be effective for ASC [10]. The initial stages of pediatric PSC involve high autoantibody positivity and immunoglobulin levels, with many scoring "definite AIH" or "probable AIH" on the AIH score. However, many patients with PSC respond poorly to immunosuppressants, unlike those with ASC in Europe and the United States, indicating that PSC differs from ASC in Western Europe. Similar to the King's College group, we recently began performing routine ERCP on children with suspected AIH to determine whether elements of PSC are present. Moreover, while IBD is sometimes observed as a complication of AIH [8], it frequently complicates PSC, so we also routinely perform lower endoscopy. In the future, it will be necessary to differentiate the pathologies of AIH, PSC, and ASC in children.

9.4 De Novo AIH

In 1998, Kerkar et al. of King's College [11] observed a previously unreported form of liver failure approximately 2 years after surgery in seven (4 %) of 180 pediatric liver transplants with no underlying autoimmune disease. The liver tissue findings were strikingly similar to those for AIH. Moreover, these patients had hyper-IgG syndrome and autoantibody positivity. Their condition did not improve with the conventional therapeutic methods used for rejection, but improved with steroids and azathioprine, with most patients recovering within 1 year. In these cases, calcineurin led to the appearance of T cell clones that induced an autoimmune response. This type of post-liver transplant autoimmune phenomenon with a pathology different from that of rejection is considered

de novo AIH. De novo AIH initially received attention in cases of pediatric liver transplant, but a similar phenomenon was later reported in adults, and it is now considered a serious complication that can cause liver transplant failure during the long-term transplant course.

9.5 Conclusion

IMLDs are not necessarily rare in children, but have not been focused on because few cases progress to end-stage liver failure during childhood. However, the number of patients with this condition has increased with the increased use of liver function tests, including those for transaminase levels, in children. The pathologies of IMLDs differ between adults and children. Suitable diagnostic criteria are needed to enable early diagnosis and appropriate treatment.

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Chapter 10
Nonalcoholic Steatohepatitis-Autoimmune Hepatitis Overlap

Atsushi Takahashi, Kazumichi Abe, and Hiromasa Ohira

Abstract Nonalcoholic steatohepatitis (NASH) patients are often positive for antinuclear antibodies (ANA). Moreover, patients with ANA-positive NASH often meet the criteria for autoimmune hepatitis (AIH). However, ANA in NASH is thought to be an epiphenomenon of NASH. Other patients have typical histological finding of both NASH and AIH in addition to meeting the criteria for both individually. This condition is referred to as having "NASH-AIH overlap," although the disease concept has not been established and the pathogenesis of AIH remains to be elucidated. However, some drugs are suspected of triggering AIH onset and these include statins that are often used to treat NASH. Furthermore, corticosteroids that comprise standard treatment for AIH also induce secondary NASH. Therefore, medication history should be confirmed before diagnosing NASH-AIH overlap.

The pathophysiological ranges of NASH and AIH are wide and thus, clinical findings of both NASH and AIH can coexist. NASH-AIH overlap should be clinically treated as coexisting AIH and NASH regardless of whether or not each is an independent disease entity.

Keywords ANA • NAFLD • NASH-AIH overlap

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10.1 ANA and NAFLD

10.1.1 Proportion of ANA in Patients with NAFLD

Autoimmune disease has been diagnosed and followed up based on the presence of antinuclear antibodies (ANA). If positive ANA is defined as ≥1:40 dilution, the frequency of positive ANA detection is 20–50 % of patients with nonalcoholic fatty liver diseases (NAFLD) or nonalcoholic steatohepatitis (NASH) [1–6]. Healthy individuals can also occasionally be positive for ANA. One study found that 31.7 % of normal individuals are ANA positive at a 1:40 dilution and that this decreases to 13.3 % at 1:80 and 5.0 % at 1:160 dilution [7]. Therefore, ANA titers should be carefully assessed when considering ANA-positive NAFLD. In fact, cutoff values vary among studies that have examined the prevalence of serum ANA in patients with NAFLD and NASH [1–6]. Moreover, immunofluorescent ANA tests using human laryngeal cancer HEp-2 cells comprise the gold standard, but they generate higher values than tissue sections. A negative cutoff titer from 1:40 to 1:160 might improve the distinction between clinically significant ANA results and positive ANA findings in normal individual [7]. High titers of ANA do not always indicate more active or severe disease [8]. Therefore, cutoff values for ANA should be established before discussing the characteristics of ANA-positive NAFLD. However, populations selected by high cutoff values might not correctly reflect all patients with ANA-positive NAFLD. Moreover, high cutoff values cause problems with diagnosing NASH-AIH overlap. In Japan, 89.1 % of patients with AIH are positive for ANA [9] and 96 % of adult patients with AIH in North America have ANA, anti-smooth muscle autoantibodies (ASMA), or both [10]. In 26.3 % of Japanese patients with AIH, the ANA titer is below the 1:40 dilution and rates of ANA positivity have recently decreased compared with those in a previous study [9]. Consequently, careful evaluation is required for patients with ANA-positive NAFLD regardless of ANA titers.

10.1.2 ANA and Pathogenesis of NAFLD

Activated natural killer T (NKT) cells increase autoantibody production [11, 12]. Hepatic NKT cell accumulation is also associated with more advanced NAFLD [13]. This evidence indicates that ANA-positive NAFLD is a consequence of hepatic NKT cell accumulation. Several publications describe increased portal inflammation in ANA-positive NAFLD, which supports this hypothesis [2, 4–6].

Visceral adipose tissue (VAT) and inflammation are associated with the pathogenesis of insulin resistance and NAFLD [14] and VAT is involved in the production of adipokines and cytokines [15, 16]. B-cell activating factor (BAFF) is a tumor necrosis factor (TNF) ligand that promotes B-cell differentiation, which in

turn causes serum immunoglobulin levels to increase [17, 18]. In addition, BAFF levels are increased in the VAT and sera of obese mice, and interaction between BAFF and BAFF receptors (BAFF-R) in VAT leads to impaired insulin sensitivity [19]. Mice transgenic for BAFF produce increased amounts of autoantibodies [20]. These findings imply that ANA production is induced by VAT via BAFF production.

Consequently, the pathogenesis, inflammation, and insulin resistance of NAFLD could be associated with ANA production. Therefore, NAFLD with ANA positivity is considered an epiphenomenon [3, 5].

10.1.3 Characteristics of ANA-Positive Patients with NAFLD (Table 10.1)

Loria et al. and Tsuneyama et al. reported that ANA-positive patients with NAFLD/NASH are older and female-dominant [1, 6], whereas others have found no significant differences in age, sex, prevalence of obesity, serum aminotransferase levels, or other biochemical findings between patients with and without ANA [2–5]. A cohort study showed an association between high ANA titers and insulin resistance [1] and another study found higher levels of gamma globulins in patients who were positive for ANA and/or ASMA [2], although other studies have not confirmed such an association [3–6].

The presence of ANA in histological liver sections is associated with a higher inflammatory grade and advanced fibrosis [2, 21]. Moreover, the grade of steatosis is significantly lower in patients with high ANA titers than in those who are ANA-negative [5, 21].

Thus, the characteristics of ANA-positive patients with NAFLD have not reached consensus because of differences in definitions of ANA or the absence of established criteria to differentiate AIH from ANA-positive NAFLD. Previous reports have presented the notion that ANA-positive NAFLD could include various entities that are not clearly distinguishable as described below.

Considering that ANA is an epiphenomenon in patients with NAFLD, studies of large patient cohorts under a definition of ANA at $\geq$1:160 dilution have not associated ANA with more histological features. This notion was supported by the findings that NKT cells or BAFF that can produce ANA is associated with the pathogenesis of NAFLD.

Considering that ANA-positive NAFLD could include NAFLD/NASH-AIH overlap, typical findings of AIH could be those of ANA-positive NAFLD, such as interface hepatitis or rosettes, etc., in addition to similar clinical findings. Such patients have been treated as having NAFLD/NASH-AIH overlap [1, 2, 4, 22]. Next, ANA-positive NASH is a different type of NAFLD, the pathogenesis of which is affected by autoimmune mechanisms but not AIH [21]. Such

Table 10.1 Proportion and characteristics of ANA-positive patients with NAFLD

Reports	Patients (n)	ANA proportion (%)	Definition of autoantibodies	Laboratory characteristics in ANA-positive patients compared to ANA-negative patients	Histological characteristics in ANA-positive patients compared to ANA-negative patients	NAFLD/ NASH-AIH overlap (%)
Loria et al. (2003) [1]	84 NAFLD	ANA 18 (21.4) ASMA 4 (4.7)	ANA $\geq$ 1:160 or ASMA $\geq$1:40	No significant differences	Severe steatosis less frequently	3 (3.6)
Adams et al. (2004) [2]	225 NAFLD	ANA 46 (20) ASMA 6 (3)	ANA $\geq$ 1:40 or ASMA $\geq$1:40	Higher levels of gamma globulin	Higher fibrosis stage Higher inflammatory grade	4 (1.8)
Colter et al. (2004) [3]	74 NASH	ANA 25 (34) ASMA 3/48 (6)	ANA $\geq$ 1:40 or ASMA $\geq$ 1:40	No significant differences	No significant differences	None
Yatsuji et al. (2005) [4]	212 NAFLD	70 (33.0)	ANA $\geq$ 1:40	No significant differences	No significant differences	4 (1.9)
Niwa et al. (2007) [21]	71 NAFLD	35 (49.3)	ANA $\geq$ 1:40	No significant differences	Increased portal inflammation Increased interface activity Increased hepatocellular ballooning	None (AIH excluded)
Vuppalanchi et al. (2011) [5]	864 NAFLD	182 (21)	ANA $\geq$ 1:160 or ASMA $\geq$ 1:40	Not shown (no difference in HOMA-IR)	Lower grade of steatosis Increased portal inflammation	None (AIH excluded)
Tsuneyama et al. (2013) [6]	54 NASH	26 (48)	Not shown	No significant differences	Increased portal inflammation	Not shown

ANA-positive NASH is associated with severe portal inflammation and interface activity. This notion supports the findings that the inflammatory cell profile of NASH is distinct from that of AIH. Ratios of CD138/CD3 among inflammatory cell profiles in the portal area are significantly lower in NASH than in AIH.

10.2 NASH-AIH Overlap

10.2.1 Histological Findings of NAFLD

The diagnosis of NAFLD is based on the presence of hepatosteatosis determined by imaging or by histology in the absence of excessive alcohol consumption and the exclusion of other liver diseases. Since NAFLD includes a wide range of conditions from simple steatosis to inflammatory steatohepatitis (NASH), liver biopsies are the gold standard for diagnosing NASH based on the presence of macrovesicular steatosis, ballooning degeneration of hepatocytes, and mixed lobular inflammation. Mallory's hyaline or pericellular fibrosis are also characteristic pathological features. Other atypical features such as little or no ballooning or no Mallory's hyaline and more portal-based chronic inflammation and fibrosis have been identified in pediatric or morbidly obese patients [23].

Evaluation of inflammation is important and a differential diagnosis of AIH needs careful interpretation. In fact, mild or moderate inflammatory cell infiltration of the portal area has been graded in Brunt's classification [24], which has been used worldwide for diagnosing NAFLD. Thus, NASH with worse than moderate inflammation in the portal area is indistinguishable from the interface hepatitis associated with AIH.

10.2.2 AIH Scoring System in Patients with NAFLD

Patients with NAFLD have been diagnosed with AIH based on a diagnostic scoring system revised by IAIHG [25] that has 13 clinical components and renders 27 possible grades. Clinical components comprise gender, laboratory manifestations of liver inflammation and cholestasis, conventional autoantibodies, viral markers, drug or alcohol exposure, HLA phenotype, concurrent immune diseases, novel antibodies, and individual histological features. A pretreatment score of $\geq$10 points or posttreatment scores of $\geq$12 points indicate the likelihood of AIH. Some of these diagnostic components are common to a diagnosis of NASH. Moreover, interface hepatitis that could also be associated with NASH is scored as three points in the revised diagnostic scoring system.

Yatsuji et al. previously evaluated the usefulness of the revised diagnostic scoring system without including liver histological findings [4]. Of 212 patients,

127 (59.9 %) had revised AIH scores of ≥10 and 97.2 % of female patients had revised AIH scores of ≥10. However, after liver biopsy, only one (0.5 %) patient could be confirmed as having "definite AIH." Finally, they concluded that the revised diagnostic scoring system without liver biopsy is not useful for diagnosing AIH in patients with NALFD.

10.2.3 Proportion of NASH-AIH Overlap

Loria et al. originally proposed the "NAFLD/NASH-AIH" overlap syndrome [1]. Thereafter, a few reports described AIH overlap in 1.9–3.6 % of patients with NAFLD and in 5.7–10 % of patients with ANA-positive NAFLD [1, 2, 4].

10.2.4 Diagnosis of NASH-AIH Overlap

Histological findings of liver sections comprise the gold standard for diagnosing NASH and AIH. However, such findings can include many, including atypical variations [23, 24, 26, 27]. Therefore, interpreting interface hepatitis in ANA-positive NAFLD is challenging. The findings indicate typical finding of AIH or excessive inflammation due to NASH. Having more specific markers for NASH or AIH should resolve this issue.

Synchronous and metachronous manifestations of pathological features of NASH and AIH characterize relatively rare NASH-AIH overlap. However, fatty changes might be induced during the clinical course of AIH after corticoid therapy [4, 28]. Other drugs are associated with AIH onset [29] such as statins [30–32]. Thus, three types of NASH-AIH overlap could exist: NASH onset after corticosteroid therapy for AIH, AIH onset after treatment for dyslipidemia in patients with NAFLD, and simultaneous NASH and AIH arising in the absence of drugs.

To diagnose or conceptualize that NASH-AIH overlap occurs during NASH onset during the clinical course of AIH after corticoid treatment would be not difficult. Diagnosing NASH with or without AIH is a clinically difficult and important issue at the time of NASH onset. In this situation, drugs, particularly those that can induce AIH should be considered. A diagnosis of NASH-AIH overlap during AIH onset is also clinically important, because progressive fatty changes can result in treatment failure [33]. However, the presence of NASH-AIH overlap at the time of a diagnosis of either NASH or AIH has not been investigated in detail.

Both NASH and AIH have diagnostic criteria and are established independent liver diseases, but disease-specific biochemical markers to differentiate them are not yet available and liver histological findings remain the gold standard for diagnosing both. Some reports describe NASH-AIH overlap, but definitions have not been determined and the disease concept has not yet been established.

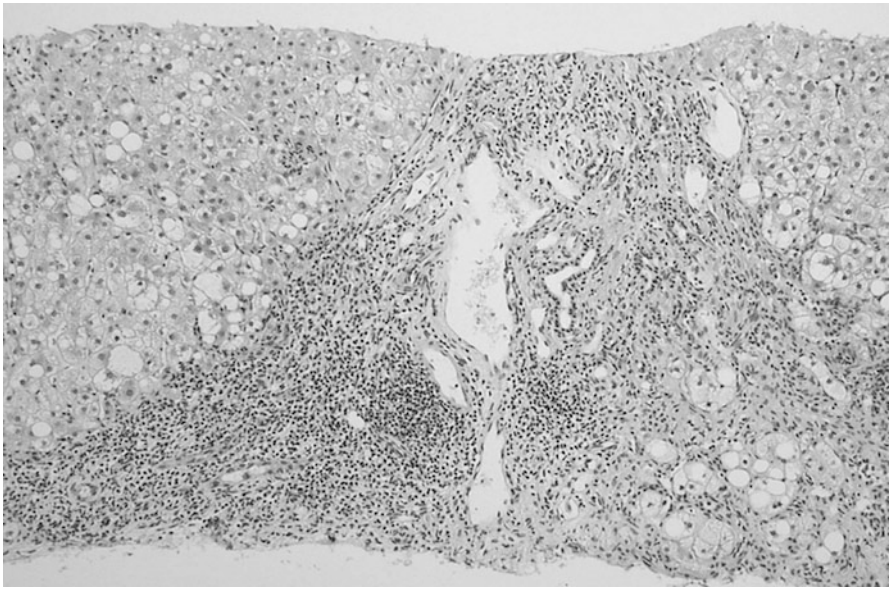

Fig. 10.1 Histological findings of NASH-AIH overlap. Histology showed typical findings of NASH (steatosis, ballooned hepatocytes, Mallory bodies) and AIH (enlargement of portal area with lymphoplasmacytic infiltrate with interface hepatitis, rosette formation)

Previous descriptions of NASH-AIH overlap are essentially based on the presence of typical liver histological findings of NASH and AIH (Fig. 10.1) in addition to satisfying the diagnostic criteria for both [1, 2, 4, 22]. However, to differentiate between NASH with interface hepatitis and NASH-AIH overlap only by liver histology is difficult. Interface hepatitis or lymphocytic infiltrates are typical features of AIH liver histology, but they are also occasionally found in liver histology of NASH without AIH [24]. Typical AIH histological findings other than interface hepatitis, such as hepatic rosette formations or emperipolesis, can be useful for a differential diagnoses, but their frequency in AIH is not so high, and these features can also exist in NASH [3]. Fatty changes are commonly identified in AIH liver histology. Moreover, patients with asymptomatic mild hepatitis or the absence of serological markers are treated as having nonclassical AIH phenotypes [27].

10.3 Treatment

Corticosteroids comprise standard and first-line therapy for AIH [34], but they can also cause secondary NASH [35]. Therefore, corticosteroids alone might be ineffective for treating NASH-AIH overlap. Moreover, progressive fatty changes in liver histology can cause treatment failure in patients with AIH [33].

Previous reports describing NASH-AIH overlap do not include any patients whose liver biochemical and histology improved without treatment for NAFLD. Therefore, the priority for treating NASH-AIH overlap would be to treat NASH except when severe hepatitis is associated with AIH. Immunosuppressive therapy with drugs such as azathioprine is also important for treating NASH-AIH overlap and this strategy might be useful for treating NASH-AIH overlap in patients with persistent NAFLD pathology.

10.4 Outcomes

Few patients with NASH-AIH have been described in the literature although many more might exist. Moreover, a standard or consensus therapy for NASH-AIH overlap has not been established. Therefore, the long-term outcome of NASH-AIH overlap has remained obscure. Prednisolone combined with diet therapy has improved biochemical or histological findings for some patients [1, 22]. However, many issues remain unresolved.

Continued corticosteroid might worsen the NAFLD or reduce the effect of diet therapy and corticosteroid withdrawal might induce AIH relapse [36–38]. The effects of combining drugs for treating NASH and corticosteroid have not been elucidated. Consequently, these problems will only become resolved when more patients with NASH-AIH are identified.

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Chapter 11
IgG4-Related Autoimmune Hepatitis

Takeji Umemura

Abstract Autoimmune hepatitis (AIH) has been broadly categorized into two distinct disease subtypes on the basis of antibody profiles. In some patients with definite AIH, we have observed abnormal serum concentrations of immunoglobulin G4 (IgG4) and extensive IgG4-positive plasma cell infiltration in liver biopsy samples. We propose that such cases should fall under a new clinicopathological entity termed "IgG4-related AIH." In this chapter, we first introduce our study on abnormal liver biopsy specimens in autoimmune pancreatitis before defining the concept of IgG4-related hepatopathy. Next, we present several cases that meet the criteria of IgG4-related AIH. Since IgG4-related AIH has a low incidence of approximately 3 %, it is still controversial whether this entity truly exists. However, we lastly describe a new possible association between posttransplant de novo AIH/plasma cell hepatitis and IgG4-related disease.

Keywords AIH • IgG4 • IgG4-related hepatopathy

11.1 Introduction

Autoimmune hepatitis (AIH) is characterized by chronic inflammation of the liver, elevated transaminase levels, hypergammaglobulinemia, serum autoantibodies, histological evidence of interface hepatitis, and a favorable response to immunosuppressive treatment [1, 2]. AIH has been broadly categorized into two distinct

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disease subtypes on the basis of antibody profiles: type 1, which is associated with the presence of either antinuclear antibodies or anti-smooth muscle antibodies in the serum, and type 2, which is much less common than type 1 and is identified by the detection of either anti-LKM-1 or anti-liver-cytosol antibody type 1.

In clinical practice, some patients do not precisely fit into a specific AIH subgroup or diagnostic category on the basis of clinical, serological, and/or histological criteria. Consequently, several conditions, including overlap syndrome and autoimmune cholangitis, have been introduced into the scientific literature to describe these variant forms of AIH. We earlier incidentally discovered an elevated IgG4 concentration of $\geq$500 mg/dL in the stored serum of a pre-therapy AIH patient. As extensive IgG4-positive plasma cell infiltration in the liver was also found, we proposed that some AIH patients had a clinicopathological entity that could best be termed "IgG4-associated AIH." In this chapter, we first introduce our study on abnormal liver biopsy specimens in autoimmune pancreatitis (AIP) before defining the concept of IgG4-related hepatopathy [3]. Next, we present two cases that meet the criteria of IgG4-associated AIH [4]. Since IgG4-associated AIH has a low incidence of approximately 3 %, it is still controversial whether this entity, which was renamed as IgG4-related AIH (IgG4-AIH) in 2012, truly exists. However, we describe a new possible association between de novo AIH/plasma cell hepatitis (PCH) and IgG4-related disease later in the chapter.

11.2 IgG4-Related Hepatopathy

In 2007, we conducted a study on changes in liver function tests and pathological liver biopsy findings in patients with AIP and prospectively compared pathological findings before and 4 weeks after prednisolone (PSL) administration [3]. We first noted various abnormalities in liver biopsy findings and classified them into five patterns: (A) the portal inflammation pattern, with intense portal inflammation with or without interface hepatitis; (B) the large bile duct damage pattern, characterized by bile ductular proliferation, neutrophil infiltration, and edematous change in the portal areas; (C) the portal sclerosis pattern, exhibiting dense portal sclerosis with scarce portal inflammation; (D) the lobular hepatitis pattern, showing lobular inflammation with hepatocellular necrosis resembling viral hepatitis; and (E) the cholestatic pattern, with canalicular cholestasis predominantly in the centrilobular area. A wide range in the number of intrahepatic IgG4-positive plasma cells infiltrating the liver parenchyma has been reported for AIP, with mean values of 2–60/high-power field (HPF). We found the incidence of cases exhibiting $\geq$10 infiltrating IgG4-positive plasma cells/HPF to be approximately 24 %.

In our pathological study of the liver before and 4 weeks after the start of therapy, we observed considerable improvement in liver findings following PSL treatment. A statistically significant difference was noted for the lobular hepatitis

pattern, but this was attributed to the small number of cases examined. The degree of IgG4-positive plasma cell infiltration also showed significant improvement after therapy. Hence, we proposed the new concept of IgG4 hepatopathy, which is now termed IgG4-related hepatopathy.

11.3 IgG4-Related Autoimmune Hepatitis

We similarly encountered an elevated IgG4 concentration of ≥500 mg/dL in the stored serum of a pre-therapy AIH patient. A tissue specimen obtained at the time of diagnosis showed interface hepatitis, rosette formation, and syncytial multinucleated giant cell change of hepatocytes. The patient was diagnosed as having definite AIH according to the diagnostic criteria of the International Autoimmune Hepatitis Group [5] and oral PSL therapy was soon commenced. However, marked IgG4-positive plasma cell infiltration in the hepatic parenchyma was evident at follow-up (Fig. 11.1). No obvious abnormalities in the extrahepatic bile ducts or pancreas had been detected by various diagnostic imaging examinations performed at the previous hospital. This case not only fulfilled the diagnostic criteria for AIH, but also had features characteristic of IgG4-related disease, such as serum IgG4 concentration ≥135 mg/dL and abundant IgG4-positive plasma cell infiltration in the hepatic parenchyma. This prompted us to assign the name "IgG4-associated AIH," which is now termed IgG4-AIH, to what we considered to be a new clinicopathological entity [4].

In a search for other cases of IgG4-AIH, we investigated serum IgG4 values and the presence of intrahepatic IgG4-positive plasma cells in 60 patients with AIH and uncovered an additional case of presumed IgG4-AIH [6]. An ensuing study on this cohort determined the prevalence of IgG4-AIH to be 3.3 % (2/60) [7]. As shown in Table 11.1, the two patients with IgG4-AIH had higher median serum IgG4 concentrations. The HLA DRB1*04:05 allele was found in one patient. Liver biopsies of the two patients revealed similar patterns of chronic active hepatitis with bridging fibrosis. Both patients showed interface hepatitis and zonal necrosis. Of particular interest was the fact that the first patient developed hepatic dysfunction, manifesting mainly as biliary enzyme elevations, as well as findings of sclerosing cholangitis in endoscopic retrograde cholangiopancreatography imaging. This occurred despite the maintenance of oral PSL at 2.5–5.0 mg/day for 5 years after the initial therapy. Although serum IgG4 concentration was normal, IgG4-positive plasma cell infiltration was found in a bile duct tissue specimen, suggesting that IgG4-related sclerosing cholangitis had developed during the follow-up period. Based on our findings, we proposed tentative diagnostic criteria of IgG4-AIH (Table 11.2). High serum IgG4 concentration (≥135 mg/dL) and marked IgG4-bearing plasma cell infiltration in the liver (≥10/HPF) appeared to be hallmark features of this disease, along with no obvious abnormal findings in the extrahepatic bile ducts or pancreas.

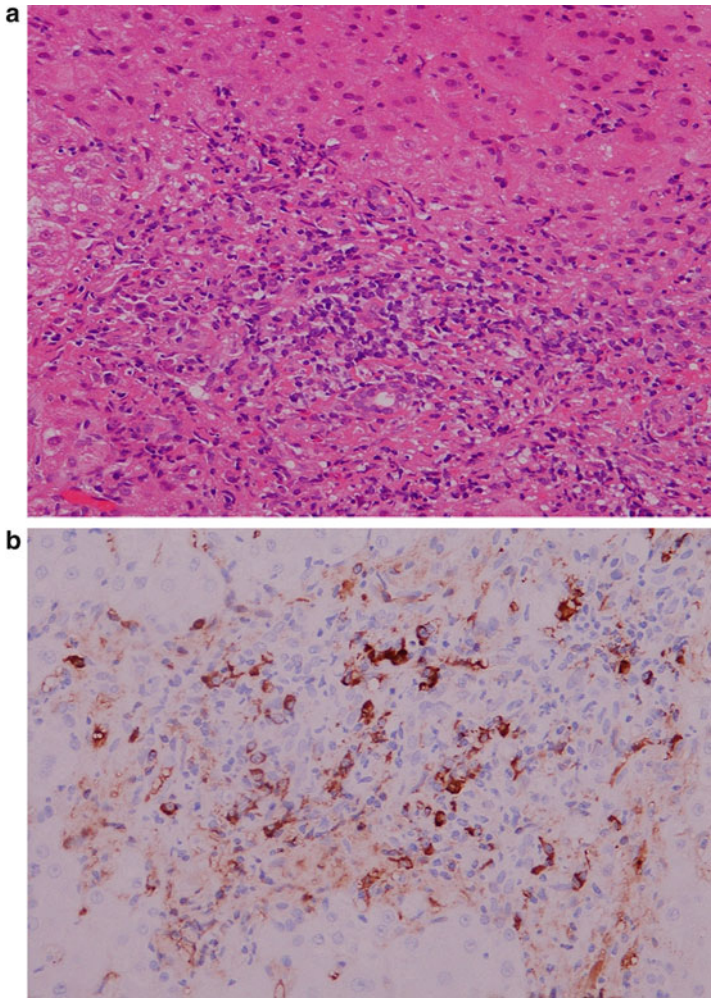

Fig. 11.1 IgG4-related hepatopathy in a patient without autoimmune pancreatitis or sclerosing cholangitis. Histological findings are comparable with those of autoimmune hepatitis. (**a**) Histological evaluation of chronic active hepatitis shows dense inflammatory cell infiltration as well as interface hepatitis in portal areas (HE stain, ×100). (**b**) In portal areas, abundant IgG4-positive plasma cell infiltration is evident (IgG4 immunostain, ×400)

In 2009, Chung et al. [8] investigated IgG4-immunostained liver biopsy samples obtained from 26 patients with AIH. Apparent IgG4-positive plasma cell infiltration was found in nine (35 %) subjects. Although they considered such cases to be of IgG4-AIH, none of the nine patients had elevated serum IgG4 and no patient met our tentative criteria for IgG4-AIH. In this regard, our two cases of IgG4-AIH appear to be distinct from theirs.

Table 11.1 Demographic, clinical, and histological characteristics of IgG4-related autoimmune hepatitis patients

	Case 1	Case 2
Age (years)	42	54
Sex	Female	Male
AIH score	18	19
AST (n.v. 7–45 IU/L)	234	642
ALT (n.v. 12–37 IU/L)	487	788
Bilirubin (n.v. 0.3–1.2 mg/dL)	2.7	8.6
IgG (n.v. 870–1,700 mg/dL)	2,403	5,622
IgG4 (n.v. <135 mg/dL)	557	642
Lobular hepatitis (zonal necrosis)	Present	Present
Rosette formation	Present	Present
Syncytial multinucleated giant cell change	Present	Present
Bile duct damage	Absent	Absent
IgG4-positive cells (/HPF)	24	29
IgG4-positive:IgG-positive cells	0.282	0.528

n.v. normal value

Table 11.2 Tentative diagnostic criteria for IgG4-related autoimmune hepatitis

Characteristic	Value
Serum IgG4 concentration	≥135 mg/dL
IgG4-positive plasma cell infiltration in the liver	≥10/high-power field
Complicating autoimmune pancreatitis or IgG4-related sclerosing cholangitits	Absent

11.4 De Novo Autoimmune Hepatitis After Liver Transplantation and IgG4-Related Disease

Recent topics in IgG4-related diseases of the liver have included de novo AIH/PCH. Posttransplant de novo AIH was initially described in 1998 [9]. De novo AIH/PCH was later recognized as plasma-cell-rich infiltrates associated with hepatic injury after liver transplantation [10]. Clinically, histopathologically, and serologically, de novo AIH/PCH resembles AIH by sharing the symptoms of necro-inflammatory activity with plasma cell infiltration in liver biopsy specimens, autoantibody production, and steroid responsiveness. While de novo AIH/PCH is well recognized, the pathogenesis of this disease is poorly understood because the concept of allograft "autoimmunity" is confusing and its potential overlap with rejection is problematic.

In 2008, Eguchi et al. [11] investigated the association of de novo AIH after living donor liver transplantation with IgG4-related disease in Japan. The main indication for liver transplantation was biliary atresia in one patient, primary biliary cirrhosis in two patients, and Budd-Chiari syndrome in one patient. Four of 72 (5.6 %) patients were suspected as having developed de novo AIH in the cohort. In one patient, low levels of infiltrating IgG4-positive cells were found in the liver, but serum IgG4 levels were within normal limits. In the other three patients, serum

IgG4 was within normal values and IgG4-positive cell infiltration was scarce. Hence, this small Japanese study did not detect any associations between IgG4 and de novo AIH/PCH after living donor liver transplantation.

On the contrary, Castillo-Rama et al. [12] have recently reported a possible association between IgG4-related disease and de novo AIH/PCH. They investigated the clinical, serological, histopathological, and IgG4-related immunohistochemical features of 20 patients with de novo AIH/PCH, 19 patients with classical AIH, and 20 patients with plasma cell-rich renal allograft rejection. Interestingly, 9 of 20 (45 %) de novo AIH/PCH recipients exhibited >25 IgG4-positive plasma cells/HPF. This frequency was significantly higher than in AIH (1/19, $P = 0.008$) and in plasma cell-rich kidney rejection (2/20, $P = 0.03$). After dividing patients into IgG4+ de novo AIH/PCH (>25 IgG4-positive plasma cells/HPF) and IgG4-de novo AIH/PCH groups, a mean age of 60 years and male predominance were common in six of the nine (67 %) patients with IgG4+ de novo AIH/PCH. Of the total of seven patients who had the HLA-DR15 allele, five (71 %) belonged to the IgG4+ de novo AIH/PCH group. This study supported our finding that IgG4-AIH may be present among AIH cases. Further studies in other ethnicities are needed to confirm the possible new concept of an association between IgG4-related disease and de novo AIH/PCH.

11.5 Clinical Significance of Proposing the New Disease Concept of IgG4-Related Autoimmune Hepatitis

In conclusion, IgG4-AIH is found in approximately 3 % of classical AIH cases in Japan and is characterized by high serum IgG4 and IgG4-bearing plasma cell infiltration in the liver. Since IgG4-AIH is clearly an IgG4-related hepatopathy, this disease should be differentiated from the classical definition of AIH. In support of this, a recent study has suggested that de novo AIH/PCH is a new possible IgG4-related disease. Further studies are needed to clarify the epidemiology and pathogenesis of IgG4-AIH and IgG4-related diseases.

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Part II
Primary Biliary Cirrhosis

Chapter 12
The Onset Mechanism of Primary Biliary Cirrhosis

Shinji Shimoda

Abstract The principal homologous antigen of antimitochondrial antibodies is pyruvate dehydrogenase complex E2 component (PDC-E2) which is present in the mitochondrial inner membrane.

The involvement of autoimmunity is considered due to the presence of both autoreactive CD4-positive T cells and CD8-positive T cells recognizing PDC-E2. Autoreactive NK cells are also involved in bile duct damage and in addition to acquired immunity, abnormalities in innate immunity are also very important regarding the onset of PBC.

In addition to the mechanism of the bile duct epithelial cells presenting autoantigens, the pathology of chronic nonsuppurative destructive cholangitis (CNSDC) is maintained by means of antigen-presenting cells such as dendritic cells, macrophages, etc., which present autoantigens discharged from damaged bile duct epithelial cells.

Keywords Autoreactive NK cells • Autoreactive T cells • Biliary epithelial cells • Toll-like receptor

12.1 Introduction

Primary biliary cirrhosis (PBC) is a chronic progressive biliary cholestasis that frequently appears in middle-aged women and is an organ-specific autoimmune disease characterized by the expression of mitochondrial antibodies that recognize oxoglutarate dehydrogenase complex such as pyruvate dehydrogenase E2

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component (PDC-E2) present in the mitochondrial inner membrane as well as by the appearance of chronic nonsuppurative destructive cholangitis (CNSDC). Various investigations have been conducted regarding the cause of PBC and suggest the involvement of hereditary predisposition and environmental factors as the common onset platform in autoimmune diseases. In this article, an explanation is provided regarding the sequence of events that have attracted attention to abnormalities in the innate immunity of PBC, and a discussion is held regarding the onset mechanism from abnormalities in acquired immunity against autoantigens.

12.2 Generic Environmental Factors Related to PBC

Due to high familial aggregation, a high concordance rate of onset in identical twins, and because complications of other autoimmune diseases are common, it was believed that hereditary predisposition is strongly correlated to the onset of PBC. From the results of the genome-wide association study (GWAS) in Western countries and in Japan [1, 2], it was clarified that the HLA class II domain is the disease-susceptible gene observed having the strongest correlation with onset. From this, it is believed that autoantigen production is strongly related to the onset. Moreover, in GWAS, many genetic polymorphisms indicated in other autoimmune diseases such as IL-12, TNF-α signal-related genes, etc. were commonly observed, leading to the belief that there is a common working disease pathway to the onset of autoimmune diseases including PBC and the maintenance of pathophysiology.

It is believed that environmental factors are involved in onset from the fact that the rate of incidence is increasing in the vicinity of the toxic waste disposal area in New York [3]. Moreover, known risk factors include past smoking history; medical history of urinary tract infection; the use of nail polish remover [4], etc. When chemical modification is carried out on PDC-E2, which is the principal homologous antigen of antimitochondrial antibodies, the antimitochondrial antibodies from the serum of patients with PBC react more sharply than to natural type PDC-E2 [5]. A PBC model mouse may be created using 2-octynoic acid (2-OA), which is known as a makeup ingredient from among xenobiotics similar to such PDC-E2 [6].

Primary bile acids increase in the bile configuration of PBC, while hydrophobic chenodeoxycholic acids also increase in addition to cholic acids. Hydrophobic bile acids work as a duplex activator to exfoliate lipids on the cholangiocyte membrane, thereby causing cell injury; additionally, they cause cell injury by inducing apoptosis via mitochondrial damage, etc. Bicarbonates are capable of preventing this; however, regarding PBC, a possibility has been suggested of cell injury occurring due to the decline in protein expression, which manages the production of bicarbonate in PBC [7]. In actual practice, a mouse model lacking the anion exchanger that carries out this $CL^{-}/HCO3^{-}$ exchange exhibits a pathophysiology similar to PBC, causing the antimitochondrial antibodies to become positive [8]. In PBC, in addition to these, other spontaneous models made by genetic modification include NOD.c3c4 mouse in which the diabetes-sensitive gene present in the chromosomes

3 and 4 of the NOD mouse is replaced with a resistance gene; dominant-negative TGF-β receptor II mouse in which TGF-β signals are selectively blocked by T cells; IL-2Rα^{-}/$^{-}$ mouse in which IL-2 signals are blocked; scurfy mouse lacking functionally controlling T cells; etc.

12.3 Autoantigens and Autoantibodies

It is surmised that in PBC, a specific mitochondrial antigen is presented in cholangiocytes from the fact that the antimitochondrial antibodies are specifically (95 % or more) observed at a high rate (90 % or more), although there is no correlation with disease activity. It has been pathologically clarified that the bile duct is apoptosed in CNSDC [9]; however, in apoptosed cholangiocytes, the mitochondrial antigens contained in microparticles with their antigenicity maintained by an cholangiocyte-specific mechanism are discharged outside the cells [10, 11] (Fig. 12.1).

The E2 of the pyruvate dehydrogenase (PDC-E2) is an autoantigen specific to PBC, along with 2-oxo acid dehydrogenase complexes (2-OADC) such as

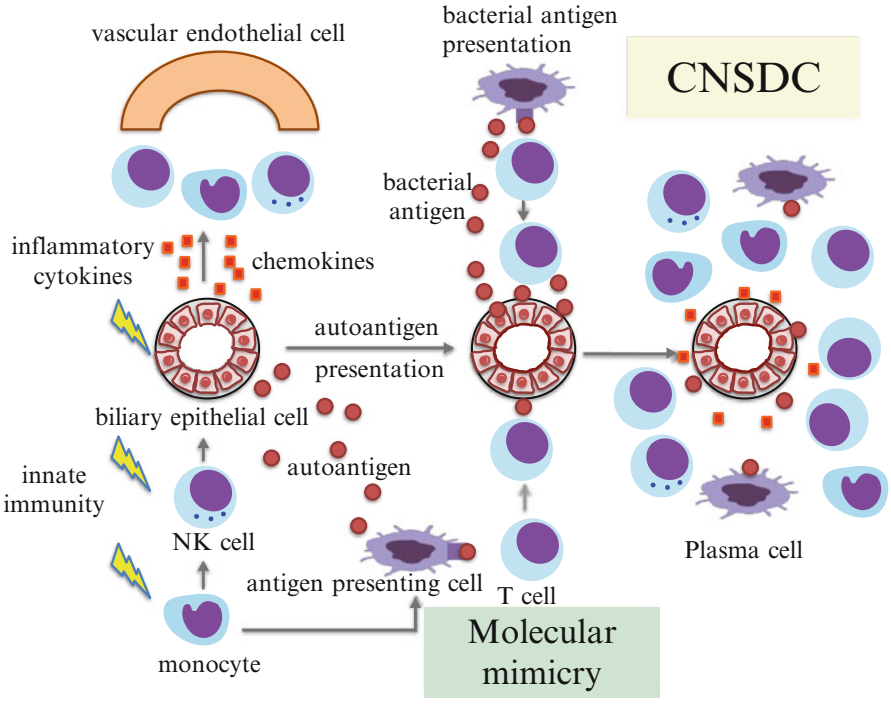

Fig. 12.1 Events related to PBC onset/maintenance of pathophysiology and the movement of cells

Table 12.1 Autoantigens identified in PBC patients

Mitochondrial antigen	
PDC-E2	E2 component of pyruvate dehydrogenase (PDC)
PDC-E3BP	E3-binding protein of PDC
OGDC-E2	E2 component of 2-oxoglutarate dehydrogenase
BCOADC-E2	E2 component of branched-chain 2-oxo acid dehydrogenase
Nuclear antigen	
Chromatin-related protein (centromere pattern)	
Histone	
Centromeres A, B, C	
Chromatin non-related protein (multiple nuclear dot pattern)	
Sp100	Nuclear dot-associated protein
PML	Promyelocytic leukemia antigen
Nuclear membrane-related protein	
Nuclear pore complex pattern	
p62	Nucleoporin
gp210	Nuclear pore glycoprotein
Nuclear envelope pattern	
Lamins A, B, C	

E3-binding protein (E3BP) and E1 component α chain (E1α) of PDC; E2 component of 2-oxoglutarate dehydrogenase (OGDC-E2); E2 component of branched-chain 2-oxo acid dehydrogenase (BCOADC-E2), etc. (Table 12.1). The PDC to become an autoantigen forms a large enzyme complex with millions of molecular weight of the compound of El, E2, and E3 components and B3BP and, further, is localized in the mitochondrial inner membrane together with OGDC and BCOADC as 2-oxo acid dehydrogenase complex (2-0ADC) in a state forming a massive enzyme complex. From among these, PDC-E2 has the strongest antigenicity.

In PBC, autoantibodies against nuclear antigens (antinuclear antibodies) such as chromatin-related antigen, non-chromatin-related antigen, nuclear membrane-related antigen, etc., are also made positive (Table 12.1). The anti-gp210 antibody with respect to the structural component of nuclear pores has high disease specificity, is positive in approximately 20–30 % of patients, has poor treatment response, and is prone to becoming positive when PBC progresses to hepatic cirrhosis; therefore, it is useful as a clinical course predicting the factor of PBC [12].

12.4 Abnormalities in Innate Immunity Related to PBC

Abnormalities in the innate immunity of PBC are becoming rapidly clarified due to the recognition of pathogens via Toll-like receptors (TLR). From the samples of the CNSDC site by laser microdissection, it was found that accelerated expression of TLR3 and type 1 IFN is observed in this site [13]. Moreover, when compared to healthy individuals by stimulating peripheral monocytes with the TLR ligands, the production of TNF-α, IL-1β, and IL-6, which are proinflammatory cytokines, was

accelerated [14]. The production of IL-1β from the attacking cells was accelerated in addition to the cholangiocytes, which become the target [15], and as a result, the surrounding environment was led to an IL-17-producing T cell (Th17) environment. Moreover, the surrounding environment of CNSDC is characterized by IFN-γ production acceleration [16]; meanwhile the bile duct epithelial cells express TLR2, TLR3, TLR4, and TLR5. The bile duct epithelial cells which immunologically become the target of inflammatory cells produce CXCL1, CXCL5, CXCL6, and CXCL8 spontaneously involved in chronic inflammation environment while producing CCL3, CCL4, CCL5, and CXCL10 involved in IFN-γ production environment by means of TLR3 ligand (polyI:C) stimulation. In PBC, these bile duct epithelial cells stimulated by mononucleosis induced by chemokine further reinforce chemokine production related to IFN-γ production [17]. Moreover, the expression of fractalkine (CX3CL1) functioning as chemokine and cell adhesion molecules specifically accelerates in PBC, and the receptor-positive cells thereof infiltrate to the periphery of the bile ducts [18]. This CX3CL1 is produced from bile duct epithelial cells in the presence of TNF-α which is produced from monocytes due to innate immunity stimulation. Further, NK cells that have migrated to the periphery of the bile ducts are activated by TLR4 ligand stimulation and, when also stimulated by type 1 IFN which is produced from the monocytes by TLR3 ligand stimulation, attack autologous cholangiocytes [19].

In PBC, senescence of the target cholangiocytes is induced in a chronic inflammation environment, and as a result, chemokine is produced by a greater amount than usual [20]. Moreover, the possibility has been suggested of abnormalities being caused in the function of autophagy that elaborately manages proteins in the cell [21] and autoantigens, which are not normally produced, being produced due to the acquired immunity-type cells. In PBC, bile duct epithelial cells/mononucleosis overreacts with respect to pathogen-associated molecular patterns (PAMPs) and various chemokines are produced via stimulation from migrated cells; subsequently, cells concentrate in the periphery of the bile ducts due to the mechanism of autocrine and paracrine secretion, thereby causing cholangitis.

Next, an inflammatory response based on immune response is caused mainly in the bile duct between hepatic lobules with bile duct epithelial cells, and it is believed that a state of chronic inflammation is subsequently constructed due to the complicated involvement of factors such as abnormalities in the inflammatory signal and senescence of bile duct epithelial cells (Fig. 12.1).

12.5 Abnormalities in the Acquired Immunity Involved in PBC

In PBC, intrahepatic small bile ducts are selectively and progressively destroyed, and as a result, chronically persisting bile congestion is generated following the disappearance of the small bile ducts. It has become clarified that the disappearance of Hering ducts is observed during the change in the initial stage of the disease [22].

Moreover, interface hepatitis is often simultaneously present in a variety of degrees, thereby causing progression of hepatic necrosis due to hepatocyte injury in addition to bile congestion, progressing to fibrosis of the portal region and hepatic cirrhosis. Advanced monocyte infiltration indicating the cause or result thereof is observed in the periphery of the injured bile ducts. Monocyte infiltration inside the bile duct epithelial cells is observed, T cells are predominant in the periphery of the injured bile ducts including infiltrating cells, and moreover, plasma cells are also scattered in the periphery of the bile ducts. Ectopic expression of class II antigens is also accelerated in the damaged bile ducts in addition to the expression of HLA class I antigens. It is believed that such a mechanism of autoimmunity involving the activation of T cells by means of the injured bile duct itself presenting autoantigens in addition to the presentation of antigens from the antigen-presenting cells is strongly involved in the formation of CNSDC.

Meanwhile, clarification of the reason for the immune reaction against mitochondrial components generally present in the human body being specifically observed in PBC, which is an organ-specific autoimmune disease, is advancing. Generally, in apoptotic cells, the intracellular protein is comprised in the apoptosis body in a digested state and then discharged outside the cells. In bile duct epithelial cells, PDC-E2 molecules, which are autoantigens, are present in the apoptosis body while still immunologically intact [23]. In PBC, apoptosis in bile duct epithelial cells is accelerated due to the action of mediators such as chronic inflammation and senescence, and the apoptotic body comprising the immunologically stored PDC-E2 molecules is discharged outside the cells. It is believed that this is incorporated by antigen-presenting cells such as macrophages, dendritic cells, plasma cells, etc., and that PDC-E2 antigens are presented or failure in immune tolerance is caused including reduced regulatory T cells, thereby causing an immune reaction with respect to autoantigens, thus causing biliary epithelium-specific cell damage.

12.6 Autoreactive T Cells

In PDC-E2, which is the principal homologous antigen of antimitochondrial antibodies, B cell epitopes, CD4-positive T cell epitopes, and CD8-positive T cell epitopes necessary for antibody recognition are located close to each other [24–26]. PDC-E2-reactive CD4-positive T cells are present in the liver and lymph node in the hepatic portal region at an incidence of about 200-fold more than the periphery [27] while PDC-E2-reactive CD8-positive T cells are present at an incidence of about tenfold more than the peripheral [28]; moreover, it is believed that although the incidence of self-reactive CD4-positive or CD8-positive T cells declines as the disease state of PBC progresses, T cells infiltrating inside the liver increase due to the relation with the mirror image thereof.

Self-reactive CD4-positive T cells are present in healthy individuals as well, although the incidence is low compared to PBC. However, the critically different

point between the self-reactive CD4-positive T cells of a healthy individual and PBC-derived self-reactive CD4-positive T cells is contact with antigen-presenting cholangiocytes, and while the PBC-derived self-reactive CD4-positive T cells are a Th1 type that mainly produce IFN-γ, most self-reactive CD4-positive T cells of healthy individuals acquire immunomodulating function producing IL-10 [29]. Differences in the affinity of antigen recognition of the T cell receptors, the difference in stimulation via cell contact from bile duct epithelial cells, or prostaglandin E2 produced by cholangiocyte, etc., are involved in this acquisition of immunomodulating function [30]. An increase in Th17-positive cells and reduction in Foxp3-positive regulation T cells are observed in the CD4-positive T cells in the vicinity of CNSDC [31]. Moreover, plasma cell infiltration was prominent in the vicinity of CNSDC [32] and may be involved in the presentation of autoantigens (Fig. 12.1).

Progress is being made in the analysis of self-reactive T cells with PDC-E2, which is the main homologous antigen of antimitochondrial antibodies, as the target, and PDC-E2 163–176 (GDLLAEIETDKATI) [25] has been identified as a CD4-positive T cell epitope, while PDC-E2 159–167 has been identified as a CD8-positive T cell epitope [26]. From an analysis using PDC-E2 163–176-reactive CD4-positive T cell clones and analog peptides, ExDK arrangement from no. 170 and EIExD arrangement from no. 168 are essential in the recognition of PDC-E2 163–176 peptides in CD4-positive T cells, and multiple bacteria-derived molecular mimicry peptides have been identified, including *Escherichia coli* that activates PDC-E2 163–176-reactive T cells in vitro [33]. Further, it has been clarified that these *E. coli* antigen-reactive T cell clones cross-reactively confirm autoantigens (PDC-E2, E3BP, OGDC-E2, BCOADC-E2) [34]. Based on these facts, it may be believed that the recognition of various autoantigens contained in 2-OADC at a T cell level is initiated by the stimulation of exogenous antigens such as *E. coli*, etc., as molecular mimicry, thereby causing clonal expansion. The formation of a granuloma, which is not often formed in lesion sites other than in infectious diseases, also suggests the involvement of exogenous antigens.

Moreover, gp210 protein also comprises a motif similar to said molecular mimicry, and in PDC-E2 163–176-reactive T cells, there are some that react with several homologous antigens of the antinuclear antibodies that become positive in PBC, including gp210-derived peptides [35]. That is, it is surmised that target antigens may spread from 2-OADC to nuclear antigens (intermolecular epitope spreading).

12.7 Conclusion

Regarding PBC, which is an organ-specific autoimmune disease, it is believed that the complicated accumulation of genetic/environmental factors, abnormalities in innate immunity, and abnormalities in acquired immunity are involved in the onset

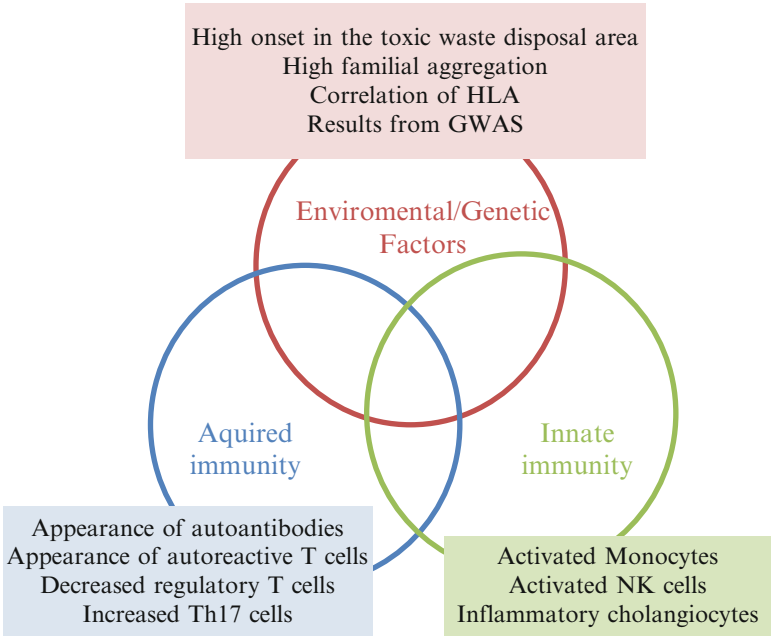

Fig. 12.2 Summary of PBC onset/maintenance of pathophysiology

and maintenance of pathophysiology, as shown in Fig. 12.2. It is believed that clarifying which abnormality is the strongest for each case and progress shall lead to the development of new drugs towards PBC treatment other than UDCA and fibrate formulation.

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Chapter 13
Genetic Factors in the Pathogenesis of Primary Biliary Cirrhosis

Minoru Nakamura

Abstract High concordance rates in monozygotic twins and familial clustering of patients indicate the involvement of strong genetic factors in the development of primary biliary cirrhosis (PBC). A recent genome-wide association study (GWAS) and a subsequent replication study in the Japanese population identified two new non-HLA susceptibility loci (*TNFSF15* and *POU2AF1*) for PBC ($P < 5 \times 10^{-8}$). In addition, *CD80*, *IL7R*, and *IKZF3-ORMDL3* were replicated as disease susceptibility genes at a GWAS significance level of $P < 5 \times 10^{-8}$. Out of 21 non-HLA susceptibility loci for PBC identified in individuals of European descent, six other loci (*DENND1B*, *STAT4*, *NFkB1*, *CXCR5*, *TNFAIP2*, and *MAP3k7Ip1*) were replicated at $P < 0.05$. These results indicated that although there are some ethnic differences in disease susceptibility loci for PBC, there are several important common disease pathways such as Th1/Th17 differentiation of T cells (CD80, IL-12A, IL-12RB2, STAT4, and TNFSF15) and B cell differentiation into plasma cells (IL7R, CXCR5, SPIB, IKZF3, and POU2AF1) between individuals of European descent and Japanese individuals. Most of these disease susceptibility genes are not specific for PBC; they are shared with various other autoimmune diseases. Further studies of environmental factors as well as gene–environment and gene–gene interactions are expected to reveal the genetic and environmental factors underlying the pathogenesis of PBC in Japanese individuals.

Keywords Disease pathway • Disease susceptibility gene • Genome-wide association study (GWAS) • Primary biliary cirrhosis (PBC) • Single nucleotide polymorphism (SNP)

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13.1 Introduction

Primary biliary cirrhosis (PBC) is a chronic and progressive cholestatic liver disease, presumably caused by autoimmune reactions against biliary epithelial cells, which lead to liver cirrhosis and liver failure [1, 2]. A higher concordance rate in monozygotic twins compared to dizygotic twins and familial clustering of PBC patients indicate the involvement of strong genetic factors in the development of PBC [3]. Although many single nucleotide polymorphisms (SNPs) associated with susceptibility to PBC have been previously identified using candidate gene methods, most of these findings have not been replicated in a distinct cohort consisting of patients of the same or different ethnicities. Only HLA haplotypes have been consistently reported to be associated with PBC [4–6]. Based on the recent progress of genome-wide association studies (GWAS), many robust candidate genes associated with PBC susceptibility have been identified in patients of European descent and in the Japanese population [7–14], which has markedly expanded our knowledge of the genetic architecture of PBC. However, our understanding of environmental factors and "missing heritability" remains mostly rudimentary.

In the present article, we summarize the results of GWAS for PBC in patients of European descent and in the Japanese population, and discuss the current understanding of disease pathways in PBC that may represent possible therapeutic targets.

13.2 Genes Associated with PBC Susceptibility Identified by GWAS in Individuals of European Descent

The first GWAS for PBC was performed in a Canadian cohort by Hirschfield et al. in 2009 [7] (Table 13.1). They identified three susceptibility loci, *HLA*, *IL12A*, and *IL12RB2*, as being strongly associated with a diagnosis of PBC at a GWAS significance level of $P < 5 \times 10^{-8}$. A subsequent GWAS and meta-analysis confirmed or identified 21 non-HLA susceptibility loci (*IL12A*, *IL12RB2*, *STAT4*, *IRF5*, *IKZF3*, *MMEL1*, *SPIB*, *DENND1B*, *CD80*, *IL7R*, *CXCR5*, *TNFRSF1A*, *CLEC16A*, *NFKB*, *RAD51L1*, *MAP3K7IP1*, *PLCL2*, *RPS6KA4*, *TNFAIP2*, 7p14, and 16q24) for PBC in individuals of European descent [7–10]. Subsequently, additional non-HLA susceptibility loci (*SOCS1*, *SIAE*, *TYK2*, *SH2B3*, *MAPT*, and *TNFSF11*) for PBC were identified by immunochip analysis in individuals of European descent [12–14].

Table 13.1 List of genes associated with susceptibility to primary biliary cirrhosis

Locus	Candidate gene[a]	Function	Odds ratio	*P* value	Population[b]	Associated autoimmune diseases[c]
1p36	*MMEL1*, *TNFRSF14*	Membrane metallo-endopeptidase-like 1, TNF signaling	1.33	3.15E−08	European	MS, CD, UC, RA, AITD, PSC
1p31.2	*IL12RB2*	IL-12 signaling, Th1 differentiation	1.61	2.47E−38	European	–
1q31.3	*DENND1B*	Guanine exchange factors (GEFs) for RAB35, phagocytosis	1.28	4.29E−12	(Japanese), European	CD
2q32.2	*STAT4*	IL-12 signaling, Th1 differentiation	1.62	2.59E−18	(Japanese), European	CeD, RA, DM, SLE, SSc
3p24.3	*PLCL2*	B cell receptor signaling	1.20	2.28E−08	European	MS
3q13.3	*CD80*	T cell co-stimulation	1.39	6.84E−16	Japanese, European	MS, CeD, VIT
3q25.33	*IL12A*, *SCHIP1*	IL-12 signaling, Th1 differentiation	1.35	3.92E−22	European	MS, CeD
4q24	*NFKB1*	Transcriptional regulation of many genes	1.26	8.48E−14	(Japanese), European	MS, UC
5p13	*IL7R*	Differentiation of B cells and T cells	1.30	2.26E−13	Japanese, European	MS, UC
6p21	*HLA* region	Antigen presentation	1.57	1.30E−48	Japanese, European	all autoimmune diseases
7p14.1	*ELMO1*	Phagocytosis, cell mobility, apoptosis	1.25	4.44E−08	European	MS, CD, PS
7q32	*IRF5*	TLR signaling	1.52	6.52E−22	European	UC, RA, SLE, SSc
9p32	*TNFSF15*	Co-stimulation of T cells, Th1 and Th17 cell proliferation	1.56	2.84E−14	Japanese	UC, CD, AS
11q13	*RPS6KA4*	Suppression of cytokine production via TLR signaling	1.23	2.06E−10	European	MS, CD, PS, SARC
11q23.3	*CXCR5*	BLC receptor, migration and adhesion of lymphocytes	1.39	7.20E−16	(Japanese), European	MS, CeD, SLE, VIT
11q23.1	*POU2AF1*	B cell differentiation, Th17 differentiation	1.39	2.38E−08	Japanese	CeD
12p13.2	*TNFRSF1A*	TNFα-NFkB signaling, apoptosis	1.27	1.18E−14	European	MS
12q24	*SH2B3*	Negative regulation of cytokine signaling	1.20	2.87E−09	European	CeD, RA, DM, VIT, AITD, PSC
13q14	*TNFSF11*(*RANKL*)	Osteoclast differentiation, regulation of apoptosis	1.33	2.18E−08	European	CD
14q24	*RAD51L1*	DNA repair	1.26	9.95E−11	European	–

(continued)

Table 13.1 (continued)

Locus	Candidate gene[a]	Function	Odds ratio	*P* value	Population[b]	Associated autoimmune diseases[c]
14q32	*TNFAIP2*	TNFα-induced protein 2	1.22	2.61E−13	(Japanese), European	–
16p13.13	*CLEC16A*, *SOCS1*	C-type lectin containing family, immunoregulation	1.29	2.19E−13	European	MS, UC, DM
16q24.1	*IRF8*	Suppression of Th17 cell differentiation	1.26	1.41E−09	European	MS, UC, RA, SSc
17q12	*IKZF3-ORMDL3*	Differentiation or apoptosis of B cells and epithelial cells	1.26	6.05E−14	Japanese, European	UC, CD, RA, DM
17q21.1	*MAPT*, *CRHR1*	Microtubule-associated protein tau, corticotropin-releasing hormone receptor 1	1.25	2.15E−09	European	–
19p13.2	*TYK2*	JAK family; IFNα, IL-6, IL-10, and IL-12 signaling	1.91	1.23E−12	European	MS, CD, RA, DM, SLE, PS
19q13.3	*SPIB*	B cell differentiation	1.46	7.97E−11	European	–
22q13.1	*MAP3K7IP1*	IL-1/TLR-NFkB signaling, TGFβ signaling	1.29	1.29E−13	(Japanese), European	CD

[a]One to two candidate genes are shown when multiple candidate genes are located at the same chromosome locus

[b]Population in whom a disease susceptibility gene ($P < 5 \times 10^{-8}$) was reported; parentheses indicate the association was replicated at significance level of $P < 0.05$

[c]*AITD* autoimmune thyroid disease, *AS* ankylosing spondylitis, *CD* Crohn's disease, *CeD* celiac disease, *DM* diabetes mellitus type 1, *MS* multiple sclerosis, *PS* psoriasis, *PSC* primary sclerosing cholangitis, *RA* rheumatoid arthritis, *SARC* sarcoidosis, *SLE* systemic lupus erythematosus, *SSc* systemic sclerosis, *UC* ulcerative colitis, *VIT* vitiligo

13.3 Identification of Genes Associated with PBC Susceptibility by GWAS in the Japanese Population

In a GWAS of PBC in the Japanese population, 1,015 samples (515 Japanese PBC patients and 500 Japanese healthy controls) were genotyped for 600,000 SNPs and data from 487 PBC patients and 476 healthy controls on 420,928 SNPs were used in the association analysis [11]. Figure 13.1 shows a genome-wide view of the single-point association data based on allele frequencies (Manhattan plot). The *HLA-DQB1* locus showed the strongest association with susceptibility to PBC (rs9275175, OR = 1.94, $P = 8.30 \times 10^{-13}$). The *TNFSF15* and *POU2AF1* loci showed evidence of association with PBC (*TNFSF15* rs4979462-T: OR = 1.63, $P = 1.21 \times 10^{-7}$; *POU2AF1* rs4938534-A, OR = 1.53, $P = 3.51 \times 10^{-6}$). In a subsequent replication study using an independent set of 1,402 and the original set of 963 samples (1,274 PBC patients and 1,091 healthy controls), the strongest associations were replicated for *TNFSF15* rs4979462-T (OR = 1.56, $P = 2.84 \times 10^{-14}$) and *POU2AF1* rs4938534-A (OR = 1.39, $P = 2.38 \times 10^{-8}$) [11] (Table 13.1).

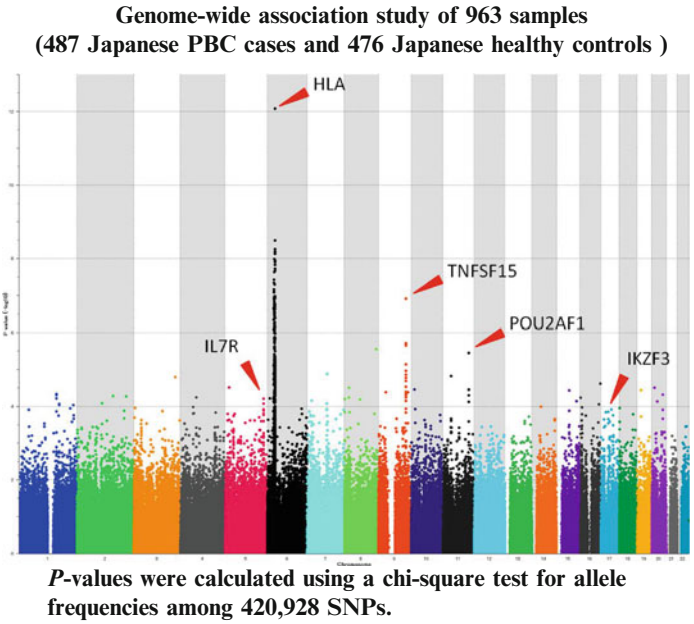

Fig. 13.1 Manhattan plot of 963 samples (487 Japanese PBC patients and 476 Japanese healthy controls). In addition to *HLA*, the most significant disease susceptibility loci in PBC ($P = 8.3 \times 10^{-13}$), *TNFSF15* and *POU2AF1* showed evidence of association with PBC ($P = 1.21 \times 10^{-7}$ and $P = 3.51 \times 10^{-6}$, respectively). In a combined study of the Japanese population, *TNFSF15* (OR 1.56, $P = 2.84 \times 10^{-14}$), *POU2AF1* (OR 1.39, $P = 2.38 \times 10^{-8}$), *IL7R* (OR 1.47, $P = 3.66 \times 10^{-8}$), *IKZF3* (OR 1.44, $P = 3.66 \times 10^{-9}$), and *CD80* (OR 1.48, $P = 3.04 \times 10^{-9}$) showed significant associations ($P < 5 \times 10^{-8}$) and *STAT4* (OR 1.35, $P = 1.11 \times 10^{-6}$) and *NFkB1* (OR 1.35, $P = 1.42 \times 10^{-7}$) showed a suggestive association

Next, we focused on the 21 loci that were reported to be associated with susceptibility to PBC in individuals of European descent [7–10]. The *IL7R* rs6890853 and *IKZF3* rs9303277 loci showed significant association (OR = 1.47, $P = 3.66 \times 10^{-8}$, and OR = 1.44, $P = 3.66 \times 10^{-9}$, respectively). *STAT4* rs7574865 showed a suggestive association with PBC (OR = 1.35, $P = 1.11 \times 10^{-6}$) (Table 13.1). Genotyping of 16 additional SNPs identified in previous studies [7–10] revealed that six SNPs located in *CXCR5*, *NFKB1*, *CD80*, *DENND1B*, *MAP3K7IP1*, and *TNFAIP2* were replicated ($P < 0.05$) in these 2,365 Japanese samples (Table 13.1). The *CD80* rs2293370 SNP showed a significant association with PBC in the Japanese population (OR = 1.48, $P = 3.04 \times 10^{-9}$), as well as *NFKB1* rs7665090 (OR = 1.35, $P = 1.42 \times 10^{-7}$). The remaining ten loci (*RAD51L1*, *PLCL2*, *IL12RB2*, *IRF5*, *SPIB*, *RPS6KA4*, *CLEC16A*, *TNFRSF1A*, *IL12A*, and *MMEL1*) did not show significant associations ($P < 0.05$) with PBC in the Japanese population (Table 13.1).

13.4 Disease Pathways in PBC

13.4.1 IL-12 Signaling Pathways (Th1 Pathway) (Fig. 13.2)

IL12A and *IL12RB2* are the strongest non-HLA susceptibility loci for PBC in the European population. This pathway for Th1 cell differentiation via IL-12/IL-12R is connected to the signaling pathway from TLR to IL-12 production in antigen-presenting cells (in which *CD80*, *IRF8*, *IRF5*, *NFkB1*, and *SOCS1* are identified as susceptibility genes for PBC in individuals of European descent) and from IL-12/IL-12R to IFNγ production in Th1 cells (in which *Tyk2*, *STAT4*, and *SOCS1* are identified as susceptibility genes for PBC in individuals of European descent). IFNγ production in turn inhibits IL-23-driven induction of IL-17-producing Th17 cells.

13.4.2 TNFSF15 and Pathways of T Cell Differentiation to Th1/Th17 (Fig. 13.2)

As described above, the Th1 cell differentiation pathway involves the activation of antigen-presenting cells via TLR stimulation followed by the production of IL-12 and stimulation of T cells by IL-12 along with co-stimulation by CD80. IL-12/IL-12R signaling is transduced via JAK2 and STAT4 in Th1-committed cells. In this pathway, TNFSF15 acts as a co-stimulator of Th1 and Th17 cell proliferation. In these pathways of Th17 differentiation, *NFkB1*, *CD80*, and *TNFSF15* have been identified as susceptibility genes for PBC in the Japanese population.

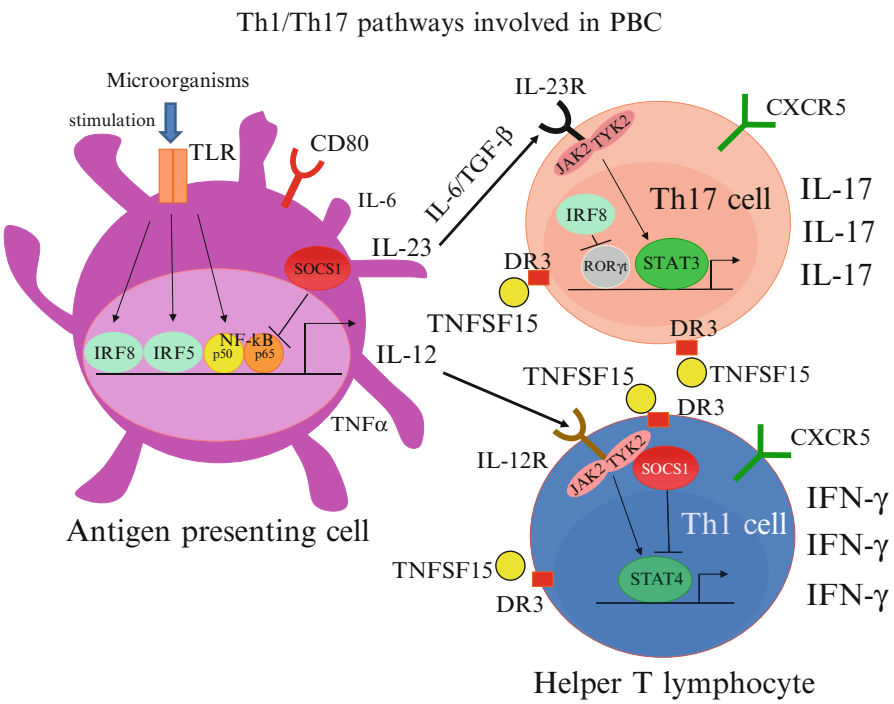

Fig. 13.2 Pathway of T lymphocyte differentiation into Th1 and Th17 cells in PBC. *IL12A* and *IL12RB2*, the most significant disease susceptibility genes for PBC in the European population, play a critical role in the differentiation of T cells into Th1 cells. Other susceptibility genes in European populations, which are shown in this figure (*IRF5*, *IRF8*, *NFkB1*, *SOCS1*, *CD80*, *TYK2*, *STAT4*, and *CXCR5*), also play important roles in Th1 differentiation. *TNFSF15*, the most significant disease susceptibility gene for PBC in the Japanese population, plays a role in both Th1 and Th17 differentiation. Other susceptibility genes in the Japanese population (*NFkB1*, *CD80*, *STAT4*, and *CXCR5*) are shown in this figure

13.4.3 Pathway of B Cell Differentiation into Plasma Cells

As shown in Fig. 13.3, IL-7R is involved in the differentiation and maturation of lymphocytes in the bone marrow [15]. Naïve B lymphocytes migrate to germinal centers of secondary lymph nodes where antigen-driven differentiation of B cells into plasma cells is initiated with the help of T follicular helper cells (Tfh). CXCR5 is essential for the migration of Tfh into the germinal center [16]. In these pathways, *IL7R* and *CXCR5* are identified as susceptibility genes for PBC in individuals of European descent as well as in the Japanese population (Table 13.1).

POU2AF1 is a B cell-specific transcriptional factor that is essential for B cell maturation and germinal center formation [17]. The E-twenty-six (Ets) transcription factor SPIB is also an essential mediator of B cell receptor signaling [18]. SPIB was recently identified as a direct target of the coactivator POU2AF1 [17],

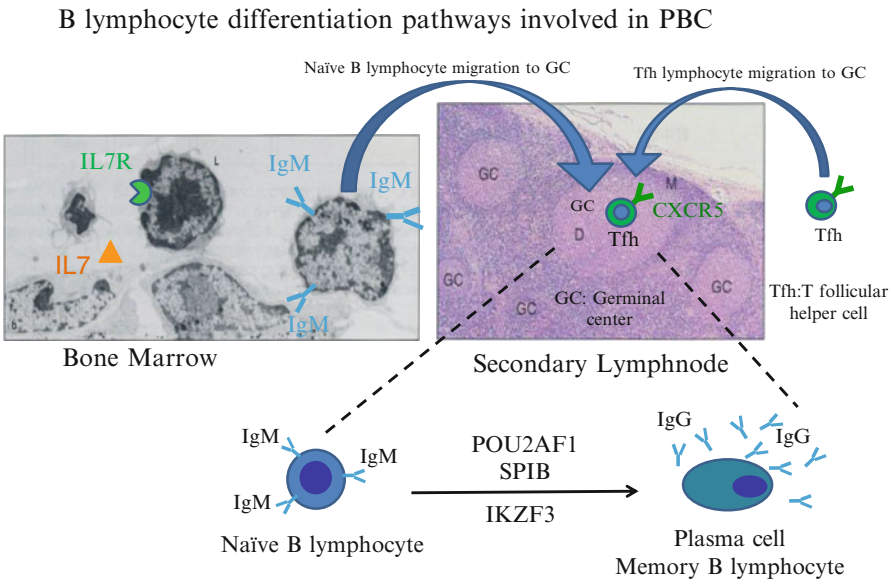

Fig. 13.3 B lymphocyte differentiation and *POU2AF1*. *POU2AF1*, a significant disease susceptibility gene for PBC in the Japanese population, plays a role in the differentiation of B cells into plasma cells. *SPIB*, a significant disease susceptibility gene for PBC in individuals of European descent, also plays a role in the differentiation of B cells into plasma cells. *IL7R*, *CXCR5*, *and IKZF3*, which are significant disease susceptibility genes for PBC in both individuals of European descent and the Japanese population, are involved in the maturation of naïve B cells in the bone marrow, migration of T follicular helper cells to the germinal centers of secondary lymph nodes, and B cell maturation into plasma cells, respectively

indicating the essential role of SPIB/POU2AF1 in the differentiation of B cells into plasma cells. IKZF3 is a transcription factor that participates in the generation of high-affinity bone marrow plasma cells responsible for long-term immunity [19]. In these pathways, *IKZF3* has been identified as a PBC susceptibility gene in both the Japanese population and individuals of European descent. *POU2AF1* and *SPIB* are identified as PBC susceptibility genes in the Japanese population and individuals of European descent, respectively (Table 13.1).

13.5 TL1A in the Pathogenesis of PBC

Aiba et al. studied the systemic and local expression of TL1A in 110 PBC patients and 46 healthy controls [20]. Serum TL1A levels were significantly higher in PBC patients with both early- and late-stage diseases compared to healthy controls. Levels were significantly reduced in early-stage PBC patients after ursodeoxycholic acid (UDCA) treatment (Fig. 13.4). TL1A was immunohistochemically localized to biliary epithelial cells, Kupffer cells, blood vessels, and mononuclear cells

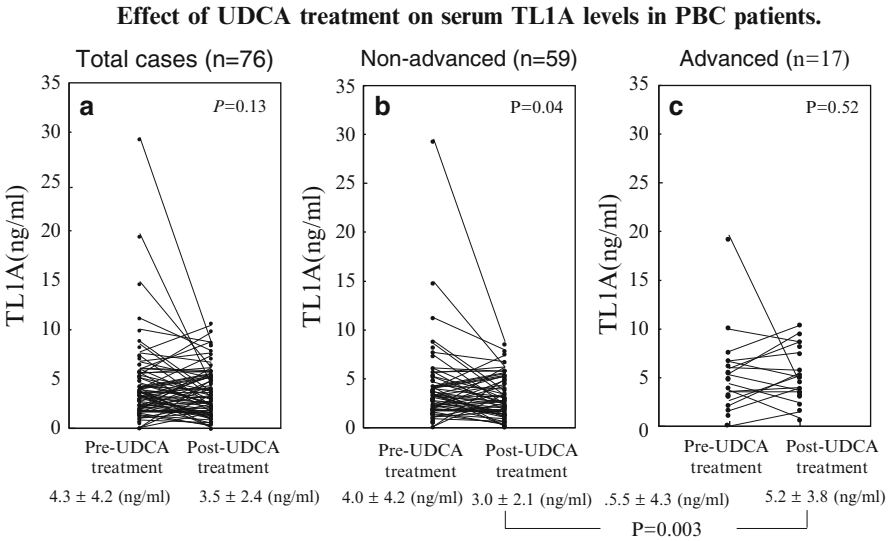

Fig. 13.4 Effect of UDCA treatment on serum TL1A levels in PBC patients. In PBC patients ($n = 76$), serum TL1A levels tended to be lower after UDCA treatment (pretreatment, 4.5 ± 4.5 ng/mL; post, 3.6 ± 2.5 ng/mL) (**a**). Serum TL1A levels were significantly lower in early-stage PBC patients ($n = 60$) after UDCA treatment (pretreatment, 4.0 ± 4.2 ng/mL; post, 3.0 ± 2.1 ng/mL) (**b**) but not in late-stage PBC patients ($n = 16$) (pretreatment, 5.5 ± 4.3 ng/mL; post, 5.2 ± 2.3 ng/mL) (**c**). Statistical analysis was performed using a two-tailed Wilcoxon's single-rank test or Mann–Whitney's *U* test

infiltrating the liver in PBC patients. In addition, TL1A messenger RNA expression was higher in liver tissue from patients with PBC compared to non-diseased liver tissue. These results indicate that TL1A may play an important role in the pathogenesis of PBC [20].

13.6 Discussion

Several GWAS have been performed in patients with PBC and controls from North America, Italy, the United Kingdom, and Japan [7–14]. Twenty-seven non-HLA genes associated with PBC susceptibility have been identified at a GWAS significance level of $P < 5 \times 10^{-8}$ (Table 13.1). Many of these PBC-related variants have also been identified as disease susceptibility genes in GWAS of other immune-related diseases including autoimmune thyroid disease (AITD), ankylosing spondylitis (AS), Crohn's disease (CD), celiac disease (CeD), diabetes mellitus type 1 (DM), multiple sclerosis (MS), psoriasis (PS), primary sclerosing cholangitis (PSC), rheumatoid arthritis (RA), sarcoidosis (SARC), systemic lupus erythematosus (SLE), systemic sclerosis (SSc), ulcerative colitis (UC), and vitiligo (VIT), with a different profile of disease-specific risk contributing to the

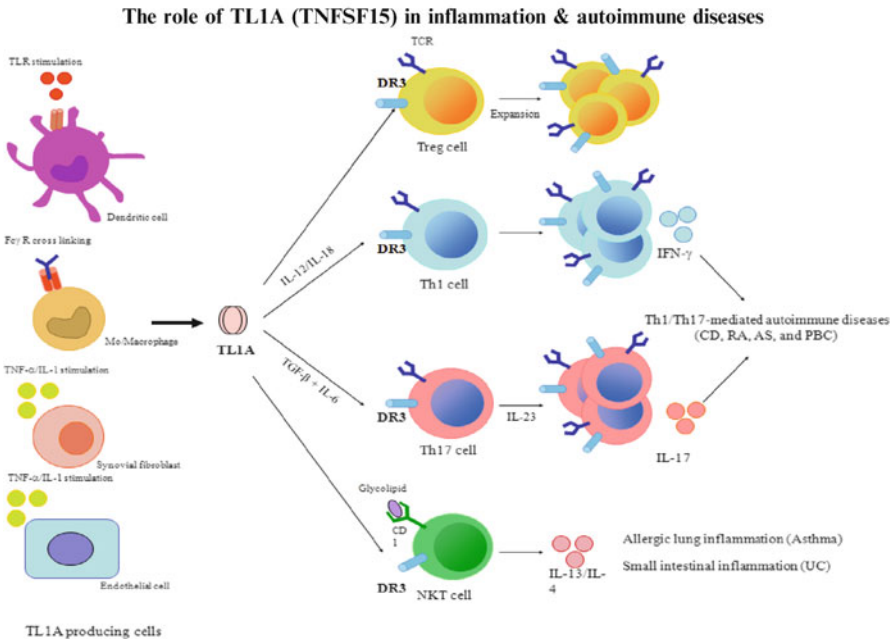

Fig. 13.5 Role of TL1A (TNFSF15) in inflammation and autoimmune disease. TL1A plays an important role in the maintenance of local inflammation by connecting innate to adaptive immune response in the differentiation and expansion of Th1/Th17 cells

pathogenesis of PBC (Table 13.1). These results indicate the presence of shared autoimmune pathways among these autoimmune diseases, instead of disease-specific immune pathways [21, 22]. Nevertheless, GWAS has markedly expanded our knowledge of the genetic architecture of PBC, which may possibly lead to the identification of molecular therapeutic targets for PBC [21–24].

GWAS in individuals of European descent strongly indicates the importance of the IL-12/IL-12R signaling pathway for Th1 differentiation in PBC, since variants of *IL12A* and *IL12RB2* have been identified as the strongest non-HLA loci associated with PBC susceptibility in this population [23, 24]. Variants of the TLR signaling pathway (*IRF8*, *IRF5*, and *NFkB1*) and the IL-12/IL-12R signaling pathway (*Tyk2* and *STAT4*) are also involved in Th1 differentiation. Pilot studies are now under way to test the safety and efficacy of the human monoclonal anti-IL-12/IL-23 (i.e., anti-p40) antibody ustekinumab in patients with PBC [21].

GWAS in the Japanese population strongly indicates the importance of TNFSF15 and POU2AF1 in the pathogenesis of PBC [11, 25, 26]. TNFSF15 (also known as TL1A) is a cytokine in the TNF superfamily produced mainly by dendritic cells (DC) and macrophages upon stimulation by TLR ligands (Figs. 13.2 and 13.5). TNFSF15 interacts with death receptor 3 (DR3, also known as TNFRSF25) not only to promote effector T cell expansion (i.e., Th1 and Th17

cells) and cytokine production (i.e., interferon-γ and IL-17) at sites of inflammation but also to induce apoptosis in cells overexpressing DR3 [27]. Increased levels of serum TL1A in untreated PBC patients that are reduced after UDCA treatment, immunohistochemical localization of TL1A to biliary epithelial cells, and increase TL1A messenger RNA expression in the PBC liver all suggest that TL1A plays an important role in the pathogenesis of PBC [20]. In addition, genetic polymorphisms in *TNFSF15* are also associated with susceptibility to other inflammatory diseases including CD, UC, ankylosing spondylitis, and leprosy (Table 13.1) [28–32]. *TNFSF15* is the strongest disease susceptibility gene for PBC as well as CD in the Japanese population [28]. These results indicate the presence of a shared disease pathway between CD and PBC in a Japanese population-specific manner.

POU2AF1 was recently reported to promote Th17 differentiation by blocking IL-2, a known endogenous repressor of Th17 cells [33]. Pathway-based analysis of GWAS recently revealed the involvement of the phosphatidylinositol signaling pathway in the development of PBC [34]. Recent studies on the molecular mechanisms of Th17 cell development and function have revealed that PI3K may play a role in the differentiation of Th17 cells [35]. Taken together, these results again indicate the importance of the Th17 differentiation pathway in the pathogenesis of PBC.

13.7 Conclusion

GWAS of PBC have revealed that other than HLA, the most significant disease susceptibility genes for PBC are *IL12A/IL12RB2* and *TNFSF15* in individuals of European descent and the Japanese population, respectively, indicating the importance of Th1/Th17 pathways in the pathogenesis of PBC [23, 26, 33, 35]. In addition, the genetic architecture of PBC is similar to those of other autoimmune diseases (i.e., a strong HLA association accompanied by other groups of loci associated with inflammatory risk).

The primary objective of GWAS is to identify common genetic variations associated with traits or diseases in an unbiased fashion. The process is based on the principle of linkage disequilibrium and the assumption that disease risk is associated with relatively common variants (allele frequency >2 to 5 %). GWAS have been very successful in identifying numerous genes associated with various traits or diseases; however, there are limitations in the analysis of "missing heritability" (i.e., rare variants, epigenetics, epistasis, etc.) [36]. The study of environmental factors including meta-genome analysis (microbiomes) is another important area to be investigated. The huge amounts of data from GWAS would be very useful for investigating gene–environment and gene–gene interactions in the post-GWAS era to come [37].

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Chapter 14
Animal Models for Primary Biliary Cirrhosis

Yuki Moritoki and Yoshiyuki Ueno

Abstract Recent PBC mouse models underwent extensive gene modifications and immunomodulations to elucidate functions of their products and signaling pathway in autoimmune cholangitis. Expanded hepatic memory $CD8^+$ T cells and elevated T helper type 1 and proinflammatory cytokines are the commonly observed in these models. B cell depletion studies revealed opposite B cell functions in the time/disease course of dnTGF-βRII mice. NKT cells positively contributed to the development of autoimmune cholangitis in the early phase in both dnTGF-βRII mice and 2-octynoic acid-conjugated BSA (2OA-BSA)-immunized mice models. Further, the depletion of the IL-12p40 subunit, comprising cytokines IL-12 and IL-23, significantly ameliorated PBC-like liver disease. In contrast, IL-12p35 depletion delayed but similarly developed cholangiopathy, but led to liver fibrosis in dnTGF-βRII mice. Inhibitory signaling, derived from exogenous costimulatory immunoreceptor-conjugated immunoglobulin, i.e., cytotoxic T lymphocyte antigen 4 (CTLA-4)-Ig, was quite efficient to improve developed cholangitis in 2OA-BSA-immunized mice. These results highlighted the pivotal roles of T cells, especially $CD8^+$ T cells, the regulatory and proinflammatory opposite functions of B cells, the indispensable role of IL-12p40 to develop cholangiopathy, and the negative regulatory roles of IL-12p35 and CTLA-4-Ig to suppress liver fibrosis and bile duct damage, respectively. These studies emphasize that failure to maintain self-tolerance in $CD8^+$ T cells and the expansion of these cells induce and promote

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inflammatory responses in autoimmune cholangitis. Further characterization of these models will support to elucidate the pathogenesis of PBC. This chapter summarizes the recent progress of understandings about the pathogenesis and pathophysiology of autoimmune cholangitis in the context of the PBC mouse model.

Keywords $CD8^+$ T cells • CTLA-4 • Liver fibrosis • Mouse model • Primary biliary cirrhosis

14.1 Introduction

Interlobular bile ducts are the primary targets in primary biliary cirrhosis (PBC), of which the term was first introduced more than six decades ago [1]. A certain condition comprising genetics, epigenetics, and environmental factors is thought to affect susceptible individuals, resulting in the development of PBC [2–4]. Compatible for this concept, some of the congenic, transgenic, gene-deleted (knocked-out), and chemical xenobiotic-immunized mice have been reported as faithful animal models with accompanying close similarity of features seen in human PBC. In addition to the enthusiastic researches on human PBC, these animals have advanced our understandings of the pathogenesis of PBC.

Five spontaneous models and one faithfully induced mouse model have been reported for human PBC in a decade [5–11]. These models overcome the limitation to access human liver tissues and have enabled to elucidate the pathogenesis of PBC in detail (i.e., contribution of each immune cell population, cholangiocytes, humoral immune components, cytokines/chemokines, and their receptors). Recent findings of PBC mouse models are summarized and compared to the features of PBC (Table 14.1).

14.2 dnTGF-βRII Mice

Transforming growth factor (TGF)-β mediates pleiotropic functions on various cells and plays a role in the central negative regulation in autoimmunity. TGF-β receptor II is essential for signal transduction of TGF-β that regulates the activation of lymphocytes. The mouse expressing the dominant-negative form of TGF-β receptor II (dnTGF-βRII) on $CD4^+$ and $CD8^+$ cells demonstrates closely resembling features of human PBC [5, 57]. dnTGF-βRII has a truncated intracellular domain of the normal receptor, resulting in the incapacity to transduce signal in both $CD4^+$ and $CD8^+$ cells after TGF-β ligation. Although anti-mitochondrial antibodies (AMAs) are not sufficient to assure specific models of PBC [58, 59], these mice demonstrate 100 % of serum AMA positivity in a time-dependent manner, and those against PDC-E2, BDOADC-E2, and OGDC-E2 inhibit its enzymatic activity in vitro. In addition, antinuclear antibodies directed against two nuclear proteins, gp210 and

Table 14.1 Comparison between human PBC and the mouse models

Index	PBC	dnTGF-βRII	IL-2Ra$^{-/-}$	NOD.c3c4	Ae2a,b$^{-/-}$	Scurfy	2OA-BSA$_{ip}$
Species	Human	Mice	Mice	Mice	Mice	Mice	Mice
Classification	N/A	Spontaneous	Spontaneous	Spontaneous	Spontaneous	Spontaneous	Induced
Background strain	N/A	C57BL/6	C57BL/6	NOD congenic	FVB/N, 129/Sv, Balb/c, SJL	C57BL/6	C57BL/6, NOD congenic
Clinical features							
Female/male ratio	9:1	1:1	1:1	1:1	1:1	(X/X females: fatal)	1:1
Age of onset	40–60 years	?	?	?	?	?	?
Genetic contribution	60 % concordance, identical twin	1	1	1	1	1	1
Environment factor	Yes	Yes	?	?	?	?	?
Serum biochemistry							
Alkaline phosphatase	+++	?	?	?	++	?	?
B cell immunity							
Immunoglobulins	IgM +−+++, IgG +	IgM +, IgA +++, IgG +	IgA +++, IgG +	IgM +, IgA ++	IgM ++, IgG ++	IgM +++, IgA ++, IgG +++	?
AMA	90–95 %	100 %	100 %	50–60 %	40–80 %	100 %	100 %
Dominant AMA target protein	PDC-E2	PDC-E2	PDC-E2	PDC-E2	PDC-E2	PDC-E2	PDC-E2
Dominant epitope	Lipoyl domain	Lipoyl domain	Lipoyl domain	Lipoyl domain	Lipoyl domain	Lipoyl domain	Lipoyl domain
Reactivity to 2OA-modified PDC-E2	+++	?	?	?	?	?	?
ANA	40–50 %	100 %	80 %	80–90 %	?	?	?
Liver histology							
Portal lymphoid infiltrates	+++	+++	+++	+++	+−+++	++−+++	+
CD4 cell	+	+	+	++	+	+	+
CD8 cell	++	++	++	+	++	++	++
B cell	+	+	+	−	+	+	+

(continued)

Table 14.1 (continued)

Index	PBC	dnTGF-βRII	IL-2Ra$^{-/-}$	NOD.c3c4	Ae2a,b$^{-/-}$	Scurfy	2OA-BSA$_{ip}$
Granuloma	+−++	−	−	+	?	−	+
Eosinophilia	+	−	−	+	+	(+)	−
Bile duct destruction	+−+++	+−++	+−++	+	+−+++	+−++	+
AE2 on cholangiocytes	↓	?	?	?	−	?	?
Liver fibrosis	−−+++	− (++ in IL-12p35$^{-/-}$)	−	−−±	−−±	−	−−± (+by poly I:C, ++ by α-GalCer,)
Cytokines/chemokines							
IFN-g	↑	↑	↑	(↑)	↑	↑	↑
TNF-α	↑	↑	↑	?	NC	↑	↑
IL-12	↑	↑	?	?	↑	↑	?
IL-12p40	−	↑	↑	?	?	↑	?
IL-23	↑	?	?	?	?	↑	?
IL-5	↑	?	?	(↑)	?	?	NC
IL-6	↑	↑	↑	?	NC	↑	?
IL-8	↑	?	?	?	NC	?	?
IL-17A	↑	↑	↑	?	?	↑	?
CXCL10 (IP-10)	↑	↑	?	?	?	?	?
Disadvantages	N/A	Moderate colitis	Severe colitis, severe hemolytic anemia	Biliary dilatation	Late onset	Short life span	Peritonitis
References	[2, 3, 12–31]	[5, 32–45]	[6, 45–47]	[7, 45, 48–50]	[8, 45]	[9, 45]	[10, 11, 45, 51–56]

2OA 2-Octynoic acid, *Ae* anion exchanger, *α-GalCer* α-galactosylceramide, *AMA* anti-mitochondrial antibody, *ANA* antinuclear antibody, *NC* no change

sp100, were detectable in all examined sera out of 21 dnTGF-βRII mice at 24 weeks of age [41]. Liver histology demonstrates lymphoid cell infiltrates in portal tracts accompanied with bile duct injury [5]. dnTGF-βRII mice show an increase in the number and frequency of the $CD44^+$ memory phenotype of $CD4^+$ and $CD8^+$ T cells, a decreased $CD4^+/CD8^+$ T cell ratio in liver lymphoid cell infiltrates, and an increment of B cells and natural killer T (NKT) cells in the liver [5]. In addition, serum levels of proinflammatory cytokines such as TNF-α, IFN-γ, IL-12p40, and IL-6 are significantly higher in this strain than in control mice.

14.2.1 T Cell Contribution in dnTGF-βRII Mice

To examine the contribution of T and B cells to the development of PBC-like disease, dnTGF-βRII mice were crossed with recombinase-deficient $Rag1^{-/-}$ mice that lack a diversified B and T cell receptor repertoire, leading to the absence of B and T cells. $Rag1^{-/-}$-dnTGF-βRII mice do not develop liver pathology, suggesting that a specific condition of T cells with impaired TGF-β-signaling in the presence or absence of B cells is involved in the pathogenesis of PBC-like disease of this mouse [5]. Thus, the pathology developed in this strain could be hypothesized due to either a structural or functional change of TGF-βRII signaling in T cells or following a breakdown of self-tolerance.

To assess the contribution of T and B cells to the development of PBC-like disease, various series of adoptive transfer studies were performed: transferring splenic $CD4^+$ and/or $CD8^+$ T cells derived from dnTGF-βRII mice into $Rag1^{-/-}$ recipients [33, 34]. Whole splenocytes in dnTGF-βRII mice were sufficient to develop features of liver disease similar to human PBC in $Rag1^{-/-}$ recipients, suggesting that the loss of self-tolerance in splenic T and B cells is sufficient to induce cholangitis, and specific abnormality in the biliary targets was dispensable for the onset of the disease, compatible to the "innocent victim" concept of cholangiocytes in human PBC [15, 60]. More importantly, adoptive transfer of $CD8^+$ but not $CD4^+$ T cells into $Rag1^{-/-}$ mice led to cholangiopathy quite similar to PBC livers, emphasizing a pivotal role of $CD8^+$ T cells in the pathogenesis of both human and murine PBCs [18, 33, 61]. In contrast, $Rag\text{-}1^{-/-}$ recipients of $CD4^+$ T cells of dnTGF-βRII mice predominantly developed inflammatory bowel disease associated with higher levels of serum interferon (IFN)-γ and tumor necrosis factor (TNF)-α. These data clarified that $CD8^+$ T cells are the primary contributors for bile duct destruction in this model [33]. Of note, breakdown of T cell self-tolerance to liver autoantigens was indispensable to develop cholangiopathy in dnTGF-βRII mice [42]. Ovalbumin (OVA)-specific $CD8^+$ T cell (OT-I) or OVA-specific $CD4^+$ T cell (OT-II) mice were utilized to develop OT-I/dnTGF-βRII/$Rag\text{-}1^{-/-}$ and OT-II/dnTGF-βRII/$Rag\text{-}1^{-/-}$ mice in which the entire T cell repertoire was substituted for OVA-specific $CD8^+$ and $CD4^+$ T cells and to examine the specificity of autoantigens in dnTGF-βRII mice. Adoptive transfer of $CD8^+$ T cells from dnTGF-βRII mice but not from OT-I/dnTGF-βRII/$Rag\text{-}1^{-/-}$ mice or

OT-I/Rag-$1^{-/-}$ mice induced cholangitis in Rag-$1^{-/-}$ recipients, confirming that the cholangiopathy is not secondarily due to nonspecifically activated $CD8^+$ T cells in dnTGF-βRII mice.

14.2.2 B Cell Contribution in dnTGF-βRII Mice

Despite the nearly universal occurrence of serum AMA as well as accumulation of B cells among liver infiltrates [62], the contribution of B cells to the pathogenesis of human PBC had remained unclear [63]. Similarly, B cell self-tolerance is abrogated in dnTGF-βRII mice [5, 41]. Thus, an expectation seemed valid that B cell deficiency should ameliorate liver disease, and dnTGF-βRII mice were crossed with B cell deficient mice (Igμ$^{-/-}$) and explored for liver inflammation as well as accompanying colitis. Contrarily, genetic B cell deprivation exacerbated both the PBC-like liver disease and colitis [34]. Also, B cell deletion enhanced the expansion of the $CD8^+$ T cell population compared to $CD4^+$ T cells and diminished the hepatic regulatory T cell (Treg) frequency in the $CD4^+$ T cell population. A putative regulatory B cell (Breg) population produces anti-inflammatory cytokine, especially IL-10 [64]; however, dnTGF-βRII hepatic B cells indicated comparable levels of IL-10 mRNA expression to those in B6 mice (Moritoki Y, unpublished data), suggesting that Breg population exists outside of the liver and suppresses liver inflammation. Two major B cell pools, the peritoneal cavity and spleen, were chosen for the B cell sources to examine the B cell suppressive function in the $CD8^+$ T cell adoptive transfer model [33]. B cells from the peritoneal cavity, but not from the spleen of dnTGF-βRII mice, were sufficient to regulate $CD8^+$ T cell induced PBC-like liver disease. These findings revealed the existence of a suppressive/regulatory B cell subset on autoimmune cholangitis in the dnTGF-βRII mice and raised a new concept of regulatory B cells in liver inflammation.

However, since the role of B cells is still controversial and disease phase dependent in autoimmunity [65], the regulatory function may not be universal in B cells for liver inflammation in dnTGF-βRII mice. Hence, to examine the effects of therapeutic B cell depletion, dnTGF-βRII mice were treated by intraperitoneal injection of anti-mouse CD20 antibodies (Abs) every 2 weeks from young (4–6 weeks) and old (20–22 weeks) age and subjected to a comparison with control Ab-treated mice. Sixteen-week anti-CD20 treatment initiated from young age demonstrated a fully depleted serum AMA, a significantly lower incidence of liver inflammation, and a fewer number of activated hepatic $CD8^+$ T cells in dnTGF-βRII mice [35]. However, colitis was significantly exacerbated in anti-CD20-treated mice. In contrast, in the aged mice treated from 20 to 22 weeks of age, anti-CD20 treatment was less effective on either liver or colon inflammation.

To summarize B cell functions in dnTGF-βRII mice, in contrast to genetic B cell depletion to exacerbate liver inflammation, anti-CD20 treatment demonstrated therapeutic efficacy to regulate liver inflammation in young but not in old dnTGF-βRII mice, suggesting time- and disease phase-dependent B cell function

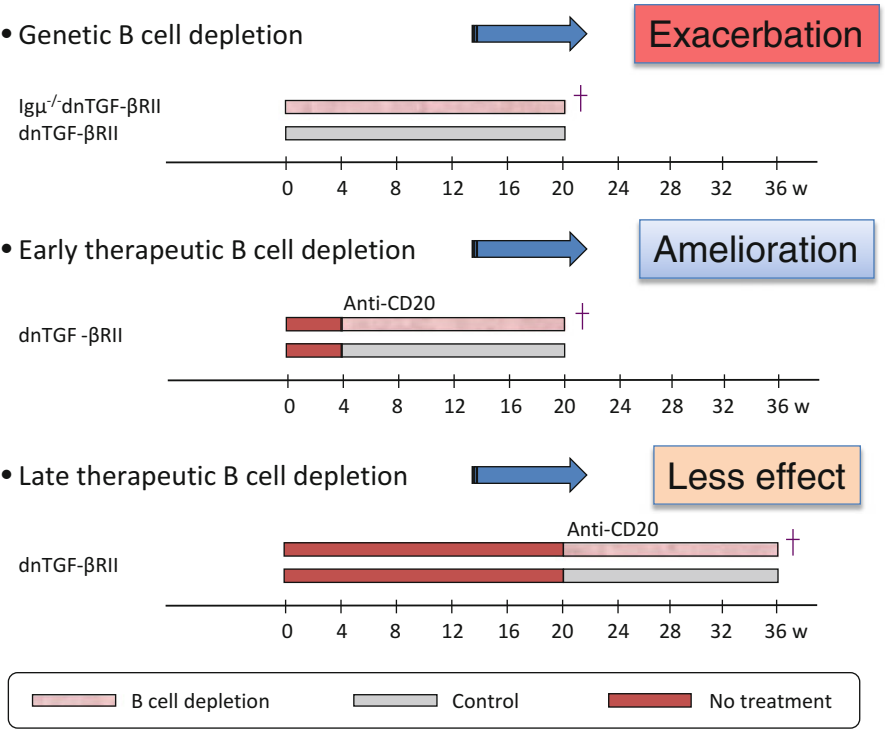

Fig. 14.1 Effect of B cell depletion in dnTGF-βRII autoimmune cholangitis. Genetic B cell depletion exacerbated autoimmune cholangitis. However, in contrast, early therapeutic B cell depletion using anti-mouse CD20 monoclonal antibodies ameliorated liver pathology. Further, late B cell depletion exhibits no significant therapeutic efficacy in liver inflammation

in autoimmune cholangitis in this strain (Fig. 14.1). Also, B cell functions for liver inflammation observed in dnTGF-βRII mice are quite similar to those recently reported in the other autoimmune disease [65]; however, B cells are collectively a suppressor population for colitis regardless of disease phase or age in dnTGF-βRII mice. These data suggest that B cells separately contribute to each target organ in autoimmunity. In addition, B cell depletion using anti-CD20 can be a possible therapeutic option for human PBC treatment due to the rare concomitance of inflammatory bowel disease. Several reports for B cell depletion using anti-CD20 rituximab in human PBC patients emerged recently [29, 30, 66, 67]. In an open-labeled study comprised of six patients with PBC, those who were refractory to UDCA treatment and administered rituximab 1,000 mg intravenous infusion on day 1 and 15 demonstrated transient decreases in memory B and T cells, an increase of the $CD25^+$ regulatory subset, an increase in mRNA levels of FoxP-3 and TGF-β, and a decrease in TNF-α in $CD4^+$ T cells. Serum levels of ALP and plasma levels of AMA, IgM, IgA, and IgG were also transiently reduced around 6 months after treatment [29]. Similarly to this, the other study with 14 PBC patients refractory to UDCA indicated a significant reduction of ALP, AMA, and IgM at 6 months after

rituximab treatment [30]. However, a case report demonstrated rapid progress in a PBC patient accompanied with coincidental gastric diffuse large B cell lymphoma (DLBCL) after eight doses of rituximab as a part of R-CHOP chemotherapy coupled with 40 Gy radiotherapy, although the patient showed biochemical, immunological, and histological improvements after rituximab treatment [67]. The other case report demonstrated persistent liver dysfunction in a PBC patient where concomitant rheumatoid arthritis was improved after treatment with 7.5 mg/week methotrexate and two doses of 1,000 mg rituximab at 2 weeks apart [66]. Taken together, the therapeutic usage of anti-CD20 seems potent to induce biochemical and immunological responses in PBC, but novel biomarkers are feasible to select adequate patients to be treated with rituximab.

14.2.3 Natural Killer T (NKT) Cell Contribution in dnTGF-βRII Mice

Natural killer T (NKT) cells bridge innate and adoptive immunity and exhibit immunoregulatory function in some autoimmune diseases [68, 69]. NKT cells are primed for proinflammatory and anti-inflammatory phenotypes under DC-derived cytokine environment such as IL-12 and IL-10, respectively [70].

The activation of invariant NKT cells is a critical factor to accelerate cholangiopathy in PBC [19, 71]. In dnTGF-βRII mice, since the absolute number and activation marker expression are augmented in TGF-β signaling-deprived CD1d-restricted NKT cells, $CD1d^{-/-}$-dnTGF-βRII mice were generated to deplete CD1d-restricted NKT cells [32]. $CD1d^{-/-}$-dnTGF-βRII mice exhibited decreased lymphoid cell infiltrates, milder biliary damage, and higher levels of serum IFN-γ compared to those of the control NKT cell sufficient $CD1d^{+/-}$-dnTGF-βRII mice. In vivo injection with α-galactosylceramide (α-GalCer) induced increases in liver cell infiltrates and serum IFN-γ in dnTGF-βRII mice. These results suggested CD1d-restricted NKT cells are a primarily proinflammatory subset inducing Th1 cytokine bias in dnTGF-βRII mice [32]. Despite the milder liver inflammation, CD1d deletion did not affect the AMA titer in this strain [32].

In the other study, anti-CD40L treatment for 8 weeks demonstrated a reduction in hepatic NKT cells and activated $CD8^+$ T cells, milder portal inflammation, and diminution of bile duct destruction in dnTGF-βRII mice [44].

14.2.4 Cytokine/Chemokine Contribution in dnTGF-βRII Mice

Since dnTGF-βRII mice demonstrated an increase in serum levels of proinflammatory cytokines TNF-α, IFN-γ, IL-12p40, and IL-6, partly due to the overexpression of microRNA-21 [5, 40], germline deletion of genes encoding

those and related cytokines was extensively performed in dnTGF-βRII mice. All four IL-12 family cytokines, IL-12, IL-23, IL-27, and IL-35, have heterodimeric constructs with α and β chains, and each cytokine shares at least one chain with another member of the family [72]. The IL-12p40 subunit is a common component of both IL-12 and IL-23, which are deprived by the deletion of the IL-12p40 subunit. IL-12p40 deletion demonstrated a marked diminution in the levels of proinflammatory Th1 cytokines including IFN-γ in livers of dnTGF-βRII mice accompanied with reductions in cellular infiltrates around portal areas and bile duct damage [36]. In contrast, IFN-γ depletion indicated no significant effect on the immunopathology of autoimmune cholangitis and colitis in this strain [36] (Yoshida K, unpublished data). These data suggest that IL-12p40 contributing signaling pathways, rather than one of its downstream cytokines IFN-γ, is a major determinant of the autoimmune cholangitis that affects dnTGF-βRII mice [36].

IL-23 comprised of IL-12p40 and IL-23p19 subunits enhances Th17 polarization in autoimmunity [73]. IL-23p19 depletion led to IL-23 deprivation, which improved colitis but not cholangitis in dnTGF-βRII mice [39]. IL-23 supports the differentiation of naïve $CD4^+$ T cells into highly pathogenic Th17 cells to produce IL-17A; however, IL-17A deletion did not affect either cholangitis or colitis in this strain [39]. IL-6 coupled with TGF-β induces Th17 cells from naïve $CD4^+$ T cells. Genetical IL-6 depletion in dnTGF-βRII mice led to a marked improvement in inflammatory bowel disease, but deteriorated biliary pathology [38]. Hepatic levels of IFN-γ and TNF-α were significantly elevated in IL-6-deficient dnTGF-βRII mice while hepatic IL-12p40 was comparable to that of IL-6-sufficient controls [38]. Taken together, the IL-12/Th1 pathway, but not coupled with IL-23/Th17 axis, is essential and sufficient to develop autoimmune cholangitis in dnTGF-βRII mice.

IL-12 heterodimeric cytokine is comprised of IL-12p40 and IL-12p35 subunits. In other words, the IL-12p35 subunit is shared in IL-12 and IL-35 coupled with an IL-12p40 subunit and an Epstein–Barr virus-induced gene 3 (EBI3) glycoprotein, respectively. IL-12p35 deletion in dnTGF-βRII mice induced a similar degree of cholangitis with delayed onset, but not colitis, while AMA titer significantly increased at 6 months of age [43]. The deficiency of IL-12 and IL-35 in the presence of IL-23 did not inhibit but delayed the development of biliary disease, suggesting that the pathogenic role of IL-23 can be enhanced in the absence of a presumably immunoregulatory cytokine IL-35 secreted from Treg [72]. Importantly, IL-12p35 deprivation led to Th1 to Th17 shift with a higher production of IL-6 and IL-17 and reduced mRNA expression of both IFN-γ/STAT1 signaling and an antifibrotic factor HGF, resulting in the development of liver fibrosis in 7 out of 13 examined mice at 24 weeks of age while none of the 14 control dnTGF-βRII mice did so. Of note, liver fibrosis is the essential feature in advanced human PBC livers but has not been previously found in spontaneous PBC mouse models [43]. IL-35 deficiency might hamper the Treg functions including the regulation of both liver pathogenesis and fibrosis. Th17 cells are developed from naïve $CD4^+$ T cells through IL-6 and TGF-β signaling; however, recent studies demonstrate that also

Table 14.2 Effects on AMA, ANA, cholangitis, and colitis in cytokine-deleted dnTGF-βRII mice

Deleted cytokine	AMA	ANA	Cholangitis	Colitis	References
Nil	++	++	+−++	+−++	[5, 36, 38, 39, 41, 43, 57]
IL-12p40$^{-/-}$	++	+	−−+	(−)[a]	[36, 41, 43], [a](Yoshida K, unpublished data)
L-12p35$^{-/-}$	+++	++	+−++, delayed	−	[41, 43]
IFN-γ$^{-/-}$	++	++	+−++	(+−++)[a]	[36, 41], [a](Yoshida K, unpublished data)
IL-6$^{-/-}$	+	±	++−+++	−	[38, 41]
IL-23p19$^{-/-}$	+++	+	+−++	±−+	[39, 41]
IL-17$^{-/-}$	++	+	+−++	+−++	[39, 41]
TNF-α$^{-/-}$	++	±	(+−++)[b]	(+++)[b]	[41], [b](Yang GX, unpublished data)

dnTGF-βRII dominant-negative form of transforming growth factor (TGF)-beta receptor type II, *IFN* interferon, *IL* interleukin, *TNF* tumor necrosis factor

Th17 cells can be induced alternatively by way of the effects of IL-6, IL-1β, and IL-23 in the absence of TGF-β [74, 75]. These "alternative" Th17 cells are thought to be more pathogenic than the "classical" Th17 cells [76]. Since the TGF-β signaling pathway is obstructed in dnTGF-βRII T cells, IL-12p35 deprivation is likely to induce more pathogenic Th17 cells.

A genome-wide association study (GWAS) identified that IL-12α (IL-12p35 subunit) and IL-12 receptor β2 gene variants were strongly associated with risk for human PBC [20, 77]. Human liver samples obtained from the patients with PBC ($n = 51$) and non-PBC ($n = 80$) control liver diseases were examined immunohistochemically for localized expression of cytokines, their subunits, and corresponding receptors with an extensive panel of antibodies directed to IL-12p70, IL-12p35, IFN-γ, IL-12RB2, IL-23p40, IL-23p19, IL-17, and IL-23R [25]. The expression of IL-12RB2 and the presence of IFN-γ-positive mononuclear cells were both observed around the damaged interlobular bile ducts in PBC livers [25].

Again, IL-12p40 depletion demonstrated significant amelioration of cholangitis in dnTGF-βRII mice, suggesting that IL-12p40 suppression serves as a novel therapeutic option for human PBC. IL-12p40 suppression mediated by monoclonal antibodies or transcription inhibitors has been clinically studied to provide therapeutic benefit in other autoimmune diseases such as psoriasis [78] and Crohn's disease [79–80].

Detection of antinuclear antibodies (ANAs) was first reported in paternal dnTGF-βRII mice [57] and, thence, extensively studied in dnTGF-βRII mice with concurrent deletions of IL-12p35, IL-12p40, IL-23p19, IL-17, IL-6, IFN-γ, and TNF-α [41]. The changes of autoantibody titers, autoimmune cholangitis and colitis are summarized in Table 14.2. IL-12p40 depletion did not affect serum levels of AMA and anti-SP100, but diminished anti-GP210, suggesting that synthesis and upregulation of IL-12p40 ais not essential for the production of AMA in this model [36, 41]. In contrast, AMA and anti-SP100 were significantly

higher, but anti-GP210 was lower in sera of IL-23p19-deleted dnTGF-βRII mice [39, 41]. IL-6 depletion reduced both AMA and anti-GP210 without any change of anti-SP100 titers in dnTGF-βRII mice [38, 41]. Further, higher levels of AMA and similar levels of anti-GP210 and anti-SP100 were detected in IL-12p35-deficient dnTGF-βRII mice [41, 43]. IL-17 depletion did not affect AMA or anti-SP100, but reduced anti-GP210 in dnTGF-βRII mice [39, 41]. IFN-γ deletion did not change titers of any AMA, anti-GP210, or anti-SP100 antibodies [41]. TNF-α depletion did not affect the degree of bile duct damage (Yang GX, unpublished data) or the titers of AMA or anti-SP100, but reduced anti-GP210 antibodies [41]. Deterioration of colitis led to shorter survival in TNF-α-deprived model mice than that of parental dnTGF-βRII mice, resulting in the difficulty of reproduction, and thus the study was discontinued (Yang GX, unpublished data). These results suggest that autoantibody-dependent cytotoxicity is not the major mechanism to induce and promote bile duct damage in the absence of these cytokines.

14.2.5 Therapeutic Immunomodulation in dnTGF-βRII Mice

Other than B cell depletion, novel therapeutic immunomodulation has been performed in dnTGF-βRII mice. First, β-glucosylceramide (GC) administration for 18 weeks from 6 weeks of age ameliorates liver inflammation and alleviated cholangitis accompanied with significant reduction of hepatic $CD8^+$ memory T cells in dnTGF-βRII mice [37]. GC is a naturally occurring glycosphingolipid and has been shown to function as a "fine-tuning factor" in several mouse models of immune-mediated disorders [81–84]. AMA is not significantly reduced in GC-treated dnTGF-βRII mice.

Second, therapeutic intraperitoneal administration of hamster anti-mouse CD40L antibodies was examined in dnTGF-βRII mice. Although anti-CD40L treatment reduced AMA titer and delayed the development of cholangitis at 12 weeks of age (after 8 weeks of treatment), however, the severity of which was not affected at 24 weeks of age (after 20 weeks of treatment) [44]. Changes in serum IgM were not indicated in anti-CD40L-treated mice while CD40L promoter methylation inversely correlates with IgM levels in patients with PBC [85]. LPS and IFN-γ stimulated human liver-derived macrophages induced apoptosis of cholangiocytes through a CD154 (CD40L)-dependent manner, which was attenuated by the antagonistic antibodies against CD154 [86]. The efficacy of blocking CD40L has been reported in the other models of systemic and organ-specific autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, and multiple sclerosis [87–90].

14.3 IL-2Rα$^{-/-}$ Mice

Interleukin-2 receptor α (IL-2Rα)$^{-/-}$ mice demonstrate low frequency but normal suppressive function of Treg [91], similar to the fact that the lower frequency and absolute number of Treg have been observed in PBC patients [92]. Also, PBC-like liver disease was observed in a child with homozygous IL-2Rα deficiency [93]. IL-2Rα$^{-/-}$ mice with C57BL/6 background show 100 % of AMA positivity against PDC-E2, 80 % of ANA positivity, lymphocyte infiltration around portal tracts, and cholangiocyte damage accompanied with intraepithelial CD8$^+$ cells in interlobular bile ducts [6]. Serum levels of IgA were markedly elevated in IL-2Rα$^{-/-}$ mice compared to those of controls [6, 46]. Serum cytokine profiles are quite similar to those of dnTGF-βRII mice, showing elevation of serum levels of TNF-α, IFN-γ, IL-12p40, IL-6, as well as IL-2. Also, similar to liver immunopathology in dnTGF-βRII mice, IL-2Rα$^{-/-}$ mice indicate an increased number and frequency of the CD44$^+$ memory phenotype of CD4$^+$ and CD8$^+$ T cells and decreased CD4/CD8 ratio of liver cellular infiltrates [6].

14.3.1 T Cell Contribution in IL-2Rα$^{-/-}$ Mice

The role of the interleukin-2 receptor α (IL-2Rα, CD25) and its relationship with Treg has been well demonstrated in murine and human autoimmune diseases such as inflammatory bowel disease as well as PBC [92–94]. Although IL-2Rα$^{-/-}$ mice develop autoimmune cholangitis concomitant with intestinal inflammation while colitis rarely coexists in patients with PBC, it was hypothesized that distinct effector mechanisms would work in selective targeting of autoimmune diseases in the colon and bile duct, and the effects of germline depletion of genes encoding CD4, CD8, or TCR (T cell receptor)-β were evaluated for the severity of colitis and autoimmune cholangitis in IL-2Rα$^{-/-}$ mice. IL-2Rα$^{-/-}$-CD4$^{-/-}$ mice, in which CD8$^+$ cells are the unique population in TCR$^+$ cells, demonstrated augmented intrahepatic biliary ductular destruction but diminished colitis. In contrast, IL-2Rα$^{-/-}$-CD8$^{-/-}$ mice lacked biliary ductular destruction but deteriorated colitis, compared with IL-2Rα$^{-/-}$ mice [46]. These results are quite similarly observed in adoptive transfer studies in dnTGF-βRII mice, where CD8$^+$ but not CD4$^+$ T cells are major contributors for biliary damage [33]. Of note, the lack of pathological changes in IL-2Rα$^{-/-}$ TCR-β$^{-/-}$ mice revealed the pivotal role of T cells to induce liver inflammation in IL-2Rα$^{-/-}$ mice, closely similar to the absence of inflammation in Rag-1$^{-/-}$-dnTGF-βRII mice [5]. Taken together, these results further argue for the key role of CD8$^+$ T cells in the pathogenesis of autoimmune cholangitis. In human PBC, precursors of PDC-E2-specific CTL, recognizing amino acids 159–167 of PDC-E2, are tenfold more frequent in the liver than those in the blood, produce IFN-γ in response to the PDC-E2, and provide cytotoxicity to PDC-E2 peptide-expressing cells [18].

14.3.2 B Cell Contribution in IL-2Rα$^{-/-}$ Mice

To date, B cell function has not been thoroughly examined in IL-2Rα$^{-/-}$ mice although which sera demonstrated reactivity to both PDC-E2 and nuclear components [6, 46]. Serum reactivity against PDC-E2 was determined in IL-2Rα$^{-/-}$ mice and CD4/CD8-deleted strains and compared to those of control C57BL/6 J mice. Frequencies of serum AMA positivity were varied: 50 % (4/8) in IL-2Rα$^{-/-}$ mice; 0 % (0/8) in IL-2Rα$^{-/-}$-CD4$^{-/-}$ mice; 75 % (6/8) in IL-2Rα$^{-/-}$-CD8$^{-/-}$ mice. Of note, bile duct damage was apparent in all (8/8)in IL-2Rα$^{-/-}$-CD4$^{-/-}$ mice that were all negative for AMA but had an increase in hepatic B cells compared to control mice. In contrast, IL-2Rα$^{-/-}$-CD8$^{-/-}$ mice demonstrated higher positivity for AMA but lacked any liver inflammation. These results suggest again that CD8^{+} T cells provide the primary contribution while AMAs themselves are not sufficient to initiate biliary disease in this strain.

Serum levels of IgA were significantly increased in both IL-2Rα$^{-/-}$ and IL-2Rα$^{-/-}$-CD8$^{-/-}$ mice, possibly corresponding with the degree of colonic inflammation accompanied with an increase in B cell infiltration within the colonic epithelia. IgM levels were reduced in IL-2Rα$^{-/-}$-CD4$^{-/-}$ mice that had minimal colonic inflammation compared to those in control mice although hyper IgM is one of the features in human PBC patients [46].

14.3.3 Cytokine/Chemokine Contribution in IL-2Rα$^{-/-}$ Mice

In addition to the severe cellular infiltration in the portal tract, both proinflammatory and T helper 1 (Th1) cytokines, TNF-α, IFN-γ, IL-12p40, IL-2, and IL-17, were elevated in sera from IL-2Rα$^{-/-}$ and IL-2Rα$^{-/-}$-CD8$^{-/-}$mice. In contrast, IL-2Rα$^{-/-}$-CD4$^{-/-}$ mice indicated elevation of those cytokines except IL-17 [46]. In IL-2Rα$^{-/-}$ mice, serum levels of IL-17 peaked around 8–13 weeks of age and hepatic CD4^{+}-positive cells grasped Th17 bias compared to Th1, which was greater than that in splenocytes [47]. Liver non-parenchymal cells supported splenic CD4^{+} T cells to secrete IL-17 with a tenfold increase than that in CD4^{+} T cell alone culture [47], suggesting a Th17 induction role in the liver microenvironment in cases of liver autoimmunity and other liver inflammatory diseases.

On the other hand, the CD45RB^{hi} transfer model of colitis has demonstrated that IL-17A is a negative regulator of Th1 development [95]. The barrier function of tight junction in mouse cholangiocytes was disrupted by Th1 and proinflammatory cytokines IFN-γ and TNF-α [96]. These results suggest that Th17 cell population is not only dispensable to develop autoimmune cholangitis, but also may reduce liver damage by inhibiting Th1 shift in autoimmune cholangitis in this model. In humans, Th17-related cytokines IL-23p19 and IL-17 were significantly elevated in sera of patients with PBC compared to those in healthy and chronic hepatitis B-affected subjects [28]. Also, IL-17-positive mononuclear cells are significantly increased in

portal tracts in diseased human livers affected by PBC, autoimmune hepatitis, nonalcoholic steatohepatitis, and chronic hepatitis C compared to those with normal livers [47]. IL-12RB2 and IL-23R were intensively expressed in cholangiocytes of PBC patients [25]. Th1/Th17 imbalance was slightly prone to Th17 in inflamed PBC livers while the ratio of Th1 and Th17, balanced in the early stage of PBC, grew into a Th17-weighted shift in advanced stage [25]. The expression of IL-6 and IL-1β was enhanced in damaged bile ducts in PBC patients [27], and IL-6, IL-1β, IL-23p19, and IL-23/IL-12p40 mRNAs were upregulated by toll-like receptor (TLR) ligand stimulation in human cholangiocytes where IL-17 induced the production of IL-6, IL-1β, IL-23p19, and chemokines (CXCL1, CXCL2, CXCL3, CXCL6, and CXCL8 and CCL2 and CCL20) [27]. These results suggest that biliary epithelia support Th17 cell development on the site of inflammation and enhance Th17 cell induced biliary damage in the advanced stage of PBC livers.

14.4 NOD.c3c4 and NOD.ABD Mice

A congenic variant of the nonobese diabetic (NOD) strain NOD.c3c4 mice has B6- and B10-derived introgressed intervals on chromosome 3 and 4 and is fully protected from diabetes [7, 48]. NOD.c3c4 mice were reported as the first spontaneous model animal developing AMA and liver pathology similar to some features of PBC [7]. These mice demonstrated serum positivity to PDC-E2 up to 50–60 %, ANA positivity of 80–90 %, and autoimmune biliary disease (ABD) comprised of lymphocyte infiltration around portal tracts with nonsuppurative destructive cholangitis and epithelioid granuloma as seen in human PBC livers [7, 19, 48]. However, the morphological feature of biliary ducts is distinct from that of human PBC because intrahepatic and common bile ducts in this strain develop biliary cysts and ductal dilatation, respectively. The biliary cyst formation was affected by T cells since pathological changes of the liver and the common bile duct were ameliorated with a 50 % reduction of liver disease penetrance by single-dose anti-CD3 treatment in NOD.c3c4 mice around 6–10 weeks of age [7]. Also, impaired Fas expression on biliary epithelial cells contributed to the development of the biliary cysts [97].

NOD.c3c4-*scid* mice, lacking T and B lymphocytes, indicated minimal biliary disease, significantly milder than those of NOD.ABD mice, which was generated from the NOD.c3c4 strain following an intercross with the NOD.B6 *Idd10/18* congenic strain, and demonstrate ABD similar to that of NOD.c3c4 mice [7, 49]. NOD.c3c4 and NOD.ABD mice were both examined for adoptive transfer of autoimmune effector mechanisms [7, 49]. ABD was reproducible in both NOD.c3c4-*scid* and NOD-*scid* mice by transfer of whole splenocytes or splenic $CD4^+$ T cells from ABD-developed NOD.c3c4 mice [7, 48]. Also, NOD.ABD splenocytes transferred ABD except biliary cyst formation into NOD.c3c4-*scid* mice, but not into NOD-*scid* mice [49], suggesting that the primary lesions mediated by the adaptive immune system are biliary duct damage and nonsuppurative destructive

cholangitis, and the genetic background of NOD.c3c4-*scid* mice is indispensable to induce ABD [49]. NOD.ABD splenic $CD8^+$ T cell alone transfer was sufficient to develop ABD, and the degree of which was comparable to that by the $CD8^+$ T cell cotransfer with $CD4^+CD25^-$ T cells, but ameliorated with $CD4^+CD25^+$ Treg.

Autoantibody production is a distinctive feature between NOD.ABD and NOD.c3c4 mice possibly due to the difference of *Idd.9.3* allele origins derived from NOD and B10 mice in NOD.ABD and NOD.c3c4 mice, respectively. ANA was detectable in sera of NOD.c3c4 mice but, however, rarely (1/30) detected in NOD.ABD mice although all mice indicated liver inflammation. In contrast, the incidence of serum AMA increased with age and liver disease severity in NOD.ABD mice (up to 44 %) and NOD.c3c4 mice (up to 50 %) [49, 50], suggesting AMA occurrence is secondary to the biliary damage in these strains.

Similar to frequent concomitance of sicca syndrome in PBC, salivary gland inflammation coexists in NOD.c3c4 mice [50]. Genetic B cell depletion ameliorated inflammation in both liver and salivary tissues at 24 weeks, while no difference of which was observed regardless of B cell presence in 8 weeks of age in NOD.c3c4 mice [50], suggesting B cells do not contribute to the initiation of the biliary and salivary diseases in this model.

14.5 Scurfy Mice

In the context of decreased frequency of $CD4^+CD25^{high}$ Treg in human PBC [92], also studied were Scurfy (Sf) mice, which have a Foxp3 gene mutation that results in a deficiency of functional Treg [98, 99]. Sf mice demonstrated serological, histological, and cytokine features characteristic of autoimmune cholangitis as seen in the other mouse models, including expanded $CD8^+$ T cell population, similar to patients with PBC [9]. This report further emphasizes to argue universal Treg requirement to suppress autoimmune diseases including autoimmune cholangitis. Of note, Sf mice develop severe immune dysregulation, as observed in human immune dysregulation, polyendocrinopathy and enteropathy, and X-linked (IPEX) syndrome which affects humans that have mutations in the Foxp3 gene [100]. One out of 12 patients with IPEX syndrome demonstrated enteropathy, skin disease, and elevation of serum total IgM, IgA, and IgA-AMA, but had no signs of cholestasis [101], although IgA and IgA-AMA have been implicated in cholangiocyte apoptosis in PBC livers [102, 103].

14.6 $Ae2_{a,b}^{-/-}$ Mice

Antigen presenting requires costimulatory signals through CD80/86; however, human and mouse normal biliary epithelial cells (cholangiocytes) lack the ability to express these molecules [104]. It has not been addressed whether cholangiocytes

present specific antigen in spontaneous PBC mouse models described above. Since human and mouse cholangiocytes express various toll-like receptors (TLRs) [105], mouse cholangiocytes are involved in immune responses as a first-line defense for microbial infection. Cholangiocytes in patients with PBC expressed similar levels of TLR subtypes, CD40, and human leukocyte antigen DRα (HLA-DRα) and secreted equivalent amounts of chemokines. Cholangiocyte-expressed chemokines enhanced transmigration of liver-infiltrating mononuclear cells (LMNCs) in PBC. Autologous LMNC cocultured with cholangiocytes from patients with PBC produced higher levels of chemokines and enhanced the expression of CD40 and HLA-DRα [60]. Cholangiocytes produced CX3CL1 in the presence of autologous LMNC, TNF-α, and a TLR3 ligand [106]. Unmodified PDC-E2 was localized in apoptotic cholangiocytes, which combined with AMA enhanced production of proinflammatory cytokines including IL-6, TNF-α, and IL-12p40 from monocyte-derived macrophages in PBC patients [107, 108]. These results suggest cholangiocytes are easily targeted on the site of inflammation by PDC-E2-reactive autoimmune cells in PBC livers.

Genetic modification of $Cl^-/HCO3^-$ anion exchanger 2 (AE2), primarily functioning in cholangiocytes, enabled to develop the other mouse model for human PBC. AE2 is involved in intracellular pH (pH_i) regulation and transepithelial acid–base transport including secretin-stimulated biliary bicarbonate excretion. Combination therapy of UDCA and corticosteroid enhanced AE2 gene expression in normal human cholangiocytes [109]. The expression of AE2 gene was attenuated in liver tissues and blood mononuclear cells in patients with PBC [110–112], possibly due to the upregulated expression of microRNA506 to interfere with AE2 mRNA in PBC livers and cholangiocytes compared to those in normal humans [31]. Ae2 gene-disrupted mice ($Ae2_{a,b}^{-/-}$ mice) demonstrated similar features seen in human PBC: enhanced production of IL-12 and IFN-γ, an expansion of $CD8^+$ T cell, and a reduction in Treg populations [8]. Since the major histocompatibility complex class I molecule H2-D1 is markedly upregulated in cholangiocytes, Ae2-deleted cholangiocytes may attract $CD8^+$ T cells and promote their attacks to biliary ducts. Of note, separately from the features of human PBC, $Ae2_{a,b}^{-/-}$ mice exhibit azoospermia, reduced gastric acid secretion, growth retardation, bone abnormalities, and deafness [113].

14.7 2-Octynoic Acid-Conjugated BSA (2OA-BSA)-Immunized Mice

The serological hallmark of PBC is the presence of antibodies to mitochondrial antigens identified as the E2 subunits of the pyruvate dehydrogenase complex (PDC) and related enzymes [63, 114], leading an expectation that PDC-E2 immunization develops mouse models for PBC; however, previous findings had been controversial [115–117]. Nonetheless, since PBC sera demonstrated high reactivity against 2-octynoic acid (OA)-modified PDC-E2 peptide [3, 118], 2-OA conjugated

bovine serum albumin (BSA) was immunized into B6 mice and a congenic variant of the nonobese diabetic (NOD) strain, NOD1101 mice. 2-OA conjugated BSA (2OA-BSA) was a potent immunodominant epitope in both strains to break down B cell tolerance against PDC-E2 and induce PBC-like liver disease, whose pathology was characterized by an abundance of lymphocytes in B6 mice and neutrophils in NOD1101 mice [10, 11]. In this model, B cells were the most dominant population in hepatic mononuclear cells and demonstrated an approximately four- to fivefold increase compared to $CD4^+$ and $CD8^+$ T cells [52]. 2OA-BSA immunization combined with either α-GalCer or a TLR-3 ligand polyinosinic-polycytidylic acid (Poly I:C) induced liver fibrosis [53, 56].

14.7.1 B Cells in 2OA-BSA-Immunized Mice

Since autoantibody-producing B cells had been believed as contributors in autoimmune cholangitis, it was a surprising fact that B cells negatively suppressed liver inflammation in dnTGF-βRII mice, confirmed in the genetic B cell depletion and $CD8^+$ T cell transfer autoimmune cholangitis model [33, 34]. Thereafter, B cell function has been examined in a few studies using anti-CD20-treated dnTGF-βRII and genetic B cell depleted NOD.c3c4 mice demonstrating amelioration of liver disease [35, 50]. In addition, anti-CD20 and CD79b pretreatment was examined in 2OA-BSA-immunized mice. B cell depleting antibodies were administered intraperitoneally 1 week before the initiation of 2OA-BSA immunization. Bile duct damage, portal inflammation, and granuloma formation were deteriorated in B cell depleted 2OA-BSA-immunized mice, accompanied with elevation of proinflammatory IFN-γ and MCP-1 in sera [54].

14.7.2 NK and NKT Cells in 2OA-BSA-Immunized Mice

NK and NKT cell depletion using NK1.1 antibodies demonstrated their roles to enhance AMA and cytokine production from T cells [51]. Enzyme-linked immunospot (ELISPOT) assay in response to pyruvate dehydrogenase complex (PDC)-E2 synthetic peptides revealed the reduction of serum AMA- and IFN-γ-producing splenic T cells in anti-NK1.1-treated mice at 6 and 12 weeks after 2OA-BSA immunization. Both anti-NK1.1- and control antibody-treated groups demonstrated mild infiltration of lymphocytes in portal tracts; however, no histological difference was detected [51]. NK and NKT cell contribution is possibly limited to the induction phase of autoimmune cholangitis in the 2OA-BSA-immunized mice model.

Invariant NKT (iNKT) cells are efficiently activated by α-GalCer, resulting in increases of IFN-γ and IL-4 in sera, productions in hepatic iNKT cells, and maturation of DCs in the liver and spleen of C57BL/6 mice [53]. Intravenous

administration of α-GalCer prior to 2OA-BSA immunization led to a profound deterioration of liver disease in 2-OA-BSA-immunized mice, including increments in AMA production, $CD8^+$ T cell biliary infiltration, portal inflammation, granuloma formation, bile duct damage, and liver fibrosis, suggesting iNKT cell activation is a critical factor to exacerbate the manifestation of PBC. It is noteworthy again that iNKT cell activation promoted liver fibrosis with higher incidence (10/13) after a 12-week 2OA-BSA immunization compared to that (1/9) of controls.

14.7.3 Cytokine/Chemokine Contribution in 2OA-BSA-Immunized Mice

2OA-BSA immunization was also examined in C57BL/6 background mice with deletion of cytokines IL-12p40, IL-12p35, IL-23p19, IL-17A, IL-17F, IL-22, and IFN-γ. The Th1/Th17 signaling pathway was examined using gene deletion of IL-12p40 (Th1/Th17 deprivation), IL-12-p35 (Th1 deprivation), and IL-23p19 (Th17 deprivation), revealing that both Th1 and Th17 cytokines are involved, and IL-12p40 is indispensable in the development of autoimmune cholangitis in 2OA-BSA-immunized mice. IL-23p19 depletion did not affect IFN-γ levels in peripheral blood as well as its secretion in mononuclear cells isolated from both the spleen and liver. However, IFN-γ levels of liver-extracted protein were diminished by IL-23p19 deletion.

Bile duct damage and portal inflammation were ameliorated by deletion of IL-17A and IL-22, but not by IL-17F deprivation. Consistently, hepatic levels of inflammatory cytokines IFN-γ, TNF-α, and IL-6 were significantly reduced in IL-17A- and IL-22-depleted mice, but not in IL-17F-deprived mice, suggesting Th17 cytokines IL-17A and IL-22 promote migration and accumulation of proinflammatory Th1 cells into biliary diseased livers. Serum reactivity against PDC-E2 was also reduced in IL-17A-deficient, but not in IL-22-depleted, mice. In contrast, IFN-γ deletion completely abrogated the development of autoimmune cholangitis and significantly decreased serum levels of AMA, suggesting a pivotal role of IFN-γ for the induction of biliary damage in this model [52].

14.7.4 Therapeutic Immunomodulation in 2OA-BSA-Immunized Mice

Coinhibitory immunoreceptor cytotoxic T lymphocyte antigen 4 (CTLA-4) has been remarked in recent PBC studies [119–122]. CTLA-4 is expressed in T cells and negatively regulates T cell activation through higher-affinity binding to CD80/CD86 than CD28 to inhibit costimulation and transmit inhibitory signals [123, 124]. CTLA-4 immunoglobulin (Ig) is one of the effective treatment options

in autoimmune diseases such as rheumatoid arthritis [125] and juvenile idiopathic arthritis [126]. CTLA-4-Ig comprised of the extracellular domain of human CTLA-4 linked to a modified portion of the Fc domain of human IgG-1 and inhibits T cell activation.

Since CTLA-4-Ig binds to both human and mouse CD80/86, its therapeutic effect was examined in 2OA-BSA mice [55]. CTLA-4-Ig pretreatment initiated 1 day before 2OA-BSA immunization completely abrogated liver inflammation, bile duct damage, and development of anti-PDC-E2 autoantibodies. Therapeutic efficacy of CTLA-4-Ig was examined on developed autoimmune cholangitis at 8 weeks after 2OA-BSA immunization. Four weeks of treatment with CTLA-4-Ig significantly diminished bile duct damage and frequency of effector T cells, but did not affect AMA titer in diseased mice, suggesting that an optimized regimen with CTLA-4-Ig is a potential therapeutic candidate for the treatment of PBC patients [55].

14.8 Summary

The spontaneous model mice reported herein have been developed by single factor disruption using gene modification primarily affecting the immune cells or the target biliary epithelia, except NOD.c3.c4 and NOD.ABD strains, demonstrating certain similarities of immunological, serological, biochemical, and histological features of human PBC, especially hepatic $CD8^+$ T cell expansion. However, of note, since these mice also have some disadvantages of complications distinct to human PBC (Table 14.1), a research objective demands a deliberate selection of a suitable mouse model.

These emerging models have enabled researchers to address issues residing on human PBC, contribute to elucidate pathogenic factors, and ultimately may propose novel therapeutic options for human PBC. Recent works imply potential usages of some biologics such as anti-CD20 (rituximab), anti-IL-12/23p40 (ustekinumab), and CTLA-4-Ig (abatacept) for human PBC.

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Abbreviations

2OA-BSA	2-Octynoic acid-conjugated BSA
ABD	Autoimmune biliary disease
Breg	Regulatory B cells
BSA	Bovine serum albumin

CTLA-4	Cytotoxic T lymphocyte antigen 4
IFN	Interferon
IL	Interleukin
PBC	Primary biliary cirrhosis
Poly I:C	Polyinosinic-polycytidylic acid
TCR	T cell receptor
TGF	Transforming growth factor
TLR	Toll-like receptor
TNF	Tumor necrosis factor
Treg	Regulatory T cells

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Chapter 15
Epidemiology and Natural History in Japan

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Abstract Multicenter national surveys of PBC have been carried out 15 times since 1980 in Japan by the members of the Intractable Hepato-Biliary Disease Study Group in Japan supported by the Health Labour Sciences Research Grant. The subjects were 8,509 patients with PBC diagnosed and followed up between January 1, 1968 and December 31, 2011 at 520 hospitals and institutions throughout the country. We investigated the epidemiological trends and long-term prognosis of PBC in Japan based on national surveys. The annual incidence expressed as number of PBC patients has almost leveled off after 1989, and almost 70 % of patients with PBC were diagnosed in the asymptomatic stage. The prevalence of PBC gradually

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increased throughout the observation period. There were no major differences in clinical findings of PBC in Japan from those in the previous reports. Recently, the annual number of deaths caused by liver disease has decreased. The 5-year survival rates were 97.9 and 80.3 %, the 10-year survival rate 93.7 and 66.7 %, and the 20-year survival rate 84.2 and 52.1 %, respectively, showing significant differences among the groups ($p < 0.0001$). As results of examining changes in survival rate of PBC patients at each clinical stage, there was significant difference between the 1980s, 1990s, and 2000s for a-PBC and s-PBC. The results of the national surveys support the view that increased a-PBC with excellent prognosis and improvement in prognosis of all clinical stages of PBC may be important for increased prevalence in Japan.

Keywords Epidemiology • National survey • Primary biliary cirrhosis • Prognosis

15.1 Introduction

Primary biliary cirrhosis (PBC) is a chronic cholestatic liver disease of unknown etiology, mainly affecting middle-aged women, and is associated with a number of immunological abnormalities. It is characterized by inflammation and destruction of interlobular and septal bile ducts, which leads to cholestasis, cirrhosis, and hepatic failure. During the more than 60 years since the term PBC was initially used by Ahrens [1], knowledge of this disease has become widespread and many studies have been performed regarding its epidemiology, clinical features, pathogenesis, and treatment.

Since1980, 15 national surveys of PBC in Japan have been carried out by the Intractable Hepato-Biliary Disease Study Group in Japan supported by the Health Labour Sciences Research Grant. We previously analyzed the collective results of surveys performed [2–4]. The subject of these surveys was a large cohort of patients with PBC followed up over 30-year period. In this review, the epidemiology and long-term prognosis of PBC in Japan based on national surveys will be discussed.

15.2 The Worldwide Distribution of PBC

Studies of incidence and prevalence in different countries and regions have generated widely varying results, ranging from 0.7 (Israel, in 1980–1990) to 49 (Scotland, in1986–1996) per million of the population and 6.7 (Israel, in 1980–1990) to 402 (the USA, in 1975–1995) per million of the population, respectively [5]. Although this may be attributed to the difference in methodology and study periods, it supports the view that either environmental or racial/genetic factors contribute to the pathogenesis of the condition.

15.3 The Nationwide Epidemiological Studies of PBC in Japan

Clinically, PBC is divided into two stages: symptomatic PBC (s-PBC) and asymptomatic PBC (a-PBC). s-PBC has several symptoms such as pruritus, jaundice, portal hypertension, esophageal varices, ascites, and/or hepatic encephalopathy. Recently, almost 70 % of PBC patients lack significant symptoms, which is defined as a-PBC. Since 1989, the medical expenses for PBC patients with specific symptoms attributable to liver disease have been a public expenditure. Under the Japanese system, patients with s-PBC were enrolled according to each prefecture in Japan. The PBC patient registration with the system recorded approximately 17,000 s-PBC patients in 2010 [6]. Therefore, the total number of PBC patients in 2010 is presumed to be 50,000–60,000 in Japan (total population was 130 million populations with approximately 105 million adults in 2010): the prevalence was 380–460 cases per one million, or 480–570 cases per one million adults in Japan. PBC has been reported throughout the world, but the incidence and prevalence of the disease seem to vary considerably between different countries. These epidemiological studies in Japan have shown that both the incidence and prevalence of PBC are similar in the USA and Europe.

15.4 National Surveys of PBC in Japan

15.4.1 Patients and Methods

Multicenter national surveys of PBC have been carried out 15 times since 1980 in Japan by the members of the Intractable Hepato-Biliary Disease Study Group in Japan supported by the Health Labour Sciences Research Grant. The subjects were 8,509 patients with PBC diagnosed and followed up between January 1, 1968 and December 31, 2011 at 520 hospitals and institutions throughout the country. The observation period was 83.3 months on average.

A questionnaire containing about 200 items regarding the history, clinical findings, and prognosis of PBC patients was sent to hospitals and institutions throughout Japan. The survival time was defined as the time from the date of diagnosis to the date of least observation. All the clinical, biochemical, and demographic records were based on the date of diagnosis in this study. PBC is diagnosed based on "Criteria for Diagnosis of PBC in Japan" by this study group [3]. The histological stage was determined according to the criteria of Scheuer [7]. The survival rate was obtained by the Kaplan–Meier method and $p < 0.05$ was defined as significant.

The results of these surveys reflect the epidemiological tendencies of all of the patients with PBC in Japan. This is because the surveys were performed using case-finding methods that were based on strict diagnostic criteria by specialist physicians with an interest in hepatology at specific hospitals and institutions.

15.4.2 Clinical Findings

15.4.2.1 Annual Incidences

Annual incidences and ratios of patients with a-PBC and s-PBC are shown in Fig. 15.1a. At one time, PBC was recognized as an uncommon liver disease in Japan. In 1974, there were only ten registered cases per year in Japan. In early surveys, annual incidence of PBC detected in Japan each year has increased as knowledge of the biochemical, immunological, and histological findings characteristic of this disease has expanded. As the routine biochemical screening of patients becomes more common, increasing numbers of patients are diagnosed in the asymptomatic stage. But it remains stable at around 250–350 incidences after 1989, and the tendency for a-PBC to comprise almost 70 % of the overall incidence did not change during theses several years (Fig. 15.1a).

15.4.2.2 Number of Patients

Regarding the number of patients, the annual number of surviving patients whose prognoses could be followed in each survey was presented as the number of patients. The prevalence of PBC gradually increased throughout the observation period (Fig. 15.1b). In 2011, the number of patients was 5,500 cases approximately.

15.4.2.3 Patient Profile

There were no major differences in clinical findings of PBC in Japan from those in the previous reports [2–4].

Figure 15.2 shows the age and gender distribution of the patients with a-PBC and s-PBC. PBC predominantly affects middle-aged women with a female/male ratio of 6.4:1 in a-PBC and 7:1 in s-PBC, respectively. The median age of disease onset is 50–60 years, but ranges between 20 and 90 years. PBC primarily affects middle-aged women, as previously reported.

The incidence of autoantibody was investigated in 6,775 patients. The positivity rates of antimitochondrial antibody (AMA), antinuclear antibody (ANA), and anti-smooth muscle antibody (ASMA) were 83.5, 47.7, and 5.3 %, respectively. Based on the PBC diagnostic criteria revised in 1992, description regarding anti-pyruvate dehydrogenase (PDH) antibody and antimitochondrial M2 antibody has been added since the 7th survey (1992). In the 7th–15th surveys, both AMA and anti-PDH antibodies were measured in only 2,824 patients. In these patients, the positivity rates of anti-PDH antibody and AMA were 76.6 and 80.7 %, respectively, and patients who were positive for either anti-PDH antibody or AMA accounted for 88.6 %.

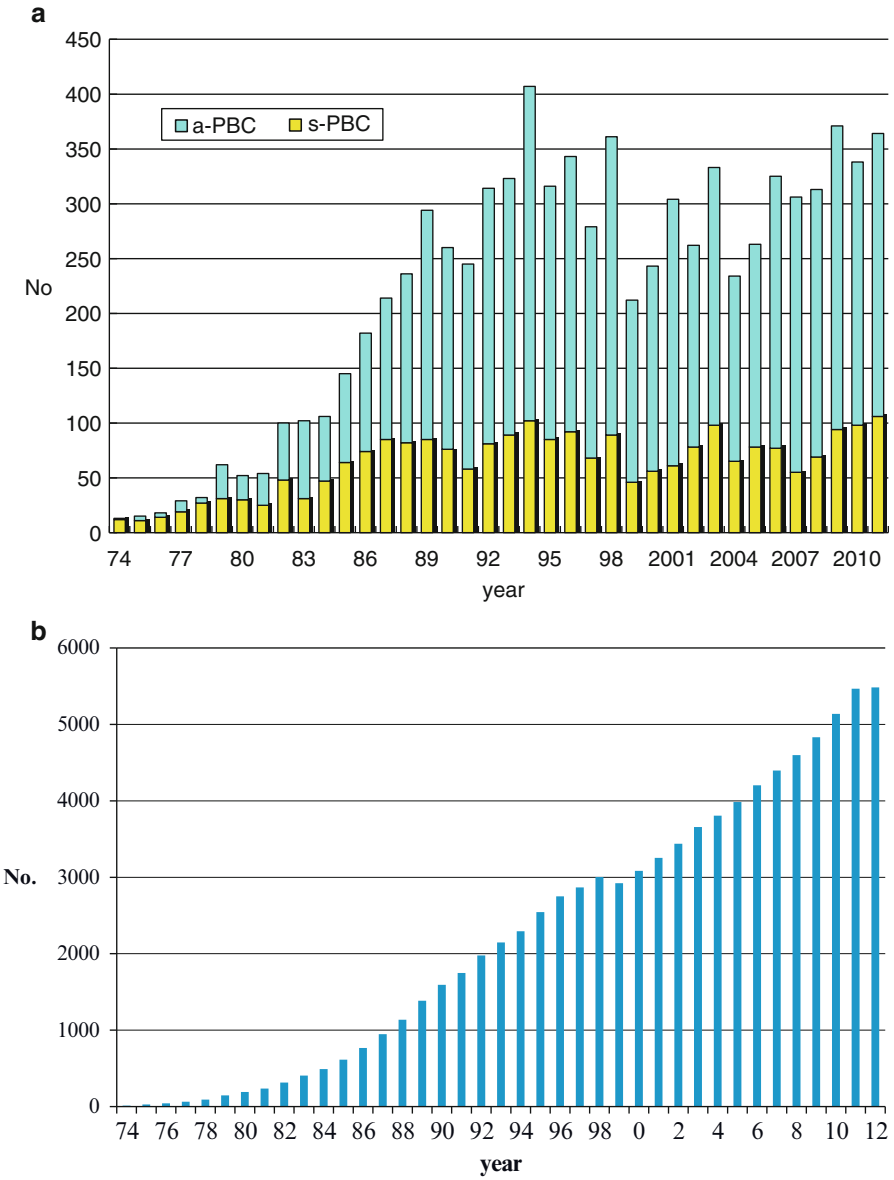


Fig. 15.1 (**a**) Annual incidences and ratios of patients with a-PBC and s-PBC from a series of national surveys in Japan (1980–2011). (**b**) Annual numbers from a series of national surveys in Japan (1980–2011). Regarding the number of patients, annual number of surviving patients whose prognoses could be followed in each survey was presented as the number of patients

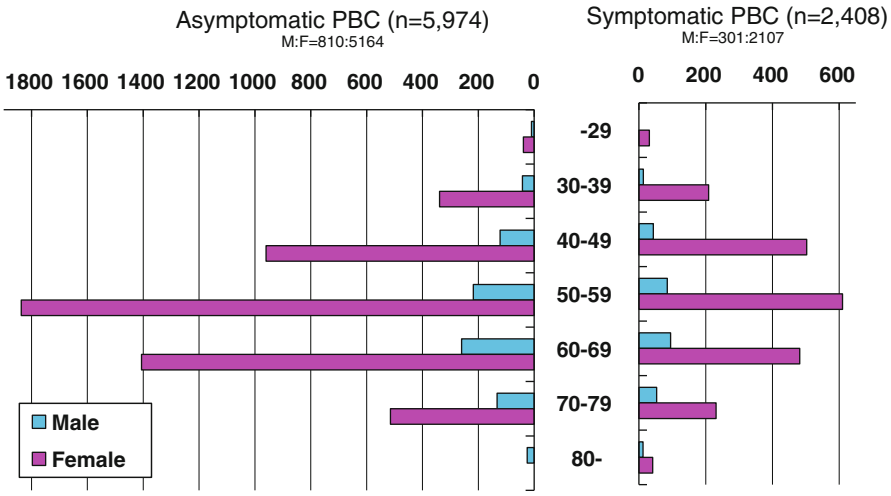


Fig. 15.2 Age and gender distribution of the patients with PBC from a series of national surveys in Japan

15.4.2.4 Complicating Autoimmune Diseases

The frequency of complicating autoimmune diseases was high in the order of Sjogren's syndrome (11.9 %), Hashimoto's thyroiditis (6.5 %9), rheumatoid arthritis (3.6 %), Raynaud's phenomenon (3.1 %), and scleroderma (2.7 %).

On comparison with previous reports [4], the frequency of complicating Sjogren's syndrome slightly decreased (from14.1 to 11.9 %), but there were no other significant differences observed. The frequencies of theses complications were higher than the general prevalence.

15.4.2.5 Complicating Malignant Tumors

Complicating malignant tumors were observed in 262 (3.3 % of all patients), and hepatocellular carcinoma (HCC) was the most frequent, occurring in 63 patients (approximately 24 %). Other malignant tumors were complicated gastric carcinoma (16 %), colorectal carcinoma (12 %), breast carcinoma (10 %), thyroid carcinoma (6 %), cervical carcinoma (5 %), hematological cancer (5 %), ovarial carcinoma (35), and lung carcinoma (3 %). These frequencies were higher than the general prevalence in Japan.

Recently, the incidence of PBC complicated by HCC has been gradually increasing.

Using data from the national surveys, we investigated the clinicopathological findings associated with HCC in PBC patients [8]. The multivariate analysis of risk factors associated with HCC by gender revealed histological stage at the time of PBC diagnosis as an independent risk factor associated with the development of

HCC in females, but not in males. In addition, histological stage at the time of HCC diagnosis was an independent risk factor for HCC in females, whereas no risk factors were identified in males. These data indicate that males are at risk of developing HCC at any histological stage of PBC.

15.4.2.6 Histological Findings

Regarding the histology of the liver at diagnosis, Scheuer's stages I and II comprised 80 % or more in all surveys.

15.4.2.7 Cause of Death

During the observation period, 1,235 deaths occurred. The cause was hepatic failure in 630 patients (51 %), gastrointestinal bleeding in 154 (12.5 %), combined hepatic failure and gastrointestinal bleeding in 138 (11.1 %), and HCC in 35 (2.8 %). Four hundred three patients (32.6 %) died of other causes. The annual number of deaths from the primary disease tended to decrease after 2000 (Fig. 15.3a). Recently, HCC is also reported to occur in PBC patients with improvements in treatment and survival. Although hepatic failure defines the prognosis in most PBC patients, the number of deaths of patients with PBC due to HCC has been gradually increasing.

15.4.2.8 Annual Changes in Managements

On investigation of annual changes in therapeutic drugs, D-penicillamine was frequently used in early surveys, but the ursodeoxycholic acid (UDCA) has rapidly increased since the effectiveness of UDCA was reported in 1987 [9] (Fig. 15.3b, c). On analysis of patients treated with UDCA, UDCA was administered to 5,754 patients, comprising 83.4 % of all patients (85.2 % of patients with a-PBC, 79.1 % of those with s-PBC). Analyzing UDCA administration by clinical stages in each diagnosis year, UDCA was administered to about 90 % of all patients with a-PBC and s-PBC after 1990. The efficacy of bezafibrate in PBC was originally reported in 1996 [10]. Bezafibrate has a different mechanism of action from UDCA; therefore, UDCA should be used as initial therapy and bezafibrate added if the efficacy of UDCA is limited. The number of patients treated with bezafibrate has gradually increased since the effectiveness of bezafibrate was reported in 1996 (Fig. 15.3c).

If large varices develop in the esophagus or upper stomach, either sclerotherapy or band ligation can be performed to obliterate varices and to prevent bleeding. As shown in Fig. 15.3d, the number of patients with this endoscopic therapy has increased.

When PBC progresses to cholestatic cirrhosis, medical treatment has little effect on disease progression and liver transplantation is the only therapeutic approach for survival. Living donor liver transplantation (LDLT) is more common in Japan because deceased donor livers are scarcely offered for transplantation (Fig. 15.3e).

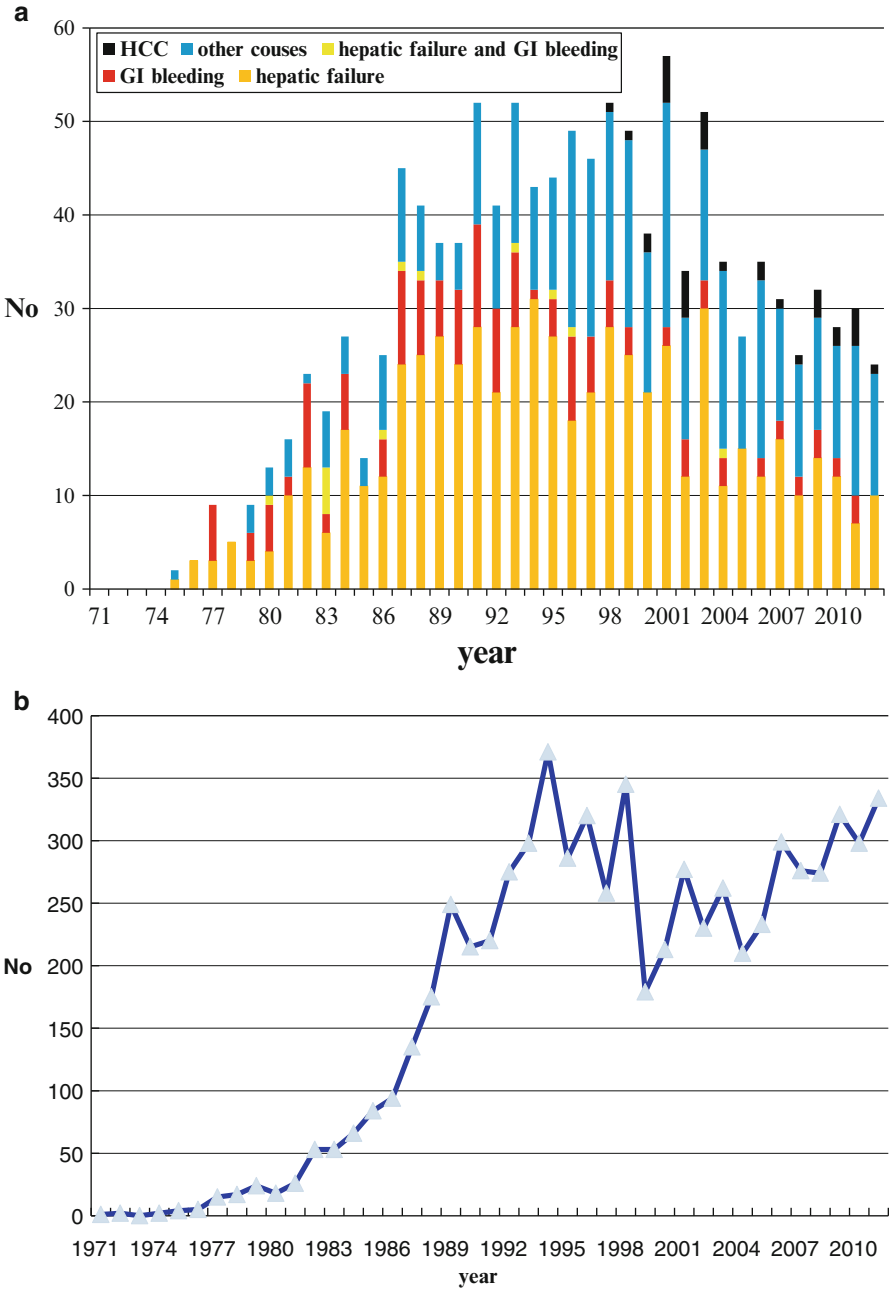
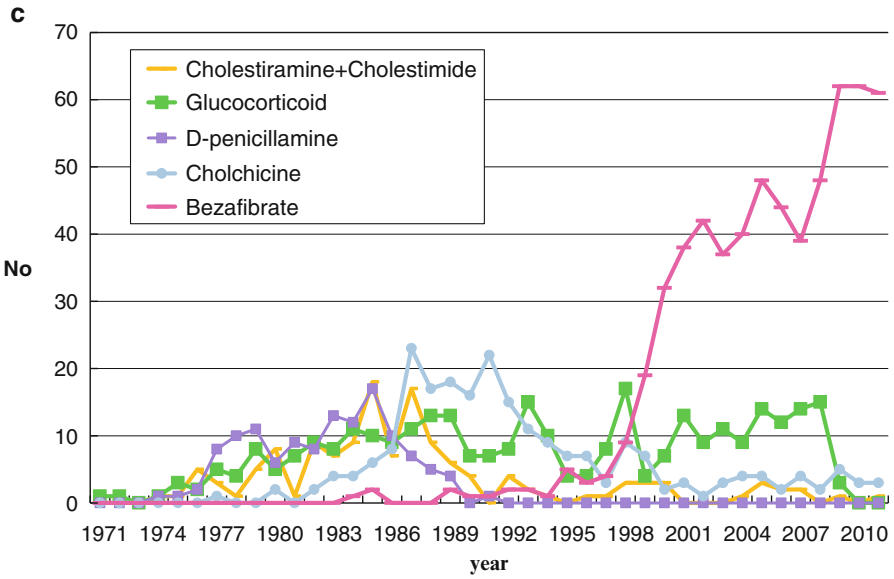


Fig. 15.3 (**a**) Annual numbers of deaths and ratios of their causes from a series of national surveys in Japan. (**b**) Annual numbers of patients with administration of ursodeoxycholic acid (UDCA) from a series of national surveys in Japan. (**c**) Annual changes in therapeutic drugs from a series of national surveys in Japan. (**d**) Annual numbers of patients with sclerotherapy or band ligation of varices in the esophagus or upper stomach from a series of national surveys in Japan. (**e**) Annual numbers of patients with administration of living donor liver transplantation (LDLT) and deceased donor liver transplantation (DDLT) from a series of national surveys in Japan



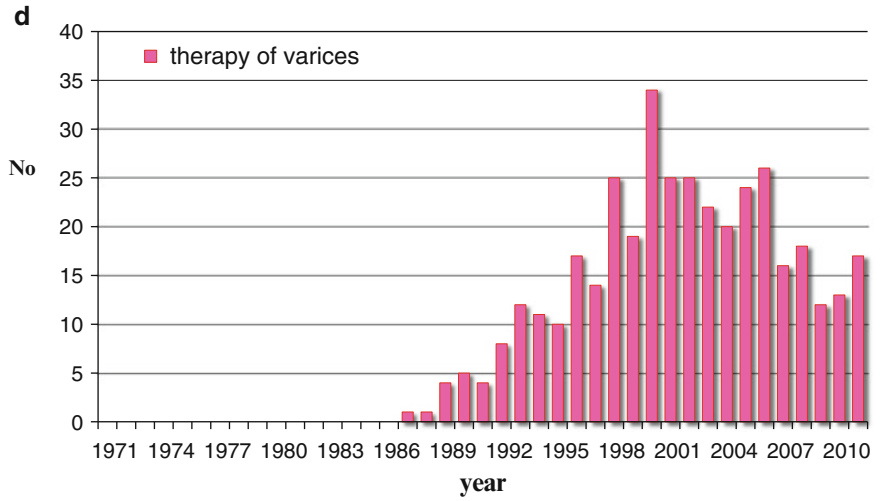


Fig. 15.3 (continued)

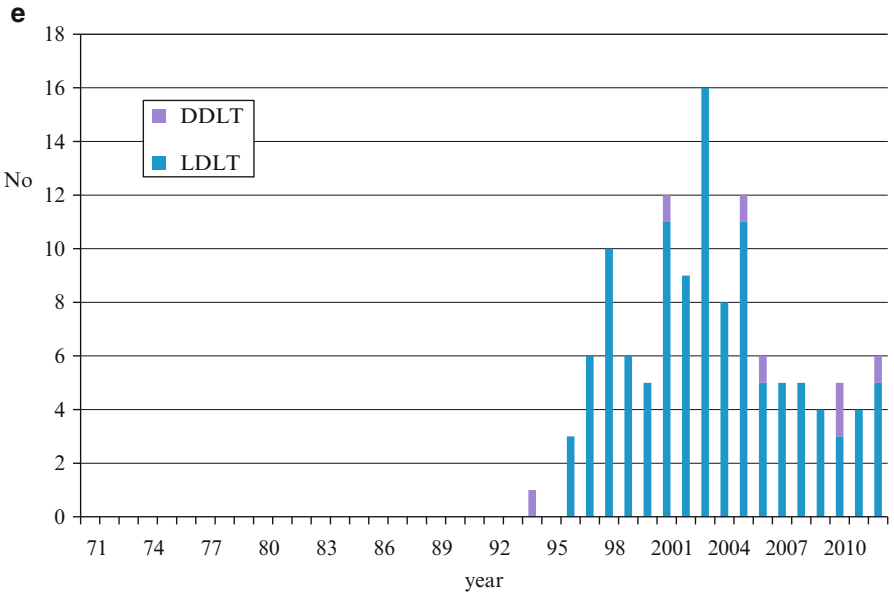


Fig. 15.3 (continued)

Table 15.1 The progression of symptoms in PBC patients from the time of diagnosis until the study endpoint ($n = 7{,}516$)

		Clinical stage at the endpoint	
		Number of cases (%)	
		Survivor/death	
		a-PBC	s-PBC
Clinical stage at the diagnosis	a-PBC ($n = 5{,}400$)	4,461 (82.6 %)	939 (17.4 %)
		4,406/55	736/203
	s-PBC ($n = 2{,}116$)	672 (31.8 %)	1,444 (68.2 %)
		658/14	983/461

15.4.3 Analysis of Prognosis

15.4.3.1 Number of Patients Showing Stage Progression and Their Survival Rate (Table 15.1)

Clinical stage had not progressed at final observation in 82.6 % of patients with a-PBC at diagnosis. In our previous study, there were two groups with different prognoses in patients with a-PBC on analysis of survival rates of patients by stage progression [4]. Most of these patients do not develop jaundice for a prolonged period and the prognosis is good. In some patients with a-PBC at diagnosis, the disease prognosis is relatively fast to symptomatic PBC accompanied by jaundice.

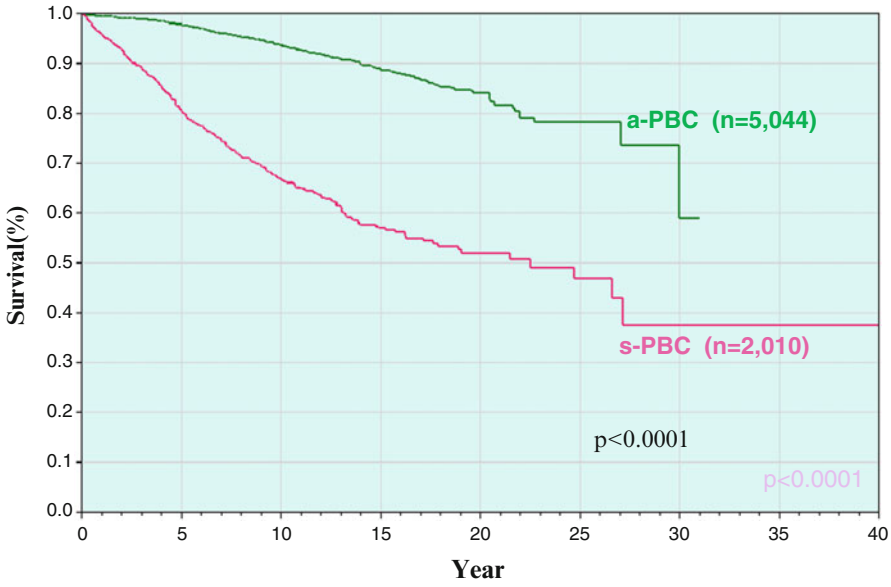


Fig. 15.4 Kaplan–Meier survival curves for the 7,054 patients with PBC at each clinical stage at the time of diagnosis from a series of national surveys in Japan. The survival rates of 5,044 with a-PBC and 2,010 with s-PBC at the time of diagnosis were obtained

15.4.3.2 The Survival Rate of Patients at Each Clinical Stage at the Time of Diagnosis (Fig. 15.4)

The survival rates of 5,044 and 2,010 patients with a-PBC and s-PBC at the time of diagnosis (7,054 patients in total) were obtained. The 5-year survival rates were 97.9 and 80.3 %, the 10-year survival rate 93.7 and 66.7 %, and the 20-year survival rate 84.2 and 52.1 %, respectively, showing significant differences among the groups ($p < 0.0001$).

15.4.3.3 Kaplan–Meier Survival Curves for Patients with PBC at the Time of Diagnosis in the 1980s Group, the 1990s Group, and the 2000s Group

As results of examining changes in prognosis of PBC patients, there was significant difference between the 1980s, 1990s, and 2000s for both a-PBC (Fig. 15.5a) and s-PBC (Fig. 15.5b). From the study of long-term prognosis of PBC in Japan based on national surveys, the prognosis of patients with all clinical stages of PBC has improved.

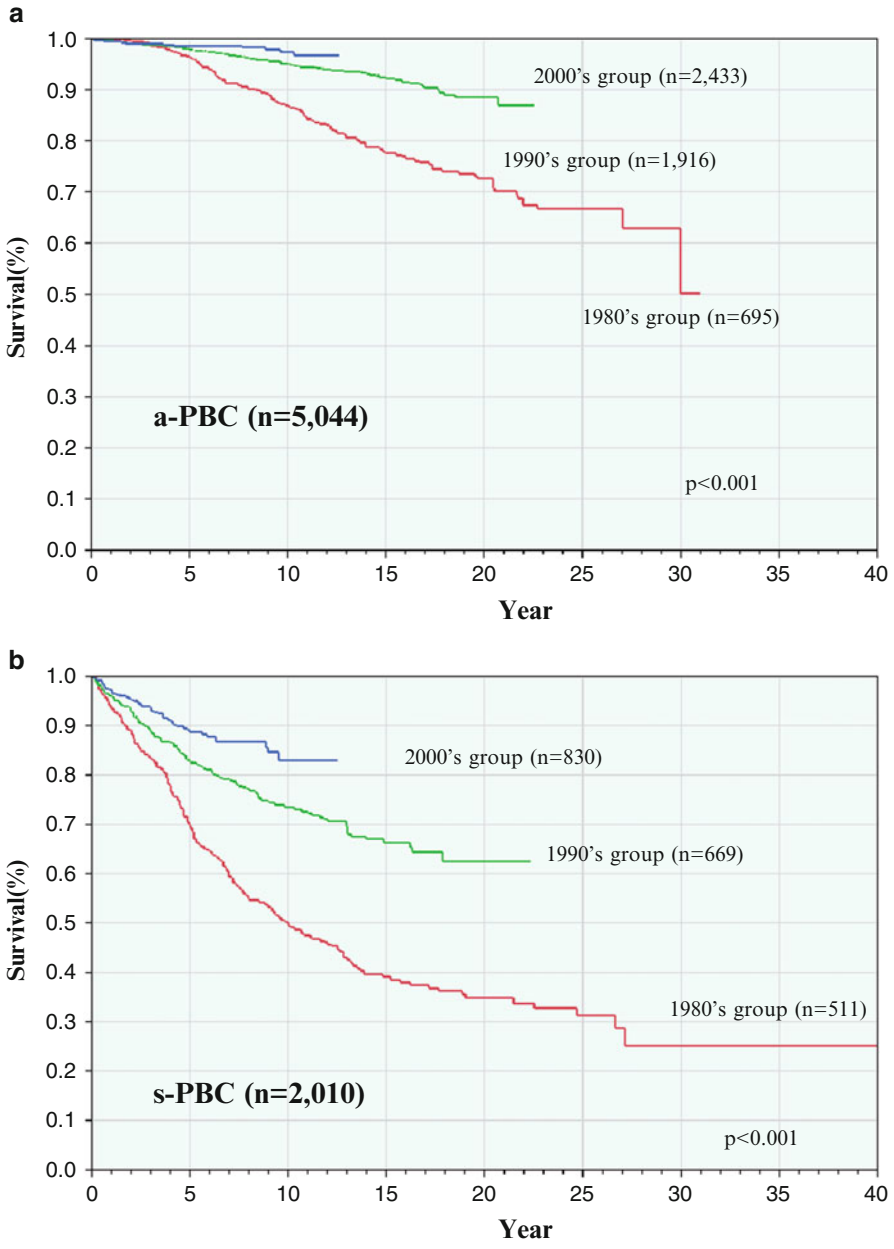


Fig. 15.5 (**a**) Kaplan–Meier survival curves for patients with a-PBC at the time of diagnosis in the 1980s group, the 1990s group, and the 2000s group from a series of national surveys in Japan. (**b**) Kaplan–Meier survival curves for patients with s-PBC at the time of diagnosis in the 1980s group, the 1990s group, and the 2000s group from a series of national surveys in Japan

15.5 Conclusion

The results of the national surveys support the view that increased a-PBC with excellent prognosis and improvement in prognosis of all clinical stages of PBC by the effects of various treatments including UDCA may be important for increased prevalence in Japan. UDCA not only improves liver test but also prolongs the time until death or liver transplantation. However, the therapeutic effects of UDCA are negligible in patients with advanced PBC and marked jaundice. In our previous study, the prognosis of s-PBC with moderate stage (serum bilirubin level: T-Bil2.0 mg/dL higher or histological stages III and IV) has not improved, even though they have been treated with medication including UDCA [11]. Although it is expected that a special form of management is necessary for these patients of advanced stage, liver transplantation is the only option for these patients.

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Chapter 16
New Histological Staging and Grading System for Primary Biliary Cirrhosis

Yuko Kakuda, Kenichi Harada, and Yasuni Nakanuma

Abstract Scheuer's and Ludwig's are the two histological staging systems for primary biliary cirrhosis (PBC) that have been used worldwide since the 1960s. In these classical staging systems, PBC livers were classified into four stages on the basis of a single characteristic histological feature of PBC such as chronic nonsuppurative destructive cholangitis and bile ductular proliferation. It is well known that these histological features are heterogeneously distributed in the liver; therefore, sampling errors are often unavoidable in these classical staging systems. In addition, there have been no categories on the grading system for necroinflammation characterizing PBC. We recently proposed a new histological staging and grading system in which we consider the degree of fibrosis, bile duct loss, and chronic cholestasis in combination for staging, in order to reduce sampling errors. Scores for the degree of both chronic cholangitis activity (CA) and hepatitic activity (HA) for grading disease activity were also proposed. Our recent study demonstrated that this new system provides more information, which is consistent with clinical laboratory data and a better reflection of the prognosis of PBC patients. Herein, the new histological system for staging and grading of PBC is reviewed.

Keywords Cholangitis • Bile duct loss • Fibrosis • Grading • Primary biliary cirrhosis • Staging

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16.1 Introduction

Primary biliary cirrhosis (PBC) is characterized histologically by nonsuppurative destructive cholangiopathy affecting interlobular bile ducts. The initial and distinctive finding of bile duct injury in PBC is chronic nonsuppurative destructive cholangitis (CNSDC). The injury is often extensive and the affected bile ducts eventually disappear, leading to chronic cholestatic features and biliary cirrhosis [1–3]. Therefore, these features are not only key for the diagnosis of PBC but are also used for the evaluation of PBC progression.

16.2 Classical Histological Staging System

16.2.1 Diagnostic Histological Findings of PBC

In the relatively early stages, PBC is characterized by florid duct lesions or CNSDC of the interlobular bile duct [4]. Epithelioid cell granulomas around damaged bile ducts, well-formed granulomas or loosely arranged epithelioid cells, are characteristic, too [5]. Bile duct loss or ductopenia (bile duct loss at $\geq$50 % of portal tracts) which follows CNSDC is a fundamental finding of PBC. This ductopenic lesion may develop and progress in the early stages and the more extensive in advanced stages.

Histopathological features that are suggestive, but not diagnostic, of PBC include mild chronic cholangitis (lymphocytic or pleomorphic), variable biliary epithelial damage, elevated IgM and predominant infiltration by IgM+ plasma cells in portal tracts, epithelioid granulomas in the hepatic lobules, atypical ductular reactions, and focal deposition of copper or copper-binding proteins [5–7]. Other pathological changes may also be indicative of PBC. Small cell changes of hepatocytes in zones 1 and 2 are frequently observed in chronic cholestatic liver disease, particularly PBC [8]. Vague nodularity of the hepatic parenchyma in similar zones is also found in PBC [9]. Prominent eosinophilic infiltration in portal tracts is also evident in some cases [10].

At relatively advanced stages of PBC, a majority of these characteristic features have disappeared, but prominent chronic cholestasis, extensive fibrosis, and regenerative nodules are apparent. PBC should be suspected on the basis of a virtual absence of interlobular bile ducts, focal lymphocytic aggregates that seem to replace the missing bile duct(s), peripheral cholestasis or chole stasis with Mallory bodies and extensive copper or copper-binding proteins deposition, a biliary pattern of fibrosis, and partial or focal preservation of the normal architecture in otherwise established cirrhosis.

In addition, hepatitic changes including interface hepatitis and parenchymal necroinflammatory changes, which also occur in other chronic liver diseases, are concurrently found variably in PBC.

16.2.2 Classical Histological Staging Systems of PBC

Several histological staging systems for PBC have been proposed since the 1960s, including those by Scheuer [11], Rubin et al. [4], and Ludwig et al. [12]. In these systems, PBC is classified into four stages, for example, in Scheuer's system, stage 1 by florid duct lesions or CNSDC, stage 2 by proliferation of bile ductules, stage 3 by fibrosis or scarring, and stage 4 by frank cirrhosis. As for Ludwig's system, stage 1 is defined by portal hepatitis, stage 2 by periportal interface hepatitis, stage 3 by bridging necrosis or bridging fibrosis, and stage 4 by cirrhosis.

The stages of the classical systems are defined on the basis of the experience of established liver pathologists. These systems appear simple and seem to be applicable; therefore, they have been widely used. However, the staging process itself is subjective because of the use of a single finding to define stages. In addition, it is well known that histological changes are heterogeneous in a PBC liver and sampling errors easily occur in liver needle biopsy; histological findings defining different stages can be seen in one liver biopsy specimen. Therefore, staging may be different in liver biopsy specimens obtained from different parts of the same liver at the same time [1]. Furthermore, the concept of cholestasis and hepatic damage due to long-standing cholestasis is lacking in Ludwig's staging.

Moreover, since the publication of the latest staging method, i.e., of Ludwig et al. [12], in 1978, considerable progress has been made in therapeutic fields. Several treatments are now available for PBC such as ursodeoxycholic acid (UDCA), bezafibrate, and combined UDCA and corticosteroid therapy for so-called overlapping syndrome or the hepatitic form of PBC. In histological fields, several features have been reported as predictors of PBC progression, such as fibrosis, interface hepatitis, ductopenia/bile duct loss, and cholestasis [13–17]. These features associated with poorer prognosis cannot be assessed individually using classical staging systems. Specifically, classical systems are lacking in the evaluation of bile duct loss and cholestasis and are insufficient to assess necroinflammatory changes in the hepatitic form of PBC or so-called overlapping syndrome.

In recent times, in chronic liver diseases such as chronic viral hepatitis [18] and nonalcoholic fatty liver disease [19], grading and staging systems have been proposed to objectively evaluate the degree of disease activity and progression. These systems are widely and routinely used because they are very useful in the estimation of therapeutic effects and the determination of therapeutic strategies. In response to the shortages of classical staging systems and advancement of the systems for chronic progressive liver diseases other than PBC, we proposed a new histological staging and grading system of PBC for needle biopsy specimens.

16.3 New Histological Staging and Grading System for PBC

Hiramatsu et al. [20] performed factor analysis on 17 representative histological findings of PBC and showed that these findings were categorized into three independent factors: factor 1 (fibrosis, fibrous piecemeal necrosis, deposition of orcein-positive granules, bile plugs, Mallory bodies, feathery degeneration, bile duct loss, and atypical ductular proliferation); factor 2 (portal inflammation, eosinophilic infiltration, lymphoid follicles, epithelioid granulomas, interface hepatitis, and chronic cholangitis); and factor 3 (interface hepatitis, lobular hepatitis, acidophilic bodies, and pigmented macrophages). When these factors were analyzed with respect to clinical data including scores used in the Mayo Clinic's prognostic model [21, 22], factor 1 lesions reflected histological progression (staging), while factors 2 and 3 lesions were related to necroinflammatory activity (grading). On the basis of these findings, we proposed a new staging and grading system (original version), which is summarized in Tables 16.1, 16.2, and 16.3.

The original version of the new staging and grading system may be suitable for surgically resected large specimens; however, it seems too detailed to be of practical use for routine liver biopsy diagnosis. Therefore, we developed a convenient version applicable to liver biopsy specimens, as shown in Tables 16.1, 16.2, and 16.4 [23]. The revised staging and grading process itself was not different from the original version.

Table 16.1 Scoring for the staging of PBC

Score	Criterion
A. Fibrosis	
0	No portal fibrosis or fibrosis limited to portal tracts
1	Portal fibrosis with periportal fibrosis or incomplete septal fibrosis
2	Bridging fibrosis with variable lobular disarray
3	Liver cirrhosis with regenerative nodules and extensive fibrosis
B. Bile duct loss	
0	No bile duct loss
1	Bile duct loss in less than one-third of portal tracts
2	Bile duct loss in one-third to two-thirds of portal tracts
3	Bile duct loss in more than two-thirds of portal tracts
C. Deposition of orcein-positive granules	
0	No deposition of granules
1	Deposition of granules in a couple of zone 1 hepatocytes at less than one-third of portal tracts
2	Deposition of granules in a variable number of zone 1 hepatocytes at one-third to two-thirds of portal tracts
3	Deposition of granules in most zone 1 hepatocytes at more than two-thirds of portal tracts

Table 16.2 Staging of PBC

Stage	Sum of scores
A. Fibrosis + bile duct loss + deposition of orcein granules deposition scores	
1 (no or minimal progression)	0
2 (mild progression)	1–3
3 (moderate progression)	4–6
4 (advanced progression)	7–9
B. Fibrosis + bile duct loss scores	
1 (no or minimal progression)	0
2 (mild progression)	1, 2
3 (moderate progression)	3, 4
4 (advanced progression)	5, 6

Table 16.3 Grading of the necroinflammatory activity of PBC (the original version)

Grade	Criteria
A. CA (cholangitis activity)	
0 (no activity)	No cholangitis but mild damage to the epithelium of the duct may be present
1 (mild activity)	Chronic cholangitis including CNSDC in less than one-third of portal tracts
2 (moderate activity)	Chronic cholangitis including CNSDC in one-third to two-thirds of portal tracts
3 (marked activity)	Chronic cholangitis including CNSDC in more than two-thirds of portal tracts
B. HA (hepatitis activity)	
0 (no activity)	No interface hepatitis and no or minimal lobular hepatitis
1 (mild activity)	Focal interface hepatitis in a few portal tract(s) and focal necrosis in the parenchyma
2 (moderate activity)	Moderate interface hepatitis in several portal tracts and variable lobular hepatitis
3 (marked activity)	Moderate-marked interface hepatitis in many portal tracts, or bridging or zonal necrosis, or both

CNSDC chronic nonsuppurative destructive cholangitis

In the new evaluation system, both original and revised versions, histological scores for staging consist of three items: fibrosis, bile duct loss, and deposition of orcein-positive granules (Table 16.2A). After each of these items is scored as 0–3, scores of the three lesions are summed in individual cases. A total score of 0 is stage 1 (no or minimal progression), 1–3 is stage 2 (mild progression), 4–6 is stage 3 (moderate progression), and 7–9 is stage 4 (advanced progression). If orcein staining is not available, the sum of the scores for fibrosis and bile duct loss is also applicable for staging, as shown in Table 16.2B.

For grading, cholangitis activity (CA) and hepatitis activity (HA) are evaluated as 0–3.

Table 16.4 Grading of the necroinflammatory activity of PBC (the revised version for liver biopsy)

Grade	Criteria
A. CA (cholangitis activity)	
0 (no activity)	No cholangitis but mild damage to the epithelium of the duct may be present
1 (mild activity)	1 evident chronic cholangitis in the specimen
2 (moderate activity)	≥2 bile ducts with evident chronic cholangitis
3 (marked activity)	≥1 CNSDC in the specimen
B. HA (hepatitis activity)	
0 (no activity)	No interface hepatitis and no or minimal lobular hepatitis
1 (mild activity)	Interface hepatitis affecting 10 continuous hepatocytes at a limiting plate in 1 portal tract or fibrous septa and mild-to-moderate lobular hepatitis
2 (moderate activity)	Interface hepatitis affecting 10 continuous hepatocytes at limiting plates in ≥2 portal tracts or fibrous septa and mild-to-moderate lobular hepatitis
3 (marked activity)	Interface hepatitis affecting 20 continuous hepatocytes at limiting plates in more than half of the portal tracts and moderate lobular hepatitis or bridging/zonal necrosis

CNSDC chronic nonsuppurative destructive cholangitis

16.3.1 Histological Findings Defining the New Evaluation System

16.3.1.1 Scoring of Fibrosis

A score of 0 indicates that there is almost no fibrosis or that the fibrosis is confined to the portal tracts without fibrous septa. A score of 1 indicates that the fibrosis extends beyond the portal area (fibrous septa) occasionally with incomplete fibrous septa. A score of 2 indicates that there is bridging fibrosis with variable lobular distortion. A score of 3 indicates cirrhosis.

Fibrosis generally reflects the progression of chronic liver disease, and fibrosis is due to prolonged chronic cholestasis and hepatocellular damage in cases of PBC [1–3]. Progression of fibrosis relates to the development of cirrhosis and is an important prognostic factor in PBC, as with other chronic liver diseases. We made the scoring system of fibrosis in reference to the staging system of chronic viral hepatitis [18] and nonalcoholic fatty liver disease [19]. Special staining, such as reticulin and Azan-Mallory stain, is useful for a more accurate evaluation of the degree of fibrosis.

16.3.1.2 Scoring of Bile Duct Loss

A score of 0 indicates that interlobular bile ducts are present in all portal tracts. Scores of 1 and 2 indicate that bile duct loss is evident in <1/3 or in 1/3–2/3 of portal tracts, respectively. A score of 3 indicates that bile ducts are absent in >2/3 of portal tracts.

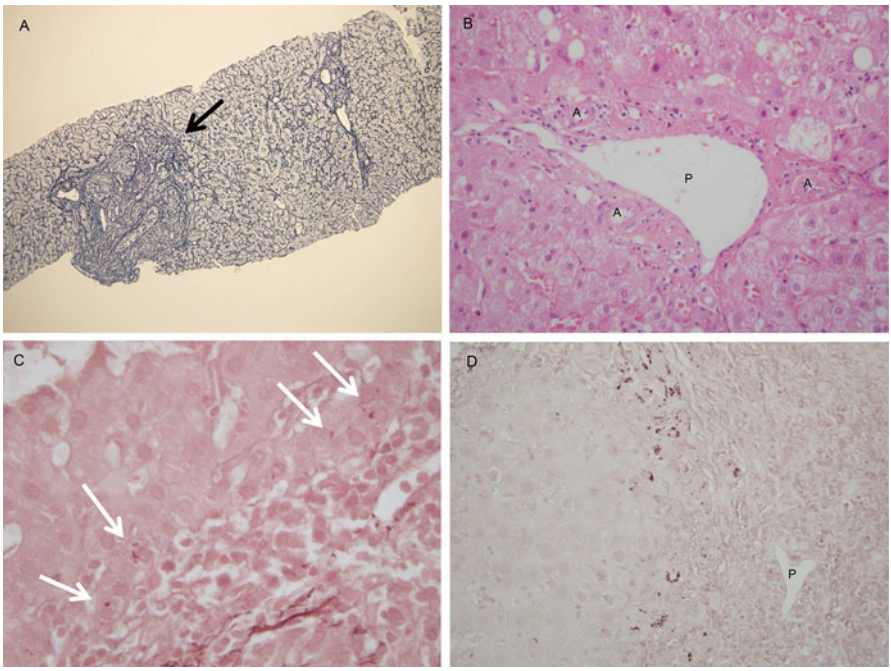

Fig. 16.1 Histological lesions defining the stage of the new evaluation system. (**a**) Portal tract showing fibrous enlargement without fibrous septa formation (*black arrow*) (fibrosis score 1) (reticulum staining, ×40 magnification). (**b**) No interlobular bile ducts are found in this portal tract (bile duct loss) [hematoxylin and eosin (H&E) staining, ×400 magnification]. (**c**) and (**d**) Orcein-positive granules are deposited in zone 1 hepatocytes around 1 portal tract. (**c**) A couple of zone 1 hepatocytes showed orcein-positive granules in their cytoplasm (*white arrows*). In this case, such deposition was found in zone 1 hepatocytes around <1/3 of portal tracts in the liver biopsy specimen (score 1) (orcein staining, ×1,000 magnification). (**d**) Orcein-positive granules are deposited in most zone 1 hepatocytes around one portal tract. In this case, such deposition was found in zone 1 hepatocytes around >2/3 of portal tracts in the liver biopsy specimen (score 3) (orcein staining, ×400 magnification). Score 2 of deposition of orcein-positive granules represents the amount of deposition in zone 1 hepatocytes in 1/3–2/3 of portal tracts between panels (**c**) (score 1) and (**d**) (score 3). *A* hepatic artery, *P* portal vein (from Kakuda et al. [28] and Harada et al. [27])

Bile duct loss is one of the characteristic features of PBC resulting from immune-mediated biliary damage. The degree of bile duct loss reflects the subsequent development of cholestasis. In addition, Kumagi et al. [16] reported bile duct loss or ductopenia (i.e., more than 50 % loss of interlobular bile ducts) as a predictor of a biochemical response to UDCA as well as histological progression of PBC. For the scoring of bile duct loss, the presence of arteries unaccompanied by ducts is a useful, yet rough, marker of bile duct loss, as shown in Fig. 16.1b. If a bile duct is ambiguous by hematoxylin and eosin staining, immunostaining of a biliary marker, such as cytokeratin 19, is helpful for the detection or identification of bile ducts in portal tracts.

16.3.1.3 Scoring of Deposition of Orcein-Positive Granules

A score of 0 indicates no deposition of orcein-positive granules in periportal hepatocytes. A score of 1 indicates deposition in zone 1 hepatocytes of <1/3 of portal tracts, as shown in Fig. 16.1c, and a score of 3 indicates deposition in zone 1 hepatocytes of >2/3 of portal tracts (Fig. 16.1d). A score of 2 is that between scores 1 and 3.

Roll et al. reported cholestasis to be an adverse prognostic factor of PBC [17]. Orcein-positive granules are copper-binding proteins that reflect chronic cholestasis [24]. They are visible as dark brownish coarse granules by orcein staining and are located in hepatocytes at mainly periportal areas. It is easy to distinguish orcein-positive granules from hepatitis B surface antigen and lipofuscin pigments [25]. The presence of orcein-positive granules is even detectable in the relatively early stages of PBC and is a sensitive marker of cholestasis. The incidence of deposition of orcein-positive granules is higher than that of the other findings representing cholestasis, such as Mallory bodies, bile plugs, and feathery degeneration. Those findings are usually evident and extensive at advanced stages. The amount of deposition of orcein-positive granules becomes more severe and extensive with progression of the disease [24]. Therefore, the evaluation of deposition of orcein-positive granules is useful for the diagnosis of PBC but also for the evaluation of PBC progression.

16.3.1.4 Cholangitis Activity (CA)

In the original and revised grading system, grade 0 indicates the absence of evident chronic cholangitis or CNSDC, although ambiguous bile duct damage and mild biliary epithelial damage are included in grade 0. In the original version, a grade of 1, 2, or 3 indicates that evident cholangitis, including CNSDC, is observed in <1/3, 1/3–2/3, or >2/3 of portal tracts, respectively. In the revised version, grade 3 indicates that at least one damaged bile duct showing typical CNSDC or florid duct lesion is found in the liver biopsy. Damaged bile ducts with periductal epithelioid granulomas are included in CNSDC. Grades 1 and 2 have 1 and ≥2 lesions of "evident chronic cholangitis," respectively.

Chronic cholangitis is a primary and fundamental inflammatory bile duct lesion of PBC. Especially, chronic nonsuppurative cholangitis (CNSDC) is characteristic. CNSDC is defined by a marked damage to the epithelium of the bile ducts; this is manifested as disarrayed epithelia with swollen or eosinophilic shrunken, surrounded entirely by marked duct-oriented lymphoplasmacytic infiltration with or without periductal epithelioid granulomas (Fig. 16.2a, b). In the revised version of the new evaluation system for liver biopsy, CA 3 indicates that at least one damaged bile duct showing CNSDC [4, 11] was found in a needle biopsy. Therefore, "evident chronic cholangitis," which needs to be distinguished from CNSDC, is defined as nonspecific chronic cholangitis surrounded entirely by mild-to-moderate duct-oriented lymphoplasmacytes (Fig. 16.2c). This is similar to the type of cholangitis encountered occasionally in chronic viral hepatitis [1]. Interlobular bile

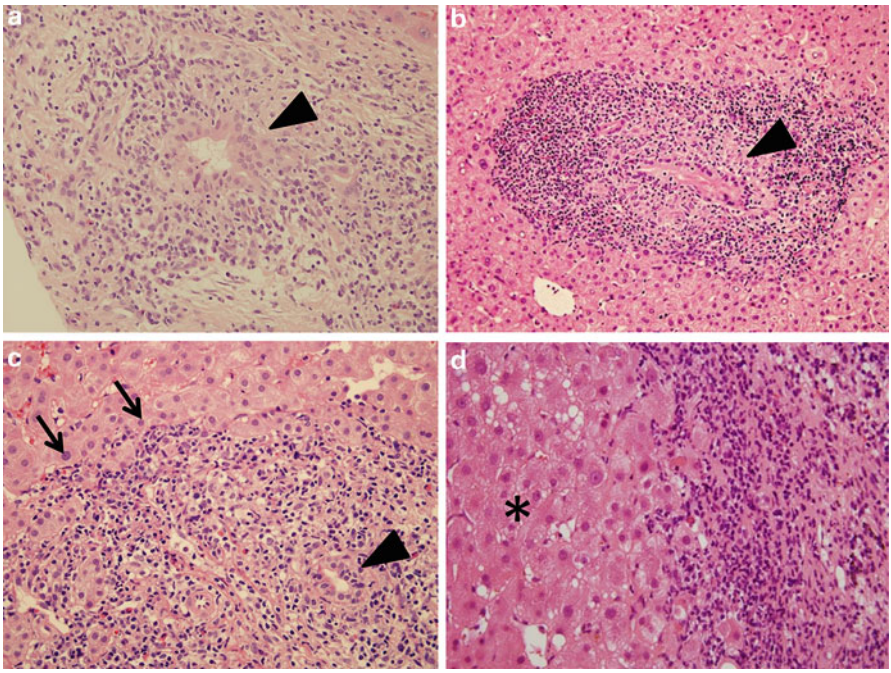

Fig. 16.2 Histological lesions defining the grades of the new evaluation system. (**a**) Chronic nonsuppurative cholangitis indicates cholangitis activity 3 (CA3) (*arrowhead*) (H&E staining, ×400 magnification). (**b**) CA3 includes granulomatous cholangitis (*arrowhead*) (H&E staining, ×400 magnification). (**c**) Evident chronic cholangitis (*arrowhead*). *Black arrow* denotes interface hepatitis affecting approximately ten hepatocytes (H&E staining, ×400 magnification). (**d**) Regenerative nodule (*asterisk*) with interface hepatitis affecting approximately 20 hepatocytes in a hepatitis activity 3 (HA3) case (H&E staining, ×400 magnification) (from Kakuda et al. [28] and Harada et al. [27])

ducts, which were surrounded by a small number of lymphoplasmacytes or were adjacent to infiltration of lymphoid cells in the portal tract, were not included in "evident chronic cholangitis."

16.3.1.5 Hepatitis Activity (HA)

In the original version, grade 0 indicates no interface hepatitis, while grades 1, 2, and 3 indicate the presence of interface hepatitis affecting $<1/3$, $1/3$–$2/3$, and $>2/3$ of the limiting plates of portal tracts, respectively. In the revised version, grade 0 means no interface hepatitis. Grades 1 and 2 indicate the presence of interface hepatitis affecting approximately ten continuous hepatocytes at the limiting plates of 1 and ≥ 2 portal tract(s) or fibrous septa, respectively. Grade 3 indicates the presence of interface hepatitis affecting more than 20 continuous hepatocytes at the limiting plate of >50 % of portal tracts or fibrous septa. As for lobular hepatitis, no or minimal lobular hepatitis is found in grade 0, mild-to-moderate lobular hepatitis in grade 1 or

2, and moderate lobular hepatitis in grade 3. Occasional zonal necrosis and bridging necrosis are regarded as grade 3 in both the original and revised versions.

Interface hepatitis is defined as lymphocytic interface activity showing damaged hepatocytes with lymphocyte infiltration affecting the interface of portal tracts or fibrous septa. This has been reported as a prognostic factor of PBC [13, 14]. In addition, PBC with prominent interface and lobular hepatitis is known as a hepatitic form of PBC or so-called overlapping syndrome [3, 26]. Thus, it is reasonable to evaluate the degree of hepatitis in PBC. In both the original and revised versions of the new evaluation system, interface hepatitis and lobular hepatitis are evaluated as hepatitis activity (HA) and are categorized into four grades. The criteria of HA in the revised version are more detailed than those in the original version because the original version showed a low kappa value in the analysis of interobserver agreement and the exact evaluation of necroinflammatory changes is important in the biopsy diagnosis of PBC [23].

16.4 The Effectiveness of the New Histological Staging and Grading System

We recently evaluated and validated a revised version of the new histological staging and grading system for liver biopsy. Harada et al. [27] investigated the distribution of each stage and grade, as well as performed the prognostic analysis of stage defined by the sum of two items (scores of fibrosis and bile duct loss) in a completely different cohort from that of Hiramatsu et al. [20]. Furthermore, Kakuda et al. [28] evaluated the new system defined by the sum of three items (scores of fibrosis, bile duct loss, and deposition of orcein-positive granules) in comparison with classical systems, namely Scheuer's and Ludwig's staging systems [11, 12].

In these two studies, we showed, first, that the distributions of cases belonging to individual stages of the new system were different from those of classical staging system(s) (Fig. 16.3). A considerable number of stage 1 cases belonging to the Scheuer/Ludwig systems were reclassified as stage 2 or 3 in the new system. The cases belonging to stage 1 of classical staging in which bile duct loss and/or deposition of orcein-positive granules was found in the absence of ductular proliferation or interface hepatitis were reclassified as stage 2 or more in the new staging system. In addition, several stage 3 cases of the Scheuer/Ludwig systems were reclassified as stage 4 because of extensive bile duct loss and/or deposition of orcein-positive granules despite the absence of cirrhotic changes. The former discrepancy may reflect histological heterogeneity in the PBC liver, and the latter may include the so-called premature ductopenic variant, which has a very rapid onset of ductopenia and severe icteric cholestasis preceding progression of fibrosis [3, 29, 30], or the patients with liver failure-type progression [31].

Importantly, the stage(s) of the new evaluation system correlated well with the prognosis of PBC patients compared with the classical system(s). Harada et al. (Fig. 16.4) and Kakuda et al. (Fig. 16.5) showed that the rates of survival or

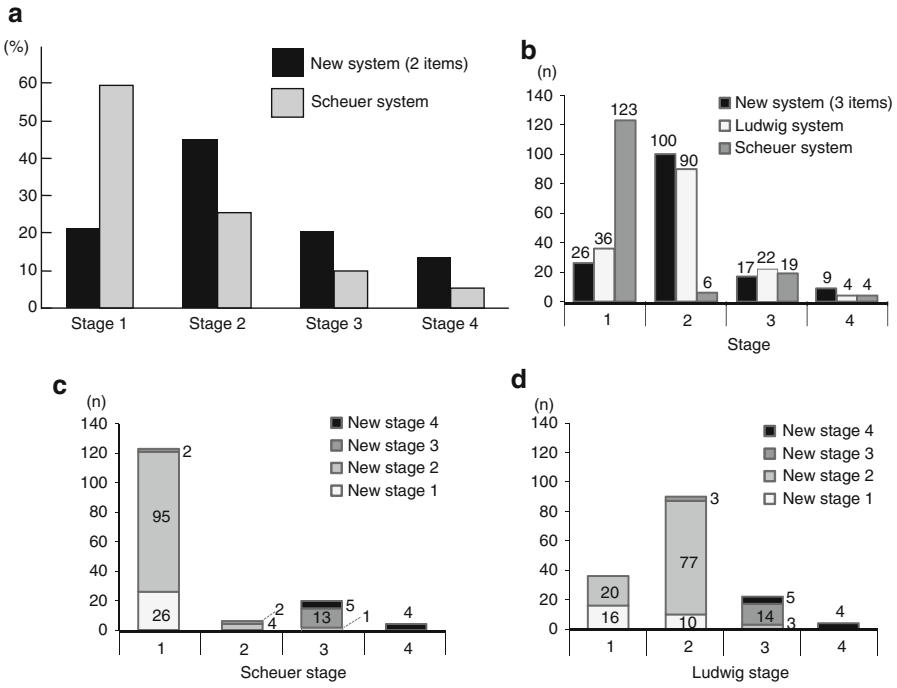

Fig. 16.3 Distribution of stages in the new staging system and classical systems. (**a**) Comparison of each stage for the new staging system using two histological criteria (fibrosis and bile duct loss) and Scheuer's system (partially modified from Harada et al. [27]). (**b–d**) Comparison of each stage for the new system defined by three items (fibrosis, bile duct loss, and deposition of orcein-positive granules) and for Scheuer's and Ludwig's systems (total 152 patients, from Kakuda et al. [28]). (**c**) and (**d**) Each population was categorized by the new system in each stage according to Scheuer's and Ludwig's systems, respectively (from Kakuda et al. [28] and Harada et al. [27], partially modified)

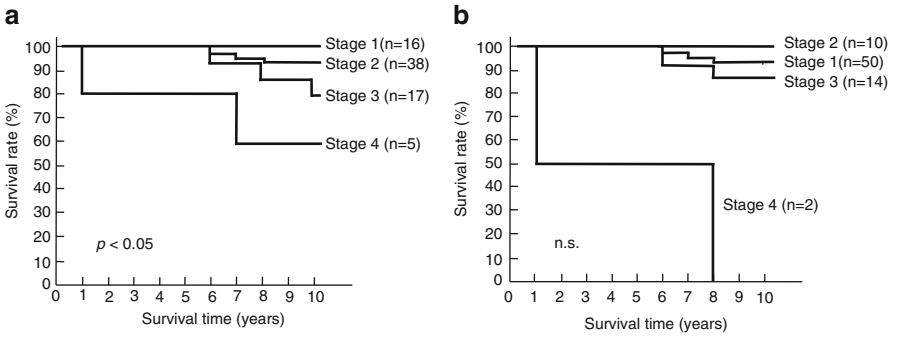

Fig. 16.4 Comparison of survival curves of stages 1–4 based on the (**a**) new staging system (defined by two items; scores of fibrosis and bile duct loss) and (**b**) Scheuer's system. In that study, endpoints were defined by death from liver failure or hepatocellular carcinoma and liver transplantation. All *p* values were calculated using the log rank test. *n.s.* not significant (partially modified from Harada et al. [27])

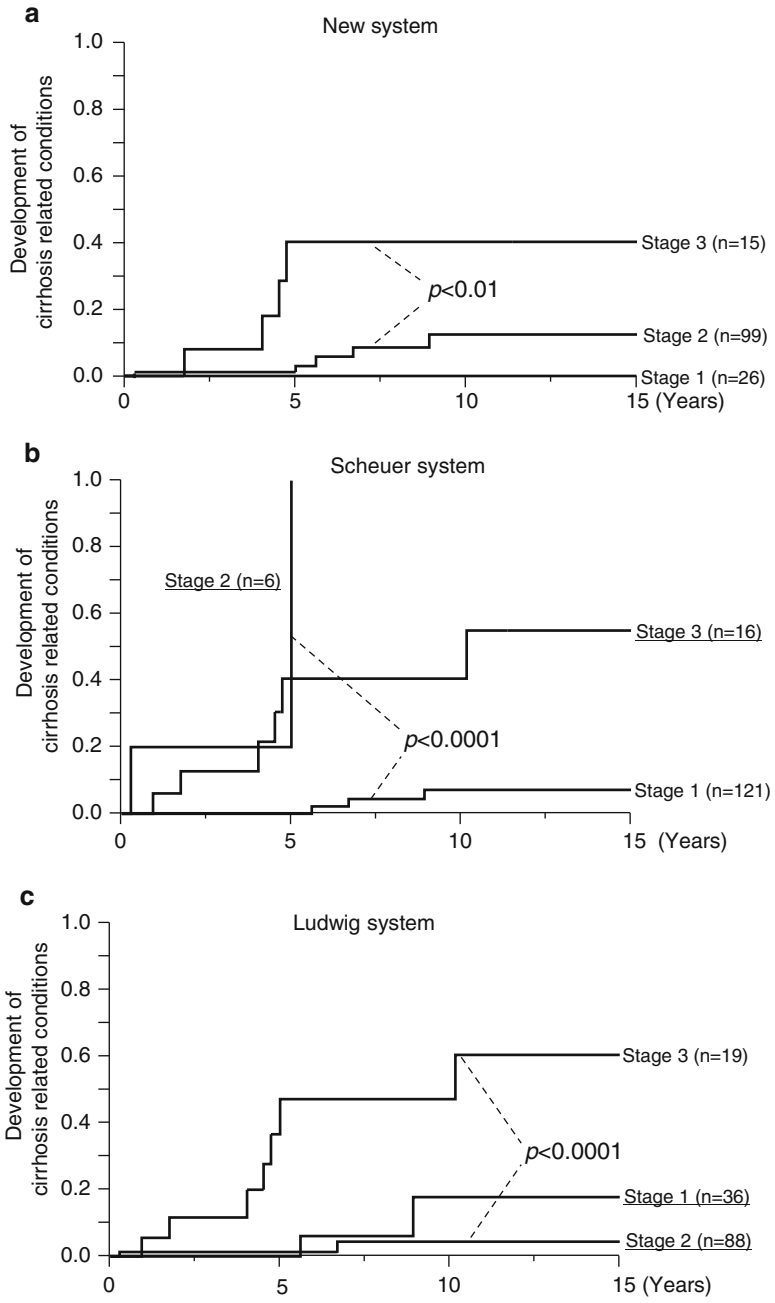

Fig. 16.5 Rates of development of cirrhosis-related conditions of noncirrhotic patients in each histological stage (total 152 patients). (**a**) New staging system (defined by three items; scores of fibrosis, bile duct loss, and deposition of orcein-positive granules). (**b**) Scheuer's system. (**c**) Ludwig's system. In that study, "cirrhosis-related conditions" defined by at least one of the following events: histologically proven cirrhosis or cirrhosis-related complications and/or symptoms, i.e., ascites, ruptured and/or endoscopically treated gastroesophageal varices, hepatic encephalopathy, hyperbilirubinemia ($\geq$2.0 mg/dL), or hepatocellular carcinoma. All p values were calculated using the log rank test (from Kakuda et al. [28])

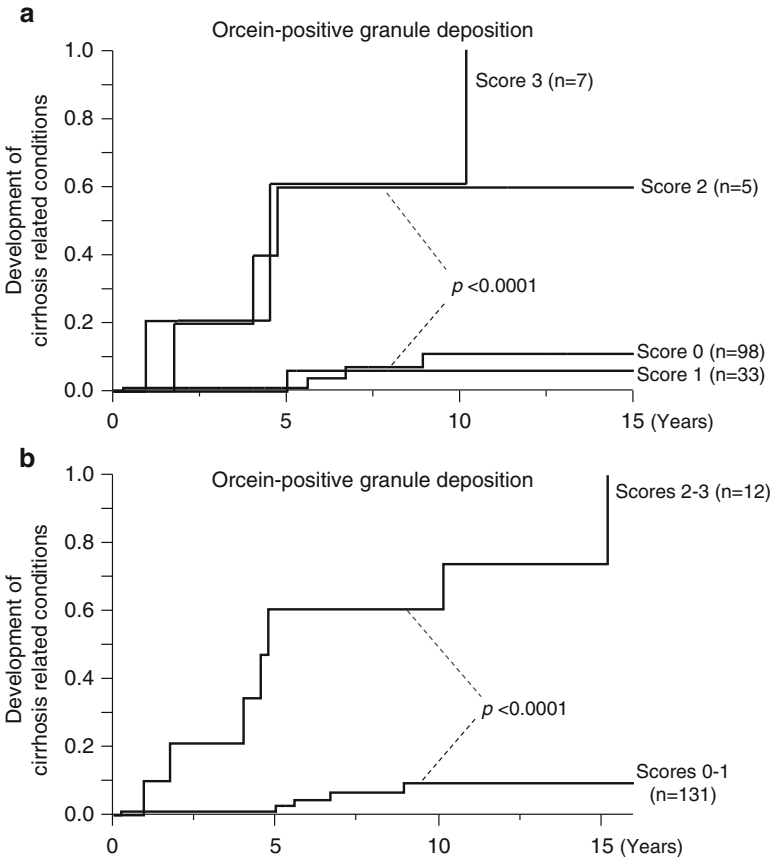

Fig. 16.6 Relationship between rates of development of cirrhosis-related conditions and the score of deposition of orcein-positive granules. (**a**) Each score and (**b**) comparison with scores 0–1 versus scores 2–3 of the deposition of orcein-positive granules. All *p* values were calculated using the log rank test (from Kakuda et al. [28])

development of cirrhosis-related conditions increased according to the stage progression of the new system, and patients with stage 1 (no progression) of the new system did not show an adverse outcome during follow-up. However, no such tendencies were observed in the classical staging systems, showing reversal between the rates of stages 1 and 2. In addition, the rate of development of cirrhosis-related conditions in patients with scores 2–3 of deposition of orcein-positive granules was significantly higher than in those with scores 0–1 ($p < 0.0001$; Fig. 16.6), which suggests that the degree of deposition of orcein-positive granules is a useful prognostic factor for PBC patients.

Moreover, using correlation coefficient analysis with blood biochemistry of PBC patients before UDCA therapy, Kakuda et al. [28] showed that the new evaluation

system combined with HA grade seems to reflect liver dysfunction such as cholestasis and the necroinflammatory activities of PBC.

Taken together, these facts suggest that the new staging and grading system can provide more accurate evaluation by taking into account pathological characteristics of PBC.

16.5 Perspectives and Conclusion

The new staging and grading system of PBC for needle liver biopsies has several advantages. In PBC, immunological processes affect hepatocytes (hepatitis) and bile ducts (cholangitis) may influence and determine the progression of PBC alone or in combination. In addition, the histopathological features of PBC are known to show heterogeneous distribution. In this context, histological evaluation using several histological features or factors in combination seems reasonable to evaluate the condition of PBC patients. However, there are several issues that remain to be solved in order to prove the usefulness and reliability of the new histological staging and grading system of PBC.

It is well known that nonresponders to UDCA have a poorer prognosis than responders [14, 15]. Further studies are necessary to investigate the relationship of this new staging and grading system as well as the clinical and biochemical response to UDCA therapy.

There have been several histological findings reportedly indicating poorer prognosis, as mentioned above. Among them, the new histological staging and grading system does not reflect the distinguishing condition of PBC patients with marked portal hypertension including those with portal hypertensive-type progression [31]. This is distinguished by nodular regenerative hyperplasia on histological examination [9, 32]. It is necessary to note with or without nodular regenerative hyperplasia in addition to the evaluation system.

Our previous investigation revealed a low kappa value of this new evaluation system at interobserver agreement analysis [23]. For staging, the kappa value was 0.385 (fair agreement) and the concordance rate was 63.9 %. For necroinflammatory activity, the kappa value and concordance rate were 0.110 (slight agreement) and 36.9 % for CA and 0.197 (slight agreement) and 47 % for HA, respectively. This means that more instruction and guidance are needed, especially for the grading of necroinflammatory activity in practice. We believe that our contribution to this book will help in this area.

The deposition of orcein-positive granules can be a predictor of poor prognosis, as shown in Fig. 16.6. The score of deposition of orcein-positive granules showed a higher kappa value than that of bile duct loss or fibrosis in the interobserver agreement study [23]. Although the staging system is defined by fibrosis and bile duct loss also showed correlation with outcome (Fig. 16.4), the score of deposition of orcein-positive granules seems necessary to evaluate PBC progression more precisely. It has been recognized for many years that there are technical problems

Table 16.5 Recommended staining method of orcein

1. Deparaffinize
2. Wash in running water (5 min)
3. A mixture of 0.25 % potassium permanganate and 0.2 % sulfuric acid (1 min)
4. Wash in running water (5 min)
5. 2 % oxalic acid (1 min)
6. Wash in running water (5 min)
7. Rinse in distilled water (5 min)
8. Orcein solution[a] (2–3 h)
9. 70 % ethanol (1 min)
10. Dehydrate, cleaning, mounting

[a]Orcein (Merck or Tokyo Chemical Industry) 0.5 g + 70 % ethanol 100 mL + concentrated hydrochloric acid 0.8 mL

with orcein staining and that there are variations in orcein staining results at every institution. However, no alternative staining has been found so far. Stains for copper, namely rubeanic acid and rhodanine, have less sensitivity than orcein [25]. We recently surveyed the methods of orcein staining at 11 institutions, including our laboratory, to standardize the staining method. Differences were observed in staining results according to the types of orcein reagents and reducers. The recommended staining method is as shown in Table 16.5.

Nowadays, a liver biopsy is not always necessary for diagnosing PBC because of the high specificity of antimitochondrial antibodies (AMA) or AMA-M2 antibodies, according to Japanese, American, and European practical guidelines for PBC management [33–35]. However, a liver biopsy remains necessary to diagnose PBC in certain patients because of AMA-negative, atypical clinical features or exclusion of other concomitant diseases such as nonalcoholic steatohepatitis. While liver biopsy is an invasive procedure, the benefits exceed its risk in many cases. Although the semiquantitative evaluation of several histological features is necessary in the new histological staging and grading system and this seems slightly time consuming and may be a little burdensome for the pathologist, it is relatively simple compared with the staging and grading of other chronic liver diseases such as nonalcoholic steatohepatitis. Furthermore, this new staging and grading system seems to be able to provide more information about the histological predictors of poorer prognosis and to evaluate the grading of hepatitis and cholangitis of PBC. Moreover, not only UDCA but also other new therapeutics aim to improve the liver histology; therefore, a more sophisticated and objective evaluation of liver biopsy histology is mandatory in this therapeutic situation and in clinical trials of PBC treatment [36]. The new histological staging and grading system aims for an objective evaluation process, and as such, many of the shortcomings inherent in the classical staging systems can be overcome. We believe that this new system can be reproducibly used for histological estimation in clinical trials.

In conclusion, the new staging and grading system proposed here may herald a new approach to the objective evaluation of histological progression and necroinflammatory activity of PBC. More routine and common usage of this system may lead to active discussion between pathologists and clinicians.

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Chapter 17
Autoantibodies in Primary Biliary Cirrhosis

Atsumasa Komori

Abstract Autoantibodies directed against a variety of mitochondrial antigens are a serological hallmark of primary biliary cirrhosis (PBC). Even with progress in the development of accurate and sensitive solid-phase assays for the detection of antimitochondrial antibody (AMA), a small but substantial number of patients are still regarded as having AMA-negative PBC. PBC-specific antinuclear antibodies (ANAs) are thus valuable as complementary serological markers for the definite diagnosis of AMA-negative PBC. Specific ANAs, particularly against proteins in the nuclear envelope, e.g., anti-gp210 antibody, possess additional and unexpected clinical significance in the prediction of PBC outcomes and possibly in disease activity; positivity for anti-gp210 antibody at the initial diagnosis is an independent predictive factor for poor prognosis and may influence the mode of disease progress. The combined use of particular ANAs in conjunction with an AMA should be advantageous for accurate and precise diagnoses and even for disease outcomes. The PBC landscape has been steadily changing with the progress in autoantibody research.

Keywords Anti-gp210 antibody • Antimitochondrial antibody (AMA) • Antinuclear antibody (ANA) • E2 subunits of the pyruvate dehydrogenase complex (PDC-E2)

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17.1 Introduction

With regard to diagnosis, what are the hallmarks of primary biliary cirrhosis (PBC)? Historically, pathognomonic chronic nonsuppurative destructive cholangitis resulting in ductopenia coupled with lobular cholestasis was used as such a hallmark. Thereafter, multilineage autoimmune reaction, specifically including the prototypic serological response against ubiquitously expressed mitochondrial antigens, was identified as accompanying the liver pathology. In addition, serum antimitochondrial antibodies (AMAs), specifically AMA-M2, were shown to have the highest disease sensitivity and specificity rates compared to other autoantibodies.

Considering these hallmarks, the fulfillment of two of the following three criteria, that is, elevation of cholestatic liver enzymes, compatible liver histology, and AMA positivity, was internationally accepted and introduced for the diagnosis of PBC [1, 2]. A product of this definition, stigmatic *AMA-negative* "probable" *PBC*, is still a matter of accurate diagnosis, and the absence of AMA detected by routine indirect immunofluorescence (IIF) does not have a specific impact on its clinical manifestation and natural history [3].

Autoantigen for AMA-M2, components of the 2-oxoacid dehydrogenase complex (2-OADC) family—which includes E2 subunits of the pyruvate dehydrogenase complex (PDC-E2)—was characterized and genetically cloned more than a quarter-century ago by Gershwin et al. [4], opening up a new era for sensitive AMA detection by solid-phase assays. However, sensitivity has not yet reached 100 %. Other autoantibodies found in PBC sera with relatively high specificity, such as antinuclear antibodies (ANAs) with multiple nuclear dots (MND) or a nuclear membrane/rim (NM) staining pattern, are gaining an importance for their complementary role in the diagnosis of PBC [5].

Moreover, several lines of evidence have indicated that the presence of ANA or a particularly specific ANA, including anti-gp210 as well as anti-centromere antibodies, was associated with distinctive outcomes among PBC patients [6]. This was not the case for AMA-M2, again highlighting the relevance of specific ANAs for disease phenotypes (Table 17.1). In addition, a recent genome-wide association study has already gone far beyond AMA to identify the specific loci responsible for distinct ANAs in PBC.

In this chapter, we review the recent literature for autoantibodies in PBC, with special attention to their roles in accurate diagnoses and in evaluations of disease status/prognosis. The use of autoantibodies as a hallmark of PBC in twenty-first-century clinical practice has already expanded from AMA, an original raison d'être for PBC.

Table 17.1 Clinically relevant autoantibodies in primary biliary cirrhosis

Autoantibody	Target antigen	Positive prevalence (%)	Presence in other diseases	Predictive for outcome
AMA	E2 subunits of 2-oxoacid dehydrogenases E1 subunits of 2-oxoacid dehydrogenases E3 binding protein of pyruvate dehydrogenase complex	90–95	–	–
ANA-MND	Sp100, PML, SUMO	~30	–	Under investigation
ANA-NM	Gp210, NUP62	~30	–	Probable
ANA-centromere	CENP A, B, and C	~30	Systemic sclerosis	Under investigation

AMA antimitochondrial antibody, *ANA* antinuclear antibody, *ANA-MND* ANA producing a multiple nuclear dot pattern, *ANA-NM* ANA producing a staining of the nuclear membrane/rim

17.2 Antimitochondrial Antibody for PBC: From Diagnosis to Pathogenesis—Still a Holy Grail?

This section is focused on two particular clinical and conceptual questions: "How frequent is AMA-negative PBC? Is AMA-negative PBC pathognomonically distinct PBC compared to PBC with AMA?"

In clinical practice, indirect immunofluorescence (IIF) AMA is measured first in patients with chronically elevated cholestatic liver enzymes in whom PBC is suspected. The detection of AMA is routinely performed on frozen sections of rodent liver, stomach, and kidney with the serially diluted serum of the patient. If a negative result is obtained at a 1:40–1:20 serum dilution as revealed in 5–15 % of clinical PBC sera, the widely accepted flowchart of AMA testing for PBC patients recommends the subsequent use of a number of solid-phase assays for AMA-M2, e.g., microtiter plate enzyme-linked immunosorbent assays (ELISAs), a membrane ELISAs, and a fluorescence bead-based assay, to further test any negative results [5].

The target antigens of AMA-M2 are components of 2-OADC; AMA-M2 recognizes the highly conserved lipoyl-binding E2 subunits of all three 2-OADC complexes: PDC-E2, 2-oxoglutarate dehydrogenase complex (OGDC), and branched-chain 2-oxoacid dehydrogenase complex (BCOADC) [7]. AMA-M2 also reacts to the E1 and E3 subunits, although to a lesser extent. Accordingly, solid-phase assays are based on either preparations of PDC from porcine or bovine heart, a mixture of recombinant human E2 subunits, or designer antigens comprised of all three E2 enzymes (MIT3 antigens) [8].

Oertelt et al. reported that 20 % of *rigorously* proven AMA-negative patients (i.e., the absence of antimitochondrial reactivity in all three of IIF, Western blotting, and ELISA assays using recombinant autoantigens) were revealed to be AMA-M2

positive by a highly sensitive fluorescence bead-based assay with MIT3 antigens [9]. Taking into account the patients who have antibodies against only the PDC-E1/E3 subunits, the rate of genuine AMA-negative PBC patients might be fewer. Still, a small (~5 %) but substantial number of PBC patients remain AMA-negative. Should we consider those patients as having pathognomonically distinct PBC?

Pioneering works by Shimoda et al. showed that the PDC-E2 peptide 163–176 is a major autoepitope for $CD4^+$ T cells under restriction with HLA DR53 [10]. They subsequently demonstrated that costimulation-independent but not costimulation-dependent PDC-E2 autoreactive $CD4^+$ T cells were disease-specific in PBC [11]. The frequency of costimulation-independent $CD4^+$ T cells specific to PDC-E2 peptide 163–176 was recently quantitated in AMA^-/HLA $DR53^+$ patients; AMA^- was confirmed by negative results with both IIF and MIT3 ELISA [12]. With the use of HLADR53-transfected mouse L cells as antigen-presenting cells, the PDC-E2 $CD4^+$ T-cell frequency and capacity for IFN-γ production were shown to be equivalent, irrespective of AMA positivity [12]. Collectively, these findings suggest that the molecular pathology of PBC always involves the mitochondria and even a highly sensitive solid-phase assay for AMA-M2 cannot confirm the diagnosis of AMA^- PBC.

Several research groups proposed that the magnitude and severity of PBC are associated with IgA class [13] and IgG3 subclass AMA [14]. However, the determination of the class (including IgM) and subclass levels for AMA awaits further verification for their current limited diagnostic values.

17.3 Antinuclear Antibody for PBC: Complementary for Definite Diagnosis or More?

The detection of ANAs by IIF in patients with PBC is routinely performed with HEp2 cells, as in other autoimmune liver diseases (AILDs). Among the different nuclear staining patterns, do some possess positive predictive values for the diagnosis of PBC? If so, what might be the underlying mechanisms between such ANAs and AMA with regard to the pathogenesis of PBC?

On the way to the thorough elucidation of AMA and their antigens, two distinct PBC-specific ANA IIF patterns were identified [5]: "multiple nuclear dots (MND)" and "nuclear membrane/rim (NM)." The MND pattern corresponds to the target antigens Sp100, Sp140, promyelocytic leukemia protein (PML), and small ubiquitin-like modifiers (SUMO), and the NM pattern was the result of reactivity against gp210 and nucleoporin p62. Though both ANA types can be demonstrated in a subset of PBC patients (~30 %), significant disease specificity was recognized [5]. These results prompted clinicians to evaluate whether PBC-specific ANAs could be useful as complementary markers in AMA^- PBC for definite diagnosis. The latest challenge has been performed with a combinatorial ELISA assay that uses purified

gp210 and Sp100 in conjunction with recombinant MIT3 antigens (PBC Screen, Inova Diagnostics, San Diego, CA). Bizzaro et al. recently reported that 44 of 100 IIF AMA-negative PBC patients revealed positive results with the PBC Screen [15]. The sensitivity was 43 % and the subdivided rate with monospecific IgG ELISA assays was determined as follows: 18 % for pMIT3, 9 % for gp210, and 23 % for Sp100. Moreover, the concordance rate among those subjects between each distinct ANA IIF pattern and the gp210 or Sp100 ELISA was greater than 90 %. As such, a diagnostic benefit for probable AMA^- PBC cases could be expected with the implementation of PBC-associated ANAs, using either cost-effective first-line IIF or solid-phase assays. In addition, the simultaneous detection of major PBC-specific ANAs and AMA by an ELISA might replace IIF as an unified diagnostic measure in the future.

The biological significance of the emergence of specific ANAs in PBC patients has been elucidated under the concept of "molecular mimicry." Hypothesizing the presence of an antigenic mimicry peptide between mitochondrial and nuclear antigens, Shimoda et al. selected T-cell clones by a peptide corresponding to PDC-E2 and then analyzed their reactivity to mimicry peptides which were originated from other mitochondrial and nuclear autoantigens [16]. Their finding that five peptides from gp210 and one from Sp100 cross-reacted with the PDC-E2-driven T-cell clones suggested that the generation of ANAs is due to the molecular mimicry of mitochondrial peptides.

Besides PBC-specific ANAs, other nonspecific ANAs have been routinely used for the diagnosis of comorbid extrahepatic autoimmune diseases, especially to confirm overlapping Sjogren syndrome and systemic sclerosis [17]. Agmon-Levin et al. comprehensively evaluated serum autoantibodies in PBC with a magnetic bead-based multiplex detection system [18], and the prevalence of Ig-ANA for different autoantigens was demonstrated as follows: anti-dsDNA, 22 %; anti-Sm, 7 %; anti-chromatin 25 %; anti-ribosomal P, 5 %; anti-RNP, 5 %; anti-SmRNP, 8 %; anti-Ro/SSA, 10 %; anti-La/SSB, 7 %; anti-centromere, 18 %; anti-Scl-70, 3 %; and anti-Jo-1, 1.5 %. After the finding that anti-SSA/anti-SSB and anti-centromere/anti-Scl-70 were recognized as specific markers for Sjogren syndrome and systemic sclerosis, respectively, *anti-dsDNA* was recently examined for its potential to differentiate autoimmune hepatitis (AIH)/PBC overlap syndrome (OS) from pure PBC. Muratori et al. reported that the prevalence of anti-dsDNA was significantly higher (60 %) in AIH/PBC OS patients than in PBC (4 %) and AIH (26 %) patients [19]. In addition, double positivity for AMA and anti-dsDNA was present in 47 % of their AIH/PBC OS patients, compared to 2 % in PBC or AIH. In light of the discrepancy that exists in the positive rate of anti-dsDNA among PBC patients between the studies by Muratori et al. and Agmon-Levin et al. [18], presumably due to the difference of procedures (i.e., IIF with *Crithidia luciliae* and multiplex detection, respectively), the clinical usefulness of such nonspecific ANAs for the diagnosis of AIH/PBC OS awaits further validation.

Finally, the abovementioned comprehensive survey for serum autoantibodies in PBC did not reveal a significant difference in the prevalence of nonspecific ANA between AMA^+ and rigorously proven AMA^- PBC (42 vs. 61 %). A comparable

result was reported by Oertelt et al.; the prevalence of positive ANA by IIF in similar AMA$^-$ PBC subjects was 45.8 % [9]. Again, even with highly sensitive fluorescence bead-based methods to detect both AMAs and ANAs, there still exist genuine AMA-ANA-negative PBC patients. Novel autoantigens other than ANAs are of importance in the full complementation for the serological diagnosis of PBC.

17.4 Antibodies Against Novel Autoantigens in PBC: Along the Way in the Quest for Complete Serodiagnosis?

With a human proteome microarray composed of about 17,000 full-length recombinant proteins, Hu et al. recently identified six novel PBC autoantigens with high sensitivities and specificities, i.e., the nuclear proteins Kelch-like protein 7 (KLHL7), KLH12, and the zinc-finger and BTB domain-containing protein 2 (ZBTB2); the outer mitochondrial membrane protein hexokinase 1(HK1) isoform I; and cytosolic HK II and ELF2C1 (Argonaute 1) [20]. Four of these six proteins displayed high positive rates in PBC ($\geq$35 %), along with high specificity over other liver diseases, including AIH. The more nuclear autoantigens that are tested in the laboratory, the better for the definite serodiagnosis of PBC. The combination of KLHL7 and gp210 led to positive signals in 47.8 % of the M2-negative PBC patients.

The IIF pattern "cytoplasmic discrete speckled (CDS)" is not common and is sometimes difficult to detect, possibly due to being masked by the presence of an AMA [21]; one of the targets of this particular IIF pattern is mRNA-processing bodies known as GW bodies (GWBs) or P-bodies, recently gaining attention for a key role in the RNA interference machinery (RNAi). Stinton et al. demonstrated by a laser-bead immunoassay that RAP55 (28 %), GW1 (12 %), and GRASP-1 (17 %) were the most common targets of proteins in GWBs, reacted against PBC sera [21]. Coupled with Hu's identification of another protein (ELF2C1) within the RNAi machinery as an autoantigen in PBC, autoantibodies against component GWBs require further investigation for their relevance in the diagnosis and/or estimation of prognosis of PBC.

17.5 The Clinical Significance of Autoantibodies in PBC: Part I—Are the Prevalence, Quality, and Chronological Fate of AMA Clues to the Pathogenesis?

As described above, positive AMA itself does not confer a specific PBC phenotype, compared with negative subjects. Nevertheless, a recent report from Dellavance et al. indicated that the AMA profile, i.e., the avidity and titer, was more robust in

definite PBC than in AMA-positive biochemically normal individuals [22]. To clarify the evolution of PBC from the initial events of the disease until pathological manifestation, AMAs still impart important information: (1) loss of tolerance, (2) exposure to endogenously or exogenously modified mimicked antigens, and (3) the maintenance of autoreactive B cells, two of which are critically influenced by genetic susceptibility.

Mattailia et al. determined the prevalence of AMA in a northern Italian healthy population, with a pMIT3-ELISA [23]. Among 1,530 people, nine (0.5 %) reacted to MIT3 by ELISA, and AMA reactivity was primarily IgM and IgA. In eight of these nine subjects, the epitope of PDC-E2 against AMA differed from patients with histologically proven PBC, but the remaining subject had an AMA profile identical to typical PBC. Regardless of the identity of the epitope, the reactivity had a wider AMA pattern after a period of 8–14 months in terms of Ig isotypes/IgG subclass as well as in terms of antigen determinant spreading.

The emergence of an AMA (anti-PDC-E2) with distinct epitope specificity was also reported in patients with multiple myeloma (MM) or chronic leukemia who underwent allogenic bone marrow transplantation and manifested good responses to the subsequent donor lymphocyte infusions (DLIs) [24], suggesting that the mechanisms leading to the loss of tolerance in these patients are likely to be distinct from those underlying PBC.

In addition, an AMA quantified by MIT3-ELISA was demonstrated to be positive in 28 (40.6 %) of 69 acute liver failure (ALF) patients, although the positivity was transient [25]. Positive AMA was found from an early post-ALF stage (days 1–4), with very high titer ($\times$2,560) in certain cases, but only one out of 14 serum samples obtained at 24 months remained AMA-reactive. Antigen specificity was dominated in PDC-E2, and the frequency of positive AMA was comparable among the ALF patients with distinct etiologies, and there were no gender-related differences.

Though such aberrant AMA production was likely to be the result of the overexpression of mitochondrial antigens in MM cells or the oxidative modification of mitochondrial antigens in severe liver injury, genetic susceptibility may additionally play a role in the sustained production of an AMA. Lazaridis et al. analyzed the prevalence of AMA in the first-degree relatives (FDRs) of PBC patients in the USA [26]; the prevalence of AMA in the FDRs was significantly higher than that in controls (13.1 vs. 1 %). Greek FDRs of PBC patients displayed similar increases in AMA, but not in a PBC-specific ANA [27]. Collectively, the above-described findings indicate that the prevalence, quality, and chronological fate of the AMAs described in healthy subjects, patients with certain non-PBC diseases, or the FDRs of PBC patients highlight the disease-specific evolution of AMA production in PBC.

17.6 The Clinical Significance of Autoantibodies in PBC: Part II

17.6.1 The Association of Antinuclear Membrane/Anti-gp210 Antibody with the Disease Phenotype of PBC

Is a particular biochemical profile or disease phenotype associated with a specific ANA, unlike AMAs? In addition, does a certain ANA emerge in the course of disease progression? Anti-NM antibodies, especially anti-gp210 antibody, and anti-centromere antibody have been investigated extensively and are considered promising, and anti-PML and anti-Sp100 (MND-associated autoantibodies) are still under investigation.

In 1998, a 200-kDa autoantigen in the nuclear envelope was reported to react with nearly 50 % of sera from patients with PBC, with high specificity [28, 29]; thereafter, the Brobel group revealed that the antigen is gp210 nuclear pore protein [30]. Over 10 years from the initial discovery, Ito et al. performed a retrospective cohort study to determine the prevalence of anti-gp210 antibody in Japanese patients and more importantly to analyze whether the positive test was associated with disease prognosis [31]. In 113 patients with PBC, the prevalences of the positive antinuclear envelope protein by IIF and by Western blotting (WB; with 1:200 diluted sera) were 27.4 and 21.6 %, respectively. Notably, of 86 early-stage (Scheuer's classification, I and II) PBC patients, five of the 17 (29.4 %) patients with positive gp-210 antibody at the initial diagnosis progressed to jaundice, whereas only six of the 69 (8.7 %) patients with a negative result did so ($p < 0.05$), indicating for the first time that the presence of anti-gp210 antibody is a prognostic marker for poor outcome of PBC at the time of diagnosis. The appearance and titer of the anti-gp210 antibody did not vary in the chronological analysis of the patients' sera.

This serendipitous finding prompted many clinicians to test the data in case-control studies or retrospective cohort analyses. Other than the study by Miyachi et al., who found no significant association between WB-detected anti-p210 antibody and the advanced stage of PBC (Scheuer's classification, stage 4) [32], six studies including that by Ito et al. have confirmed that the advanced clinical stage (i.e., four analyses using histology with either the Scheuer or Ludwig classification, and two studies that used hepatic reserve) is associated with an increased prevalence of positive anti-gp210 antibody [31, 33–38] (Table 17.2). In addition, although the inclusion criteria (early stage or all stages) and the mode of detection for the antibody (IIF, WB, or ELISA) varied among the studies, five of these six retrospective cohort studies found that the presence of anti-gp210 antibody at the time of diagnosis is a prognostic marker for poor outcome [6, 31, 36–38].

Notably, Nakamura et al. established an in-house gp-210 ELISA with human gp210 C-terminal 25-mer peptide (SPNALPPARKASPPSGLWSPAYASH) and analyzed 23 patients with their 1:100 diluted sera for chronological changes in

Table 17.2 Association of the presence of antinuclear pore/envelope or anti-gp210 antibody and poor outcome of PBC

Author (reference)	Year	Ethnical background	Number of patients	Stage[a]	Observational period (median, range) or (mean ± SD)	Primary endpoint[a]	Method of detection	Antibody	OR (CI)[b]
Ito [31]	1998	Japanese	86	SI and SII	80.9 ± 52.9[c]	Jaundice	IIF/WB	Anti-gp210	$p < 0.05$ (Fisher's exact test)
Yang [39]	2004	North American (Toronto:T/ Mayo:M)	403	All	5.7[d] (1–12.3):T 11[d] (1–16):M	Liver failure	IIF	IIF NPC	1.44 (0.88, 2.36)
Wesierska-Gadek [36]	2006	Italian	127	All	11.2[d] (4.1–13.5)	Liver-related death or OLT	WB	Anti-NPC	2.70 (1.20, 6.00)
Nakamura [6]	2007	Japanese	217	SI and SII	60.5[c] (1–292)	Liver-related progression (i.e., hepatic failure-type progression)	ELISA	Anti-gp210	7.09 (2.65, 20.21)
Gao [37]	2008	Chinese	140	All	32[c] (2–50)	Hepatic failure-type progression	ELISA	Anti-gp210	9.85 (1.07, 90.90)
Sfakianaki [38]	2010	Greek	147	All	89.5[c] (1–240)	Liver-related death	IIF	Anti-NEA	$p = 0.043$ (Breslow test)

[a]Sub-analysis with early-stage patients was selected, if present in each study
[b]Logistic-regression analysis, otherwise indicated
[c]month
[d]year

the titer of anti-gp210 antibody [35]. Two groups of patients were revealed: patients in whom the anti-gp210 antibody titers were sustained at a high level (more than 50 units per mL) during the observation period (group A) and those in whom the antibody titers decreased to normal or to a low level (less than 50 units per mL) after the initiation of ursodeoxycholic acid (UDCA) treatment and remained low (group B). Remarkably, the prognosis—evaluated by the cumulative rate of end-stage hepatic failure—was comparable between the patients negative for anti-gp210 antibody at the initial diagnosis and group B, whereas group A was revealed to have significantly worse prognoses. The fluctuating nature of anti-gp210 antibody in the clinical course of PBC was inconsistent with the previous report by Ito et al. [31], probably due to a difference in the sensitivity of the quantification for anti-gp210 antibody, namely, between the solid-phase analysis with the antigenic peptide and Western blotting.

Nakamura et al. further extended the analysis to determine whether the positive anti-gp210 antibody in early-stage PBC patients who fulfill Scheuer's classification 1 or 2 plays a role in distinct outcomes of PBC, namely hepatic failure-type progression (liver transplantation or death of end-stage liver failure) and portal hypertension-type progression (emergence of esophagogastric varices) [6].

They found that positivity for anti-gp210 antibody in the early-stage patients at initial diagnosis was associated with a high risk of hepatic failure-type outcomes, whereas that of anti-centromere antibody was associated with a high risk of portal hypertension-type outcomes: the odds ratios (95 % confidence interval) were 33.777 (5.930, 636.745) and 4.202 (1.307, 14.763), respectively. Although precise details in the clinical course were lacking in that report, the anti-gp210 antibody-related outcome might be reminiscent of a particular clinical phenotype of PBC, i.e., the pre-ductopenic variant that follows a rapidly progressing clinical course even in younger patients, ultimately leading to hepatic failure with profound jaundice [40].

Collectively, the above-described findings showed that the presence of anti-gp210 antibody is positively associated with the disease progression, and the decrease in titer probably has an association with the treatment response to UDCA. Moreover, positivity to anti-gp210 antibody at the time of diagnosis is an independent prognostic factor for poor prognosis of PBC and may influence the landscape of PBC outcomes. Prospective cohort analyses using the same method of antibody detection are of particular importance in the future, along with analyses of the biological implications of these associations.

17.6.2 The Association of Anti-centromere and Anti-MND Antibodies with the Disease Phenotype of PBC

In 2004, Yang et al. first demonstrated in a retrospective analysis that the presence of anti-centromere antibody among PBC patients (in a Toronto and Mayo cohort) was associated with liver failure and that the time to liver failure was shorter in

anti-centromere antibody-positive patients than in the negative patients [39]. Conversely, in their attempt to determine the significance of comorbid systemic sclerosis (SSc) on the outcome of PBC, Rigamonti et al. observed that the risk of liver transplantation- or liver-associated death was significantly lower in PBC patients with SSc—in whom the prevalence of anti-centromere antibody was 70 %—than in those without SSc; liver disease had a slower progression in the PBC-SSC patients compared with matched PBC-alone patients [41].

The role of anti-centromere antibody in the outcome of PBC was then reevaluated by Nakamura et al., giving an answer albeit in a twisted manner; the presence of anti-centromere antibody confers a significantly high risk of portal hypertension-type progression in PBC patients, whereas it tends to provide greater probability of survival free of liver transplantation [6].

In 2013, a single-center prospective observational study in China confirmed that the presence of anti-centromere antibody is an independent predictor of hepatic decompensation in PBC patients, although this study lacked an analysis for the "hard" endpoint, i.e., liver transplantation- or liver-associated death [42]. Compared with anti-gp210 antibody, the specific role of anti-centromere antibody in the outcome of PBC requires further validation.

Lastly, how relevant is the presence of PBC-specific MND-associated autoantibodies to the outcome of PBC? Mytilinaiou recently established a robust and accurate immunoassay for the simultaneous detection of anti-PML and anti-Sp100 autoantibodies [43]. PML and Sp100 autoantibodies were present in approx. 23 % of AMA-positive PBC patients with highly specificity; anti-PML/Sp100 double-positive cases were more frequently observed in cirrhotic patients, accompanying a positive correlation between anti-Sp100 levels and Mayo risk score.

17.7 Autoantibodies in PBC for Future Clinical Practice

Neither the clinical practice guidelines for PBC published by the American Association for the Study of Liver Diseases nor those for chronic cholestatic liver diseases issued by the European Association for the Study of the Liver recommend the combinatorial implementation of a particular ANA, such as antibodies against gp210, centromere, and Sp100 for special assessments of prognosis [1, 2], possibly due to unresolved issues or pitfalls (Table 17.3). Instead, the on-treatment response to UDCA, evaluated using independently proposed criteria, is of particular interest to clinicians to predict the outcome of PBC. Nakamura et al. recently reported that patients' biochemical response to treatment is also influenced by the anti-gp210 antibody status at baseline [44]. Though the biological plausibility for such causality is yet to be determined, a combination of the presence of particular autoantibodies and early treatment response may help clinicians stratify the risk of poor outcome in patients and facilitate appropriate designs for future clinical trials with difficult-to-treat PBC patients.

Table 17.3 Unresolved issues or pitfalls in the predictive autoantibodies in PBC

Low negative predictability
Overinterpretation, resulting in unnecessary treatment modification (e.g., add-on with fibrates)
Variable serum levels during disease course
Appearance and disappearance during disease course
Lack of correlation between antibody titer and disease severity or outcome
Lack of validated cutoff level
Lack of standardized assays

As mentioned in the previous section, the emergence of autoantibodies in autoimmune disease is influenced by genetic susceptibility to the loss of tolerance and to the maintenance of autoreactive B cells. On the contrary, disease development and progression itself should also be determined by host and environmental interactions. Accordingly, the causal association between autoantibodies and prognosis in PBC could be either modified or confounded by genetic background.

Nakamura et al. observed in a Japanese multicenter retrospective cohort that *HLA-DRB1* polymorphisms are significantly associated with not only the disease development and progression of PBC but also the production of anti-gp210 and anti-centromere antibody [45]. They also postulated that the specific combination of *HLA* allele and autoantibody status in patients might confer a significant risk for different patterns of progression (i.e., jaundice or non-jaundice type). The associations of disease susceptibility, tendency to progress, and the production of anti-gp210 antibody with *HLA* polymorphism were analyzed elsewhere [46, 47].

Precision medicine in PBC to predict patients' outcomes and to perform tailor-made interventions could be introduced as genome-wide association study progress; surrogate markers themselves should be regulated by multiple upstream signal transductions and upon causal genetic background. We anticipate that in-depth analyses of the web of causality involving autoantibodies in PBC could lead to breakthroughs in future treatment.

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Chapter 18
Diagnosis and UDCA Treatment of Primary Biliary Cirrhosis

Atsushi Tanaka

Abstract The diagnosis of PBC is largely based on three features: persistent elevation of cholestatic liver enzymes (ALP and/or GGT), detectable anti-mitochondrial autoantibodies (AMAs), and liver histology. The clinical guidelines from the AASLD, EASL, and Japan agree that the diagnosis of PBC can be made in patients who met two criteria from the above three clinical features. In atypical cases in whom serum AMAs are negative or elevation of AST and/or ALT is remarkable a liver biopsy is mandatory. The role of ursodeoxycholic acid (UDCA) for treatment of PBC is well established by several clinical trials, and UDCA is the only approved drug for PBC worldwide. There are, however, some patients with PBC who are refractory to UDCA treatment, and the prognosis is shown to be worse in these cases. The second-line treatment is strongly warranted and bezafibrate is a robust candidate for this, although long-term effect of bezafibrate has not been demonstrated.

Keywords Anti-mitochondrial antibodies • Bezafibrate • Clinical guidelines • Pyruvate dehydrogenase complex • Ursodeoxycholic acid

18.1 Introduction

Primary biliary cirrhosis (PBC) is a chronic and progressive cholestatic liver disease, potentially resulting in liver failure and mortality without liver transplantation [1]. The diagnosis of PBC is usually not a hard task because of presence of autoantibodies highly specific for PBC, anti-mitochondrial autoantibodies (AMAs) [2], while atypical cases, for instance, AMA-negative PBC or PBC with features with autoimmune hepatitis (AIH), are present. As for treatment, several lines of

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evidences demonstrate that ursodeoxycholic acid (UDCA) is effective for improvement of long-term prognosis of patients with PBC, and currently UDCA is the only accepted first-line medical treatment for PBC in several clinical guidelines [1, 3, 4]. In this chapter the diagnosis and treatment with UDCA will be discussed.

18.2 Diagnosis of PBC

In most of cases the diagnosis of PBC is not problematic, because of the presence of anti-mitochondrial antibodies (AMAs), highly specific for PBC. The algorism for making a diagnosis of PBC is shown in Fig. 18.1. The diagnosis of PBC should be suspicious when there is elevation of cholestatic enzymes (ALP and/or GGT). It is important to rule out other etiologies including excess consumption of alcohol beverages and drug-induced liver injury with extensive medical interviews. Imaging studies also should be done to deny biliary obstruction or dilatation caused by gallstones or malignancies. Positive testing of AMA and/or liver histology confirms the diagnosis of PBC.

18.2.1 Symptoms

Patients with PBC are suffering from a variety of symptoms, even in early stages. These symptoms include general fatigue, pruritus, jaundice, and sicca syndrome. In patients with advanced PBC symptoms associated with cirrhosis may be present. However, a large part, approximately 70–80 %, of patients with PBC are recently diagnosed as asymptomatic PBC (aPBC), lacking any of the abovementioned symptoms. Nationwide surveys in Japan with a cohort of 8,509 patients with PBC

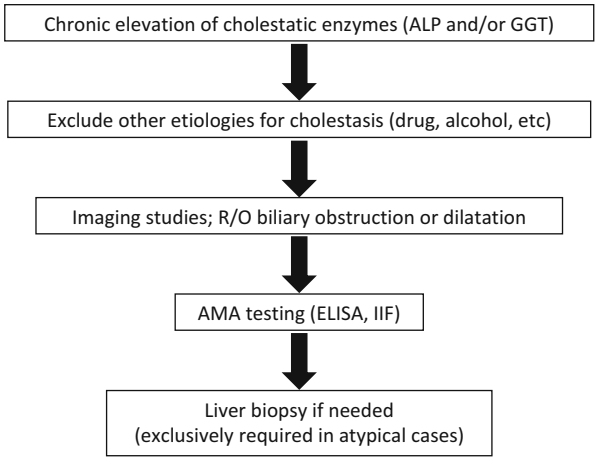

Fig. 18.1 Diagnostic algorithm of PBC

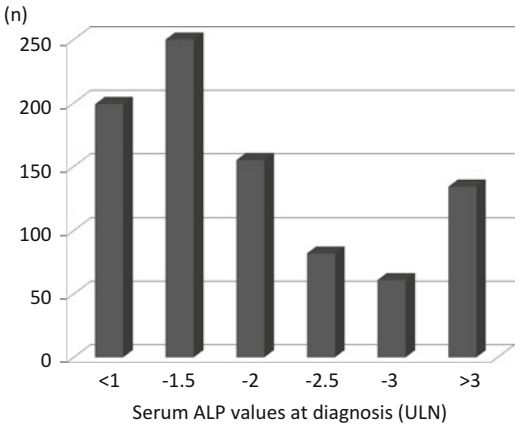

Fig. 18.2 The distribution of serum ALP values at diagnosis in patients with PBC (shown as xULN [6])

started in 1980 demonstrated 71.8 % of patients developed an aPBC [5]. Indeed symptoms in PBC are frequently nonspecific for the disease and it is extremely difficult to make a diagnosis or even suspicion of PBC based on symptoms only. If patients with cholestasis have other symptoms, i.e., fever, abdominal pain, or loss of body weight, cholangitis and/or malignancy of the biliary tract rather than PBC should be suspected. Nevertheless, physicians should always keep attention on and manage symptoms which patients with PBC may have after making a diagnosis, since these symptoms may greatly reduce the quality of life (QOL) of patients.

18.2.2 Biochemistry

In typical cases with PBC, cholestatic enzymes, both ALP and GGT, are elevated, while biomarkers of hepatocellular injuries, AST and ALT, are within normal ranges or marginally increased. The elevations of cholestatic enzymes in PBC are not temporary and persisting with mild fluctuations for years in many cases. Abrupt boost or gradual elevation over time is uncommon in PBC and rather suggests other progressing diseases such as malignant diseases. As the disease is progressing, biochemical indicators for cirrhosis become evident as other liver diseases, reflecting on decreased protein synthesis in the liver or portal hypertension.

It should be kept in mind that some cases with PBC present atypical elevation of liver enzymes. In Fig. 18.2, we demonstrated the distribution of serum ALP values at diagnosis in patients with PBC accumulated in Japanese nationwide survey. While the patients in whom ALP was elevated more than ULN were more than 80 % of the whole, approximately 20 % of patients had serum ALP levels within normal limit at diagnosis. In these cases only serum GGT level was elevated as cholestatic enzymes. Although physicians tend to consider alcoholic liver disease as an etiology for GGT elevation with normal ALP levels, these findings suggest that PBC should be also considered as an alternative candidate for GGT elevation.

Table 18.1 Diagnostic criteria of PBC addressed by the Intractable Hepatobiliary Disease Study Group in Japan [4]

With histological findings
1. Biochemical evidences of cholestasis accompanied by histological evidence of chronic non-suppurative destructive cholangitis (CNSDC)
2. Presence of AMA accompanied by histologically compatible features of PBC, even without CNSDC
Without histological findings
3. Presence of AMA accompanied by clinical features and course of classical and cholestatic PBC

Another atypical presentation of PBC is elevation of AST and/or ALT. It is well known that some PBC cases had a feature of autoimmune hepatitis (AIH), another autoimmune liver disease, and in these cases autoimmune reactions are targeted to hepatocytes as well as bile duct cells. The terminology and definition of these cases, which have both clinical features of PBC and AIH, are not well defined [7] and will be discussed in another chapter of this book.

18.2.3 Imaging Studies

In patients with PBC the diseased sites are intrahepatic small bile ducts and therefore imaging studies, including CT, US, and cholangiography, give us no specific features of the liver, especially in early-stage PBC. In advanced stages the findings of chronic liver diseases are obtained, although they are not specific to PBC either. However, imaging studies should be done in all patients with elevated ALP and/or GGT, to exclude the possibility of gallstones or malignancy which results in obstruction or dilatation of biliary tract and needs to be treated in emergency.

18.2.4 Anti-mitochondrial Antibodies (AMAs)

AMAs directed the enzymes called 2-oxo acid dehydrogenase complex (2-OADC) located at the inner membrane of the mitochondria. Among several enzymes in the 2-OADC, E2 components of pyruvate dehydrogenase complex (PDC-E2), branched chain 2-oxo acid dehydrogenase complex (BCOADC-E2), and 2-oxoglutarate dehydrogenase complex (OGDC-E2) are specifically recognized by AMA in patients with PBC [8]. In Table 18.1, the frequency of autoantibodies directed to each autoantigen in PBC sera is shown. As seen, PDC-E2 is the most frequently recognized autoantigen by PBC sera, followed by BCOADC-E2 and OGDC-E2.

AMA is highly specific for PBC. AMAs are detected in approximately 90 % of patients with PBC, irrespective of disease stage, and rarely found in sera of patients with other diseases. Therefore the clinical guidelines from AASLD, EASL, and

Table 18.2 Mitochondrial autoantigens recognized by AMA

Terminology	Approximately molecular weight (kD)	Frequency of recognition with PBC sera (%)
PDC-E2	74	95
BCOADC-E2	52	53–55
OGDC-E2	48	39–88
PDC-E1α	41	41–66
E3 binding protein	55	95

Japan agree that the diagnosis of PBC is made without histology if AMA is detectable in a patient with cholestasis (Table 18.2). In clinical setting AMAs are examined in two ways: indirect immunofluorescence (IIF) and ELISA. The latter testing is frequently called "AMA-M2," according to the trade name of the kit. The AMA-M2 kit employs recombinant fusion protein of all three autoantigens, PDC-E2, BCOADC-E2, and OGDC-E2 as antigens, and it is reported that both sensitivity and specificity are superior to ordinal AMA using IIF, which may react with other mitochondrial antigens. Therefore, it is recommended that AMA-M2 using ELISA should be tested first and AMA using IIF should be sought if AMA-M2 was negative and PBC was still clinically suspicious.

Although AMA is almost exclusively present only in patients with PBC, the role of AMA in immunopathology of PBC still remains unclear. Lleo et al. demonstrated that PDC-E2 is expressed on the surface of biliary epithelial cells as a part of the apoptotic bodies and recognized by circulating AMAs, leading to stimulation of macrophages and innate immunity, suggesting the link between AMA and autoimmunity in the onset of PBC [9].

Less than 10 % of patients with PBC lack detectable AMA in sera, called "AMA-negative PBC." Several lines of evidence indicated that clinical and histological features between AMA-positive and AMA-negative PBC are quite similar. Indeed, Shimoda et al. demonstrated that CD4-positive T cell clones specific for mitochondrial autoantigens exist even in patients with AMA-negative PBC, indicating that AMA may be present below the detectable threshold in these patients [10].

18.2.5 Other Autoantibodies

Antinuclear autoantibodies (ANA) are detectable in nearly half of patients with PBC. In particular, anti-centromere antibodies (ACA) are relatively specific to PBC, detectable in up to 30 % of patients with PBC [11]. Another ANA targeted to antinuclear pore protein is anti-gp210 antibody, which is also relatively specific to PBC. ACA and anti-gp210 antibody should be sought in patients with persistent elevation of cholestatic liver enzymes and yet AMA negative.

ACA and anti-gp210 antibody are useful for diagnosis of PBC and furthermore are reported to be of value for the prediction of prognosis of PBC [4]. In general

PBC is largely divided into three clinical types: jaundice/hepatic failure type, portal hypertension type, and slow progression type. While detectable ACA are demonstrated to be a marker of portal hypertension type, positivity of anti-gp210 antibodies is indicative for jaundice/hepatic failure type.

18.2.6 Liver Histology

Liver histology confirms the diagnosis of PBC, especially if the findings of chronic non-suppurative destructive cholangitis (CNSDC) are obtained. However, as previously mentioned, a liver biopsy is not essential for diagnosis of PBC in typical cases where cholestatic enzymes are predominantly elevated and AMAs are detectable. On the other hand, a liver biopsy should be done in (1) atypical cases with AMA negative or elevation of AST/ALT and (2) cases where activity and stage of the disease need to be assessed. The characterization of liver histology in PBC will be discussed elsewhere in this book.

18.2.7 Diagnostic Criteria

So far, three diagnostic criteria have been established from AASLD [1], EASL [3], and Japan [4]. All these guidelines agree that a diagnosis of PBC can be made with two of the following three criteria, biochemical cholestasis (elevation of ALP), detectable AMA, and liver histology, while the necessity or importance of liver biopsy is somewhat differently mentioned among them. The guideline from EASL clearly stated that "A liver biopsy is not essential for the diagnosis of PBC" in patients who met two criteria, biochemical cholestasis and the presence of AMA. On the other hand, the guideline from Japan does not definitely state that a liver biopsy is not needed, and the criterion without histological findings is added to the other two criteria with histological findings. In any case, a liver biopsy is not always required for diagnosis of PBC, especially in cases where both chronic cholestasis is evident and serum AMA is detectable.

18.3 Treatment with UDCA

18.3.1 UDCA Treatment

UDCA is the only approved drug for PBC by the Food and Drug Administration (FDA) as well as the health insurance framework of Japan. UDCA exerts its anticholestatic and anti-inflammatory effects through the activation of hepatic

Table 18.3 The criteria for determining biochemical response to UDCA

	Enrolled patients	Time for determination	Criteria	Endpoint	References
Barcelona criteria	All PBC	1 year	ALP; <40 %	LT-free survival	[21]
Paris criteria	All PBC	1 year	TB <1 mg/dL	LT-free survival	[18]
Rotterdam criteria	Advanced PBC	1 year	Normalized TB	LT-free survival	[20]
Ehime criteria	All PBC	6 months	Normalized GGT	LT-free survival	[23]
Paris II criteria	Early PBC	1 year	Normalized TB	Progression to cirrhosis	[19]

transporters including the canalicular bile salt export pump (BSEP), canalicular multidrug resistance protein 3 (MDR3; ATP-binding cassette transporter B4 [ABCB4]), and basolateral multidrug resistance-associated protein 4 (MRP4 [ABCC4]) [12]. UDCA is administered for patients with PBC at a dose of 13–15 mg/kg/day. In Japan, however, 600 mg/day is an initial dose in most cases since dose-finding study in Japan demonstrated that the efficacy of 600 mg/day and 900 mg/day was comparable and better than those of 300 mg/day [13]. Several lines of randomized controlled study and meta-analyses demonstrated that UDCA treatment is clinically effective for improvement of long-term effect of patients with PBC [14–16]. Recent cohort studies also indicated that good "biochemical responses" to UDCA are significantly associated with improved survival rate or progression to cirrhosis [17–21]. As a result, the role of UDCA as the first-line treatment for PBC is well established.

18.3.2 When and How to Determine Biochemical Responses to UDCA

On the other hand, there are not a few number of patients who exhibit suboptimal response to UDCA treatment [22], and the outcome of these patients has been reported to be worse [18, 20, 21]. Therefore, it is important that the treatment response to UDCA is determined in every case treated with UDCA, and the second-line treatment regimens, although not well established, should be considered for nonresponders to UDCA. In this regard, it remains controversial when and how to determine biochemical responses to UDCA.

In Table 18.3, the criteria for determining biochemical response to UDCA are summarized. As shown, several retrospective studies regarding this problem, four from Europe and one from Japan, have been reported [17–21]. All three studies from Spain, France, and the Netherlands set the endpoint and the time for determination of biochemical response as liver transplantation-free survival and at the point of 1 year from the commencement of UDCA, respectively [18, 20, 21]. The biochemical parameters used for determination of the criteria varied in these studies, including ALP, AST, albumin, and total bilirubin. Both two studies from

Spain and France demonstrated that among the whole PBC cohorts the patients who met each biological criterion at 1 year after the commencement of UDCA had good prognosis compared to those who did not [18, 21]. Another study from the Netherlands divided the whole PBC cohort into three groups depending on the progression, and the prognosis predicted by the biochemical response was significantly different in the advanced PBC and not different in the early PBC when the UDCA response was determined using albumin and total bilirubin [20]. Their result sounds reasonable because total bilirubin and albumin reflect fibrosis of the liver and seemed not to be sensitive to predict the prognosis in early-stage PBC patients. On the other hand, Japanese group demonstrated the biochemical criteria using GGT to determine UDCA response for the prediction of the prognosis defined as LT-free survival [23]. They are unique compared to the previous studies from Europe in terms of the fact that they determined the response earlier, at 6 months after the commencement of UDCA. It is probably because Japanese physicians prefer to use bezafibrate as a second-line treatment for cases refractory to UDCA and therefore tend to determine the response to UDCA earlier. Furthermore, along with the recent increase of asymptomatic PBC in early stages, a group from France demonstrated another criterion for patients in the early stages [19]. They suggested that when setting the endpoint as progression to cirrhosis, not LT-free survival, the determination of UDCA response using ALP, AST, and total bilirubin at 1 year after the commencement of UDCA was useful for predicting the prognosis.

18.3.3 Management of Cases Refractory UDCA

Then, what should we do if the patient was judged as an UDCA refractory case?

As mentioned, in Japan the starting dose of UDCA was 600 mg/day in most cases. This is because the dose-finding study concluded that 600 and 900 mg/day demonstrated similar results [13]. However, in the guidelines, the dose of UDCA was not fixed but dependent on the body weight, 13–15 mg/kg/day [1, 3, 4]. Currently it is not rare that patients with PBC, in early stages in particular, are sometimes overweight. Therefore, 900 mg/day of UDCA may be necessary in some cases for developing expected effects of UDCA. Therefore it is strongly recommended that the dose of UDCA should be increased up to 900 mg/day if the optimal results are not obtained with 600 mg of UDCA in a patient weighing more than 50 kg.

Alternatively, bezafibrate would be the choice for the cases refractory to UDCA. Bezafibrate (BF), ligands of the nuclear peroxisome proliferator-activated receptor alpha (PPARα) and other isoforms [24], originally labeled as drugs for hyperlipidemia and used for prevention of cardiovascular diseases, has been shown to demonstrate biochemical efficacy of BF in patients with suboptimal response to UDCA [25–35]. As a result, BF has been empirically recognized as a second-line therapy for PBC in Japan [4]. However, most of these clinical reports had

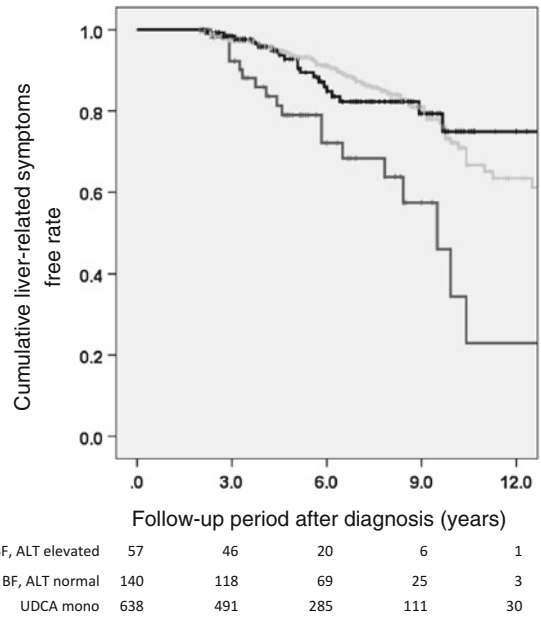

Fig. 18.3 The cumulative liver-related symptom-free rate of asymptomatic patients with PBC (cited from Tanaka et al. [36]). (*Black line*) patients with UDCA monotherapy. (*Dark gray line*) patients treated with BF in addition to UDCA and elevated ALT. (*Light gray line*) patients treated with BF in addition to UDCA and normal ALT

substantial limitations which might impair supporting the role of BF as an alternative treatment in PBC. Very recently, using a large-scale database of patients with PBC accumulated by nationwide prospective cohort study in Japan, we demonstrated that normalization of ALT with additional BF treatment to UDCA significantly decreased the occurrence rate of liver-related symptoms in asymptomatic PBC patients with suboptimal response to UDCA (Fig. 18.3) [36]. This result would provide a rational evidence for BF use to the cases refractory to UDCA. The use of BF for patients with PBC will be discussed elsewhere in this book.

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Chapter 19
Bezafibrate Treatment of Primary Biliary Cirrhosis

Shinji Iwasaki

Abstract Hepatologists commonly encounter difficulty in treating patients with primary biliary cirrhosis (PBC) who are refractory to ursodeoxycholic acid (UDCA). Even when UDCA treatment is initiated in the early stages of the disease, approximately 20–30 % of patients show persistently abnormal levels of hepatobiliary enzymes and undergo a progressive course, eventually leading to the icteric stage or liver transplantation. Several reports, mainly from Japan, have shown the beneficial effect of the fibric acid bezafibrate in UDCA-resistant patients. According to both case studies and pilot studies, bezafibrate lowers the biliary enzyme levels below the upper limit of the normal range in 60–70 % of patients who respond poorly to UDCA alone. Interestingly, IgM also decreases in a parallel manner with biliary enzymes. The main putative mechanisms of bezafibrate involve increased output of phosphatidylcholine into the bile through the upregulation of multidrug resistance protein 3 (MDR3) P-glycoprotein and a consequent reduction in the cytotoxicity of hydrophobic bile acids. Fenofibrate, another fibric acid derivative, demonstrates equivalent clinical efficacy to bezafibrate with a similar molecular mechanism. More than a dozen reports regarding the efficacy of fibrates, along with an understanding of the molecular basis of bile acid metabolism, produce the expectation that large-scale, randomized clinical trials would demonstrate the full impact of bezafibrate on cholestasis.

Keywords Bezafibrate • MDR3 • Phosphatidylcholine

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19.1 Introduction

Ursodeoxycholic acid (UDCA) replaces the cytotoxic hydrophobic bile acids that accumulate during endogenous bile acid recycling under cholestatic conditions and alleviates bile duct damage by virtue of its hydrophilic properties. It has been widely used throughout the world since the early 1990s as the first choice of treatment for primary biliary cirrhosis (PBC) [1].

Long-term monotherapy with UDCA appears sufficient for approximately 60–70 % of patients with early-stage PBC [2–4]. However, according to data from the Japanese national survey on PBC, 20–30 % of UDCA-treated asymptomatic patients continued to exhibit abnormal biochemical parameters and progressed to the icteric stage [5]. Long-term observational data from Europe also suggests that the survival rate of PBC patients treated with UDCA alone was statistically lower than that of sex- and age-matched controls [6]. Therefore, there is still a need for additional therapeutic approaches, particularly in patients refractory to UDCA.

Since a Japanese group first reported the efficacy of bezafibrate in non-cirrhotic patients with PBC in 1999 [7], a growing body of case reports and pilot studies has demonstrated that bezafibrate or a combination therapy of UDCA with bezafibrate is effective in patients who have shown incomplete responses to UDCA alone [8–17]. Most of these reports, however, are of insufficient study size and relatively short observation periods. Now that UDCA-bezafibrate combination therapy has been shown to be effective, at least in improving biochemical parameters and lowering IgM levels, it is expected to represent a new therapeutic option for improving the prognosis of PBC. Recent understanding at the molecular level of bile acid metabolism supports this contention. One of the putative mechanisms by which bezafibrate alleviates cholestasis is believed to be through the increased expression of phosphatidylcholine-specific flippase (multidrug resistance protein 3 (MDR3) in humans, mdr2 in mice, also called ABCB4) on canalicular membranes. This lipid transporter increases phosphatidylcholine output into the bile, thereby forming micelles with and reducing the cytotoxicity of the bile acids.

The initial pathological event of PBC is thought to be autoimmune destruction of bile duct epithelial cells. As bile duct damage advances, cholestasis plays a more important role in tissue damage than autoimmunity. Thus, when designing a strategy for the treatment of PBC, detoxification of the accumulated bile acids should be the major objective. Although the actions of bezafibrate and UDCA are not specific [14, 18] for PBC, they should be important agents for lessening the harmful effects of organic detergents on cells, thereby preventing further disease progression in PBC patients.

In this chapter, we discuss and review the following issues: the putative mechanisms of bezafibrate treatment, clinical studies from Japan regarding bezafibrate treatment of PBC, and our experience in the medical treatment of 89 PBC patients.

19.2 Mechanism of Action of Bezafibrate on Cholestasis (Fig. 19.1)

19.2.1 History of Fibrate and Cholestasis

Fibrate has a long history in Japan, with clofibrate first approved for hyperlipidemia in 1965 and bezafibrate approved in 1991. A large-scale clinical trial revealed that long-term bezafibrate treatment reduced the risk of cardiovascular events in patients with hyperlipidemia without major side effects [19] and bezafibrate has been widely used in Japan ever since.

The potential role of fibrate therapy in cholestasis was first suggested in 1993. Day et al. reported that bezafibrate reduced serum alkaline phosphatase (ALP) activity in patients with hyperlipidemia and prevented cholestasis. The authors speculated that the benefits were due to reduced hepatic ALP production [20]. Although some clinical researchers considered using bezafibrate to treat cholestatic liver diseases, the idea was never tested because the underlying mechanisms and clinical significance of lowering ALP activity were not well understood.

Recently, membrane transporters along the enterohepatic bile acid recycling route (present on hepatocytes, biliary epithelial cells, and enterocytes) and their regulation by the nuclear receptors PPARα and farnesoid X receptor (FXR) have

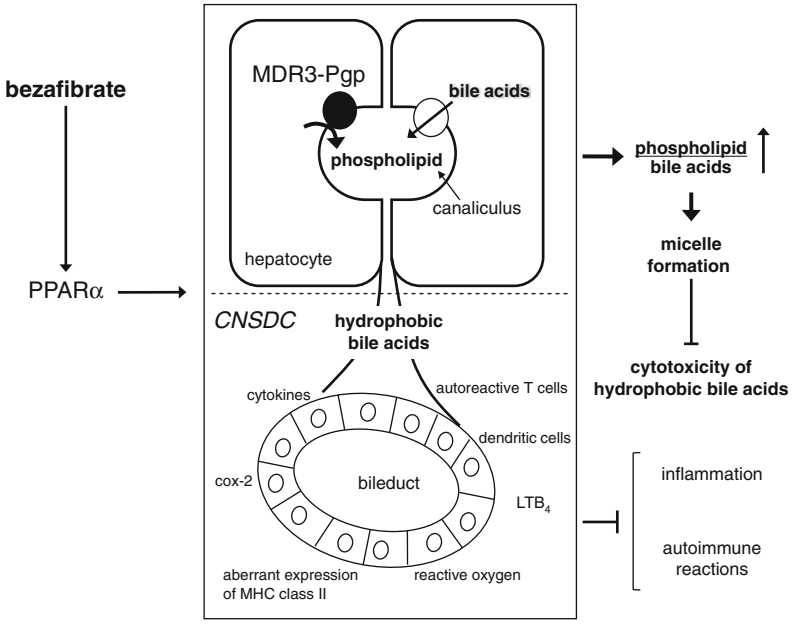

Fig. 19.1 A schematic summary of putative mechanisms of bezafibrate in treatment of PBC

been thoroughly investigated. Accordingly, our understanding of the pathological changes associated with bile formation has been enhanced, enabling the extrapolation of the putative mechanisms underlying the anti-cholestatic effects of bezafibrate [21, 22].

19.2.2 Cytoprotective Properties of Phospholipids

Bile acids are recycled several times a day, with a total amount of bile acid production of up to 20 g/day. The cells bordering the route of the bile stream are continuously exposed to harmful concentrations of hydrophobic bile acids. Therefore, these cells are equipped with several different cytoprotective mechanisms that prevent bile salt-induced toxicity. The concentration of bile acids in hepatocytes is mainly controlled by FXR, which either activates the expression of the *BSEP* (bile salt export pump, ABCB11) and *MRP2* (multidrug resistance-associated protein 2, cMOAT, ABCC2) genes or represses the *NTCP* (sodium-taurocholate cotransporting polypeptide), *OATP* (organic anion transporting polypeptides), and *CYP7A1* (cholesterol 7alpha-hydroxylase) genes [21, 22]. On the other hand, non-hepatocytes possess other protective mechanisms. HCO_3^- secretion, regulated by anion exchanger 2 (AE2) [23], maintains pH levels higher than the pK_a of bile salt monomers (the so-called HCO_3^- umbrella hypothesis). In addition, in large bile ducts, membrane-bound and secreted mucins provide a protective coating for cholangiocytes. However, the most important mechanism is the formation of mixed micelles of bile salts and phospholipids in the extracellular environment of the bile. Under physiological conditions, the ratio of bile acids to phospholipids is maintained at benign levels by the coordinated regulation of the nuclear receptors PPARα and FXR. There are many reports that emphasize the importance of this mechanism.

In mammals, phosphatidylcholine (PC) biosynthesis is completely dependent on phosphatidylethanolamine *N*-methyltransferase (PEMT). When *Pemt*$^{-/-}$ mice were fed a choline-deficient diet, hepatic PC decreased by 50 % and mice died within 5 days because of the complete absence of PC in the bile. On the other hand, animals with a double knockout of *Pemt* and *mdr2*, the gene for PC-specific flippase, survived more than 90 days. The double knockout mice adapted to choline deprivation via various recycling mechanisms, including the induction of phospholipase A2, choline kinase, and phosphocholine cytidylyltransferase, as well as the suppression of choline oxidase [24].

Tsuboi et al. described the cytoprotective effects of lecithin against bile salt-induced bile duct damage in vitro. Immortalized mouse cholangiocytes were cocultured with various hydrophobic bile acids to induce cellular apoptosis. Addition of lecithin inhibited bile acid-induced apoptosis in a concentration-dependent manner, accompanied by enhanced multidrug resistance-associated protein 3 (*MRP3*) expression and suppression of apical sodium-dependent bile salt transporter (*ASBT*) [25].

These findings indicate that bile phospholipid provides physiological protection of cells from damage by hydrophobic bile acids by virtue of micelle formation as

well as by the possible alteration of membrane transporter expression. Furthermore, hereditary abnormalities in transporter genes, or cholestasis caused by acquired factors, result in the deterioration of these cytoprotective mechanisms [22].

19.2.3 MDR3 Controls Phospholipid Concentration in the Bile

In humans, biliary phospholipids are transported into the bile via MDR3 P-glycoprotein (also called phosphatidylcholine flippase), which is equivalent to rodent *mdr2* P-glycoprotein. Mice with homozygous disruption of the *mdr2* gene completely lack phospholipids in the bile and develop portal inflammation, ductular proliferation, hepatocyte degeneration, and fibrosis [26]. deVree et al. identified mutations in the *MDR3* gene in patients with progressive familial intrahepatic cholestasis (PFIC) type 3 [27], which shows similar hepatic histological characteristics to that of *mdr2* knockout mice.

It should be noted that these pathological features are similar to those found in PBC liver. Recent advances in basic research clarified that hereditary abnormalities in ATP-binding cassette transporters are related to a broad spectrum of cholestatic liver diseases such as PFIC, benign recurrent intrahepatic cholestasis, and intrahepatic cholestasis of pregnancy [22]. Although the role of genetic impairment or variation in such ATP-binding cassette transporters in the pathogenesis of PBC is not clear, recent data suggest that such variation might be related to PBC disease severity or susceptibility.

Interestingly, no abnormalities were found in *mdr2*(+/−) heterozygous mice, even though the maximal phospholipid output in these mice is reduced to 60 % of that observed in (+/+) mice [26]. Similarly, *MDR3* (+/−) human subjects do not appear to have liver disorders. The mother of a patient with PFIC3, who is thought to be heterozygous for mutant *MDR3*, developed recurrent cholestasis during pregnancy [27]. Presumably, an unknown pregnancy-related mechanism decreased the phospholipid/bile acid ratio. These observations lead to the speculation that a phospholipid output of 60 % of normal is sufficient to counteract the detergent action of the bile acids, and increasing the amount of phospholipid output could represent a therapeutic strategy for cholestatic liver disease.

19.2.4 Bezafibrate Upregulates MDR3 Expression and PC Output into the Bile

These data prompted us to speculate that fibrate could have therapeutic benefits on cholestatic liver diseases, because one of the most important actions of fibrate is the augmentation of MDR3 expression via PPARα and the subsequent increased

phospholipid output into the bile [28]. Therefore, bezafibrate is expected to increase the phospholipid concentration in the bile and restore the phospholipid/bile acid ratio to harmless levels. Direct evidence was reported recently indicating that bezafibrate induces MDR3-Pgp expression in cultured human hepatocytes and humanized liver in chimeric mice [29], with expression levels of MDR3 in PBC liver reported to be unchanged or not deficient [30–32]. To date, there are no reports of genetic abnormalities in MDR3 or bile acid transporter genes, and a recent genome-wide association study comparing PBC patients and normal subjects did not identify PBC-specific SNPs in those genes [33]. Therefore an imbalance of the phospholipid/bile acid ratio is thought to be mainly due to the accumulation of bile salts in cholestasis rather than congenital genetic abnormalities. Furthermore, bezafibrate may induce further upregulation of MDR3 expression to compensate for the excess levels of hydrophobic bile acids in the cholestatic environment.

Recently, Honda et al. reported a novel mechanism for bezafibrate using DPX2 cells, a derivative of the Hep2 cell line, which suggests that bezafibrate is capable of acting as a dual agonist of PPARs (α, δ, γ) and PXR and modulates their target genes, resulting in the upregulation of CYP3A4, downregulation of CYP7A1, and enhancement of canalicular MDR3, MDR1, and MRP2 expression [17]. Although further studies are required, their study breaks new ground in the elucidation of the precise molecular mechanisms of bezafibrate and may lead to the development of new therapeutic agents.

19.2.5 Bile Salt Transporters and Fibrate

Under cholestatic conditions, MRP3 and MRP4 become important as alternative basolateral transporters for bile acid efflux. Bezafibrate may upregulate these export transporters to eliminate toxic bile salts, and it has been reported that clofibrate alters the expression of these transporters in mice [34]. Another potential beneficial effect of bezafibrate on the enterohepatic circulation of bile salts is the regulation of ileal bile acid-binding protein (I-BABP) expression. I-BABP acts as a bile acid carrier and contributes, together with the liver fatty acid-binding protein (FABP), to the regulation of bile acid metabolism. Bezafibrate has been shown to upregulate the expression of I-BABP in human intestine-derived Caco-2 cells [35].

There has been almost no data regarding the effects of bezafibrate on FXR. When PPARα knockout mice were administered bezafibrate, CYP7A1 expression was suppressed and BSEP expression was enhanced [36]. Because these expression profiles are consistent with FXR activation, it is expected that bezafibrate could influence signaling networks involving nuclear receptors and transcription factors.

19.2.6 Anti-inflammatory Effects of Fibrate

Fibrates, including bezafibrate, are capable of acting as ligands of PPARα. PPARα plays a pivotal role in mitochondrial energy metabolism and the maintenance of cellular homeostasis. PPARα also regulates leukotriene B_4 (LTB_4) inactivation, which determines the extent and duration of inflammation [37].

In smooth muscle cells in human atherosclerotic lesions, PPARα inhibits interleukin-1-induced production of interleukin-6 and prostaglandin and the expression of cyclooxygenase 2 (Cox2) [38]. This observation is interesting because Cox2 is reported to be expressed in PBC biliary epithelial cells [39].

PPARα plays an important role in immunological reactions, such as MHC expression and antigen presentation in dendritic cells. Aberrant expression of MHC class II on biliary epithelial cells is one of the most important phenomena in the pathogenesis of PBC [40]. Bezafibrate may have certain effects on autoimmune reactions involved in chronic non-suppurative destructive cholangitis (CNSDC) of PBC.

Oxidative stress is considered to be another important mechanism in bile duct damage in PBC. Inoue reported that rat liver Cu^{2+},Zn^{2+}-superoxide dismutase (SOD) gene expression was enhanced by bezafibrate administration, which was correlated with the expression of PPARα mRNA level [41]. Consequently, bezafibrate may have an antioxidant effect in liver inflammation. Recent study showed that bezafibrate improved oxidative stress, hepatic stellate cell activation, and fibrogenesis in murine nonalcoholic steatohepatitis model and directly inhibited hepatic fibrogenic response induced by TGF-β1 in vitro [42].

The above findings suggest the possibility that bezafibrate may contribute to the attenuation of inflammation or autoimmune reactions in the PBC liver through PPARα activation.

19.3 Pilot Studies Examining the Effectiveness of Bezafibrate on PBC (Table 19.1)

The first study that suggested the effectiveness and putative mechanism of bezafibrate on PBC originated from Japan in 1999 [7], and more than a dozen case studies and pilot studies [8–17] have been subsequently reported. Most of these studies were from Japan and no reports were from the USA, probably because bezafibrate has not been approved for use by the FDA.

Overall, the reports concur on the beneficial effects of bezafibrate on the improvement of biochemical changes, including levels of ALP, abnormal γ-glutamyltransferase (GGT), aminotransferases, cholesterol, and immunoglobulin M (IgM), as well as the manifestation of cholestasis. In most studies, biliary enzymes were reduced to 50–70 % of the pretreatment levels. Most interestingly, as reported in all eight studies that included UDCA-resistant patients, bezafibrate

Table 19.1 Summary of prospective clinical studies and case series testing the efficacy of bezafibrate in patients with primary biliary cirrhosis

			Number of patients			Treatment outcome of additional BF or BF alone								
Author (year)	Study design	Study duration	UDCA	UDCA + BF	BF	UDCA resistant	ALP	GGT	ALT	Bil	IgM	cho	TG	Pruritis	Fatigue
Randomized controlled trials															
Nakai (2000) [12]	Single center	12 months	13	10	0	23	↓*1*2	↓*2	→	→	↓*1*2	NS	NS		
Kurihara (2000) [11]	Single center	12 months	12	0	12	0	↓*1*2	↓*1*2	↓*1*2	NS	↓*1*2	NS	NS		
Kanda (2003) [10]	Single center	6 months	11	11	0	22	↓*1	↓*1	→	→	↓	→	NS	↓	NS
Itakura (2004) [9][a]	Single center, crossover design	6 months	7	9	0	0	↓*1	↓	↓	→	↓	→	→		
Iwasaki (2008) [8]	Multicenter	52 weeks	25	20	0	0	↓*2	↓*2	↓*2	→	↓*2	↓*2	↓*2		
	Multicenter	52 weeks	10	12	0	22	↓*2	↓*2	↓*2	→	↓*2	↓*2	↓*2		
Pilot studies and case series															
Iwasaki (1999) [7]		12–21 months	0	7	4	9	↓	↓	NS	NS	↓	NS	NS	↓	↓
Ohmoto (2001) [13]		12 months	0	10	0	10	↓	↓	↓	NS	↓	NS	NS	Symptoms were improved in all patients	
Akbar (2005) [16]		12 months	0	10	6	10	↓*2	↓*2	NS	→	↓*2	↓*2	NS		
Kita (2006) [14]		6< months	0	12	0	12	↓*2	↓*2	↓	NS	↓*2	NS	NS		
Takeuchi (2011) [15]		6< months	22	15	0	12	↓*1	↓*1	→	→	↓*1	↓*2	↓*2		
Honda (2013) [17]		3 months	0	19			↓*2	↓*2	↓*2	NS	↓*2	↓*2	↓*2		

UDCA ursodeoxycholic acid, *BF* bezafibrate, *1 significant compared to UDCA alone, *2 significant compared to the data before treatment

[a]The data from the first period of the crossover trial

appears to show a beneficial effect in patients who have been refractory to previous UDCA monotherapy.

Three studies showed that the IgM lowering effect of bezafibrate was significantly stronger than that of UDCA. Four studies evaluated bezafibrate monotherapy and reported its effectiveness in PBC patients in improving hepatobiliary enzymes and IgM. As for symptoms, such as malaise and pruritus, three studies using bezafibrate and UDCA combination therapy reported improvement of symptoms.

A UDCA dose of 600 mg/day was used in all of the Japanese studies. This standard dose in Japan is thought to be subtherapeutic compared to the typical recommended dose of 13–15 mg/kg/day used in other countries. Therefore, it is difficult to precisely compare the Japanese data with those from European countries or the USA with respect to UDCA therapy.

Although the great majority of these studies used biliary enzyme levels as a measurement of treatment effects, the definition of refractoriness or resistance to UDCA therapy is not consistent. Most of the definitions of suboptimal biochemical responses to UDCA proposed in European countries use ALP levels [2, 3, 43], and the most recent study from France reported that ALP <1.5 times the normal upper limit after adequate UDCA treatment is the biochemical criterion used to identify early-stage PBC patients who are at very low risk of long-term development of liver failure [44]. The Ehime group analyzed the Japanese population and proposed that abnormal GGT, or inadequate decreases in GGT following UDCA treatment, should be a more important criterion for determining treatment outcomes [45]. Taking these studies into consideration, satisfactory improvement of biliary enzymes could be considered as a good prognostic marker.

Results from these pilot studies are encouraging, because in all studies bezafibrate improved or normalized biliary enzyme levels, which are surrogate markers of prognosis in patients with PBC. However, a long-term, large-scale, multicenter randomized control study using international unified criteria is definitely required to prove the genuine efficacy of bezafibrate as an additive treatment for PBC. Indeed, the systematic review of six trials with 151 Japanese patients by Cochrane showed only a possible beneficial effect of bezafibrate on hepatic biochemical data compared with the control group. Furthermore, this study did not demonstrate an effect of bezafibrate, versus no intervention, on mortality, liver-related morbidity, adverse events, and pruritus, because of several limitations and a high risk of bias [46].

19.4 Bezafibrate in Clinical Practice

Bezafibrate is recommended for all PBC patients that have been refractory to UDCA alone. Although several definitions of a suboptimal biochemical response to UDCA are proposed [2, 3, 43], in clinical practice all patients with abnormal biliary enzyme levels, in spite of adequate UDCA therapy, deserve consideration for additive therapy with bezafibrate. In our experience, almost all patients with early-stage

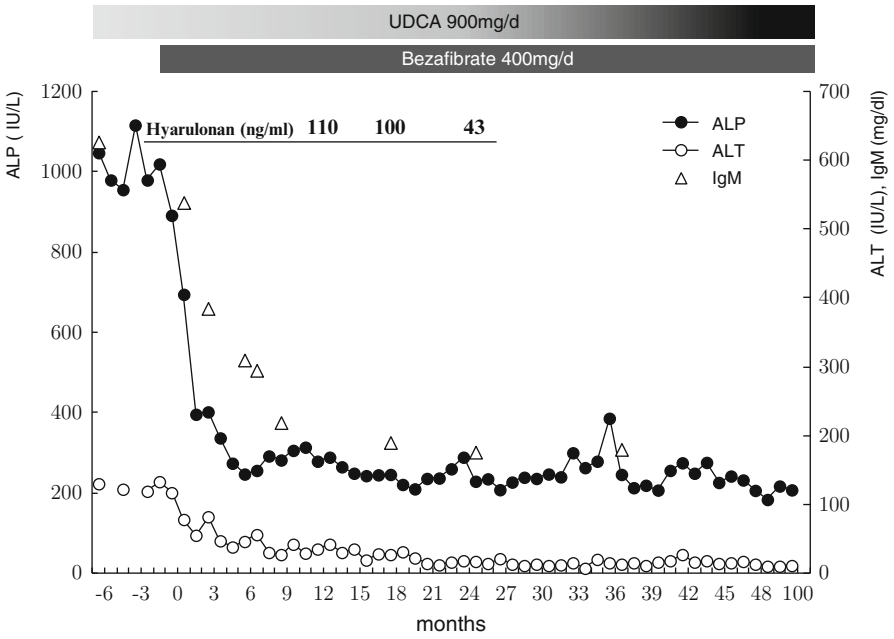

Fig. 19.2 Clinical course of 56 years of male patients with stage II PBC. After addition of bezafibrate to 900 mg/day of UDCA, hepatobiliary enzymes and IgM were normalized within 6 months and were maintained under the upper limit of normal range until the age of 74, except 19 weeks of bezafibrate discontinuation at the age of 68 (identical to the second case of Fig. 19.3). Needle biopsy at the age of 74 in 2014 revealed progression of disappearance of interlobular bile ducts, but inflammation in portal area has been improved and no bridging fibrosis was observed. In total, his histological stage remained in stage III

PBC exhibited good responses to bezafibrate, but no or little biochemical improvement was observed in patients with advanced disease. Careful attention may be required for patients with diminished hepatic functional reserve because exacerbation of ALT was observed after bezafibrate administration in two icteric patients.

The standard dose of bezafibrate is 400 mg/day, usually administered twice a day. In patients with serum creatinine >1.5 g/dL, the dose should be reduced to 100–200 mg/day. Coadministration with UDCA is recommended because the pharmacological mechanisms of these drugs are different and complementary to each other, with possible additive or even synergistic effects. In particular, for patients with high biliary enzymes or high-grade histological inflammation, it is recommended to initiate UDCA and bezafibrate simultaneously or to evaluate the effectiveness of UDCA for 3–6 months after commencing UDCA treatment, since these patients are at a high risk of developing cirrhosis.

When bezafibrate is effective, biliary enzymes and transaminase rapidly decline by 3–6 months, frequently reaching levels near or below the upper normal range. The typical clinical course of the patient is shown in the figure (Fig. 19.2). IgM also decreases in a parallel manner with biliary enzymes.

Bezafibrate is usually well tolerated, but rhabdomyolysis or renal dysfunction can occur. Careful attention is necessary, especially in the elderly and patients with renal dysfunction. Concomitant use of statins should be avoided, because of an increased risk of rhabdomyolysis or myopathy.

Fenofibrate is another fibric acid used for the treatment of PBC. In 2002, Ohira reported on the efficacy of fenofibrate comparable to that of bezafibrate in patients with PBC [47]. Since then, similar results have been obtained in four other studies. The hypothesized mechanism is the same as for bezafibrate, and upregulation of MDR3/ABCB4 by way of PPARα has also been demonstrated in rats [48]. Comparison of individual fibric acid derivatives, in terms of efficacy and adverse events in the treatment of PBC, should be a topic for future research.

19.5 Response to UDCA Monotherapy and UDCA/ Bezafibrate Combination Therapy: Single-Center Results from 89 Patients [49]

19.5.1 Single-Center Results of Medical Treatment of 89 PBC Patients

Eighty-nine patients with anicteric (total bilirubin, <2.0 mg/dL) PBC were analyzed retrospectively. All patients were diagnosed based on biochemical and histological findings as having definite PBC, and UDCA monotherapy was initiated from 1995 to 2004 at Kochi Medical School. In 28 of 89 patients (31 %), hepatobiliary enzymes did not return to the normal limit following at least 6 months of UDCA administration, after which bezafibrate adjunct therapy was started. In 19 (67.9 %; 10 stage I, 4 stage II, 4 stage III, and 1 stage IV) of these 28 UDCA-resistant patients, hepatobiliary enzymes improved to below normal limits following coadministration of bezafibrate and have remained within normal limits for an extended period of time. Of the three patients who underwent serial liver biopsies, two patients showed histological improvement in portal inflammation and fibrosis. The nine patients (32.1 %; 4 stage I, 4 stage II, and 1 stage IV) who did not have a complete response to combination therapy exhibited disease progression. Among them, one died of liver failure and two had to undergo liver transplantation.

19.5.2 Predictive Factors Related to Responsiveness to Adjunct Bezafibrate

To explore the factors that influenced the efficacy of combination therapy with UDCA (600 mg/day) and bezafibrate (400 mg/day), we analyzed the patients' clinical, biochemical, and immunological parameters. When the responder group

was compared with the nonresponder group using univariate analysis, serum bilirubin (group of complete response to bezafibrate, 0.59 ± 0.17 mg/dL, vs group of incomplete response to bezafibrate, 1.05 ± 0.42 mg/dL; $p = 0.0004$), aspartate aminotransferase (AST)(group of complete response, 47.2 ± 22.4 IU/L, vs group of incomplete response, 66.3 ± 13.1 IU/L; $p = 0.03$), and GGT levels (group of complete response, 118 ± 66.3 IU/L, vs group of incomplete response, 354 ± 252 IU/L; $p = 0.0007$) were found to be significantly associated with responsiveness to adjunct therapy with bezafibrate. Subsequent multivariate analysis, however, revealed that none of the factors was significantly related to responsiveness to the addition of bezafibrate. This is likely due to the small patient size.

19.5.3 Discontinuation of Bezafibrate Resulted in Exacerbation of Biochemical Findings

Of the 19 patients who had been refractory to prior UDCA monotherapy and responded completely to adjunct bezafibrate with long-term biochemical normalization, bezafibrate was discontinued and reverted to UDCA monotherapy in five patients. The duration of combination therapy with UDCA and bezafibrate ranged from 5 to 12 years. Several weeks after discontinuation of bezafibrate, biochemical rebound was observed in all five patients and bezafibrate treatment was restarted. Renormalization of biochemical data was observed in all patients in response to the resumption of bezafibrate treatment (Fig. 19.3).

Overall, UDCA was effective in terms of normalization of biochemical data in about two thirds of patients with anicteric PBC, which was largely consistent with other reports. Among the remainder of UDCA-resistant patients, two thirds responded to bezafibrate. Although the number of patients is small and histological evaluation is lacking, our observation strongly suggests that bezafibrate is indispensable for maintaining biochemical parameters within the normal range in a cohort of patients who are refractory to UDCA.

On the other hand, it is reasonable to speculate that an underlying inflammatory process in the bile duct, which is likely to be autoimmune in nature, remains active in patients with normal biochemical parameters during UDCA and bezafibrate combination therapy, because withdrawal of bezafibrate produces an immediate biochemical rebound even in the patient with >12 years of treatment.

19.6 Conclusions and Outlook

PBC has two aspects of the disease process, the first being liver-specific autoimmunity and the other being cholestasis. Although many studies have been conducted since the discovery of the anti-mitochondrial antibody, the significance

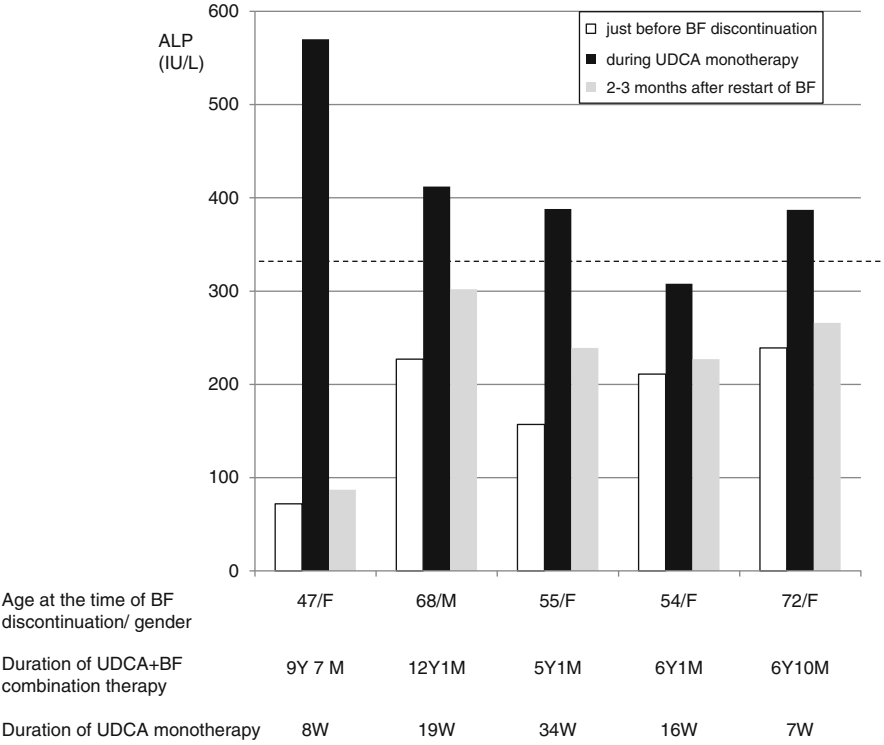

Fig. 19.3 Discontinuation of bezafibrate resulted in exacerbation of ALP and renormalization was observed in all patients in response to resumption of bezafibrate treatment. The *dotted line* indicates the upper limit of normal range. *BF* bezafibrate, *UDCA* ursodeoxycholic acid

and relationship of this antibody to PBC pathology remains unknown. An autoimmune process is probably the initial event that is important for the development of this disease. However, subsequent cholestasis modifies the disease pathology, obscures the elucidation of the disease mechanism, and complicates therapeutic strategies. Although clinical trials involving several immunosuppressants targeting autoimmunity have been conducted, most of these trials failed or could not demonstrate beneficial effects in PBC.

To date, accumulating knowledge of the molecular basis of bile acid metabolism has provided insight into the pathophysiology of cholestasis and clues for therapeutic approaches. In fact, several novel therapeutic approaches are under investigation, such as agonists for FXR and the G protein-coupled bile acid receptor TGR5 [50, 51]. Bezafibrate already has greater than a decade of history in clinical and basic research as a PPARα ligand and therapeutic agent for PBC. Large-scale, prospective, multicenter randomized controlled trials are definitely required to evaluate the full impact of bezafibrate on cholestasis, as noted by others [52, 53].

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Chapter 20
Management of the Patients with Feature of Autoimmune Hepatitis

Kazumichi Abe, Atsushi Takahashi, and Hiromasa Ohira

Abstract Primary biliary cirrhosis (PBC) and autoimmune hepatitis (AIH) differ in clinical, laboratory, and histological features, as well as response to therapy. Some patients with PBC present with characteristic features of AIH; however, there is no formal designation for conditions in which features of PBC and AIH overlap (PBC-AIH overlap syndrome). The pathogenesis of PBC-AIH overlap syndrome is not well understood and its diagnosis is challenging. The International Autoimmune Hepatitis Group (IAIHG) scoring system for the diagnosis of AIH has been widely used to describe PBC-AIH overlap syndrome, but has not been proven to be appropriate for this purpose. Due to the low prevalence of this disorder, recommendations on therapy focus on treatments available for each individual disorder and on findings from retrospective, non-randomized studies. The aim of this report is to provide a current overview of the clinical and histological features, diagnosis, treatment, and prognosis of PBC-AIH overlap syndrome.

Keywords Corticosteroids • Paris criteria • PBC-AIH overlap syndrome • Primary biliary cirrhosis • Revised IAIHG scoring system • Simplified IAIHG scoring system

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20.1 Patients with Primary Biliary Cirrhosis with Autoimmune Hepatitis Features

20.1.1 Characteristics

Primary biliary cirrhosis (PBC) and autoimmune hepatitis (AIH) are classically viewed as distinct liver diseases. However, patients presenting with clinical, biochemical, serological, and/or histological features characteristic of both diseases, either simultaneously or consecutively, have been reported [1, 2]. This condition has been given several names, such as "PBC-AIH overlap syndrome," "PBC with AIH features," "PBC hepatic form," or "hepatitic variant of PBC." Of these, "PBC-AIH overlap syndrome" is the most commonly used and will be used in this report.

Although PBC-AIH overlap syndrome was first reported in the 1970s [3, 4], little is known about its pathogenesis. In one study, characteristic HLA haplotypes of AIH, HLA-DR8, DR3, or DR4 were present in 17 of 20 patients with PBC-AIH overlap syndrome [2]. In some cases, the diagnosis can change during follow-up. For example, among 35 patients with variant forms of PBC that were identified in a Swedish study, transitions between variant forms of PBC were observed over time, although the diagnosis remained unchanged in 16 of 25 PBC-AIH overlap syndrome cases [5]. In patients with PBC, features of AIH may develop sequentially [6–8]. Among 282 patients with PBC in one study, AIH developed in 12 (4.3 %) patients after 6 months to 13 years [6]. Importantly, the development of AIH cannot be predicted from baseline characteristics and initial response to ursodeoxycholic acid (UDCA). In some cases, PBC may occur after the diagnosis of AIH [9].

Several studies have reported on the clinical characteristics of PBC-AIH overlap syndrome (Table 20.1). The mean age of onset, based on these studies, is 43–55.9 years, with 80–100 % of patients being female. Levels of AST or ALT, ALP, and IgG and ANA or ASMA positivity were significantly higher in patients with PBC-AIH overlap syndrome compared to patients with PBC alone [6, 10–16].

20.1.2 Diagnosis

Three criteria are used to determine whether patients with PBC have AIH features [10, 17, 18]. The first criterion is the International Autoimmune Hepatitis Group (IAIHG) scoring system, the original draft of which was validated in two independent populations of patients diagnosed with AIH. This system was subsequently revised [19, 20], and the score compatible with either probable or definite AIH has been used in several recent studies to identify potential cases of PBC-AIH overlap syndrome [11, 21]. Despite this, the revised scoring system is not suitable for diagnosing PBC-AIH overlap syndrome [22], because AMA positivity and biliary changes, both of which are essential in PBC, are assigned negative scores

Table 20.1 Baseline characteristics of patients with PBC-AIH overlap syndrome

Author (reference)	*N*	Female (*n*, %)	Age (years)	AST/ALT (ULN) or (U/L)	ALP (ULN) or (U/L)	IgG (ULN) or (mg/dL)	ANA/SMA positive (*n*, %)
Poupon [6]	22	21 (95)	43	5.8 (3.1–16)	3.2 (0.8–21)	1.6 (0.9–2.5)	17 (77)
Muratori [14]	15	12 (80)	51	5.1 (2–38)	2.5 (1–13)	1,935 (1,400–3,743)	12 (80)
Kuiper [15]	15	12 (80)	43	11.2 (1.9–104.7)	2.6 (1.6–9.5)	1.9 (0.6–3.3)	10 (67)
Silveira [11]	26	16 (100)	55.6	112 ± 95	$1{,}073 \pm 712$	ND	8 (27)
Neuhauser [12]	43	41 (95)	54.3	102 ± 49	$1{,}014 \pm 692$	$2{,}042 \pm 621$	23 (64)
Yokokawa [13]	16	14 (88)	55.9	240.5 (59–3,250)	594 (377–1,637)	3,344 (1,800–4,371)	16 (100)
Tanaka [10]	33	31 (94)	54.6	230 (23–1,490)	579 (171–6,866)	2,516 (1,260–5,150)	29 (88)
Yoshioka [16]	28	26 (93)	55	198 (99–1,872)	537 (291–3,792)	2,405 (1,810–5,080)	28 (100)

ND not detected, *ULN* upper limit of normal, *ANA* antinuclear antibodies, *SMA* smooth muscle antibodies

Table 20.2 Simplified diagnostic criteria for AIH

Variable	Cutoff	Points
ANA or SMA	≥1:40	1
ANA or SMA	≥1:80	2
or LKM	≥1:40	
or SLA	Positive	
IgG	>Upper normal limit	1
	>1.1 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

ANA antinuclear antibodies, *SMA* smooth muscle antibodies

Table 20.3 Diagnostic criteria for PBC-AIH overlap syndrome (*Paris criteria*)

PBC criteria
1. ALP >2× ULN or γGT >5× ULN
2. AMA ≥1:40
3. Liver biopsy specimen showing florid bile duct lesion
AIH criteria
1. ALT >5× ULN
2. IgG >2× ULN or a positive test for anti-smooth muscle antibodies (ASMA)
3. Liver biopsy showing moderate or severe periportal or periseptal lymphocytic piecemeal necrosis

ULN upper limit of normal

(i.e., −4 and −3, respectively) [20]. The second criterion relies on a simplified scoring system proposed by the IAIHG to assist in the early diagnosis of AIH (Table 20.2) [23], which does not assign negative scores for AMA positivity and biliary changes. A recent study demonstrated the utility of the simplified scoring system for diagnosing PBC-AIH overlap syndrome [12]. The third criterion, i.e., the "Paris criteria," relies on the presence of at least two of the three accepted criteria of both diseases to support a diagnosis of PBC-AIH overlap syndrome (Table 20.3) [1], and this system has been adopted in many studies as inclusion criteria for this disease [13–15, 24–26]. In the two largest studies using Paris criteria, the prevalence of PBC-AIH overlap syndrome among patients with PBC was in the range of 4.8–9.2 % [1, 24]. In studies applying the revised IAIHG scoring system, findings of PBC-AIH overlap syndrome varied considerably, from 2.1 % to 19 % [12, 21, 27–29]. Application of the simplified IAIHG scoring system to 368 patients with PBC resulted in a reduction of the proportion classified as PBC-AIH overlap syndrome from 12 % (as determined by the revised IAIHG scoring system) to 6 % [12]. Kuiper et al. reported the sensitivity and specificity of Paris criteria for diagnosing PBC-AIH overlap syndrome to be 92 % and 97 %,

respectively; lower percentages were reported when the revised and simplified IAIHG scoring systems were used [15]. In contrast, Tanaka et al. reported the sensitivity and specificity for corticosteroid administration to be 92 % and 25 %, respectively, with Paris criteria, the latter being very low due to the overdiagnosis of patients with PBC-AIH overlap syndrome. The simplified scoring system had better specificity, as revealed by the sensitivity and specificity for corticosteroid administration of 92 % and 75 %, respectively [10].

20.1.3 Histological Findings

Several histological staging systems have been proposed for diagnosing PBC since the 1960s, including those by Scheuer [30], Rubin et al. [31], and Ludwig et al. [32]. The presence of degenerating bile duct epithelium with focal bile duct obliteration and granuloma formation, termed a "florid duct lesion," is highly suggestive of PBC, but not invariably present in all cases. Granulomatous cholangitis was present in only 32 % of 258 biopsies from patients with PBC [33]. Moreover, the grading of necroinflammatory activity as a reflection of autoimmune-mediated pathogenesis of PBC is not reflected in these classical staging systems [34]. "Interface hepatitis" is defined as lymphocytic interface activity showing damaged hepatocytes with lymphocyte infiltration at the interface of portal tracts or fibrous septa [35, 36]. Severe lymphocytic interface hepatitis is present in up to 25–30 % of patients with PBC [21, 37, 38]. Previous reports have reported the prevalence of interface hepatitis in livers of PBC-AIH overlap syndrome cases to be 69 % [11], 92 % [26], and 93 % [12]. Recently, Nakanuma et al. proposed a new grading and staging system for PBC that takes into account the histological findings of cholangitis and hepatitis activity (CA and HA) for grading, as well as those of fibrosis, bile duct loss, and chronic cholestasis for staging [34]. HA grade 0 corresponds to no interface hepatitis. Grades 1–3 correspond to the presence of interface hepatitis in $<1/3$, 1/3–2/3, and $>2/3$ of portal tracts, respectively. The degree of lobular hepatitis is also taken into account for HA grading [34]. Another study has shown that HA scores of patients with PBC having AIH-like histological features were significantly elevated compared with those having PBC-like features [10]. Given the lack of specific markers for AIH, there is a tendency for clinicians to place more focus on histological findings. When assessing liver biopsies, limitations associated with sampling error should always be acknowledged [25].

20.1.4 Treatment

The low prevalence of PBC-AIH overlap syndrome has made controlled therapeutic trials impossible. Thus, therapeutic recommendations are mainly based on experiences with treating either PBC or AIH and on findings from retrospective,

non-randomized studies [17]. While UDCA is a safe and life-extending therapy for most patients with PBC [39], corticosteroids with or without azathioprine markedly improve survival in patients with AIH [39, 40]. Several studies have demonstrated a positive response to immunosuppressive therapy in patients with PBC-AIH overlap syndrome [1, 2, 6, 14, 25, 41], but treatment with UDCA alone is also sufficient in some cases [24].

In the largest long-term follow-up study, 17 strictly defined patients received UDCA alone or UDCA in combination with immunosuppressants and were followed for 7.5 years [25]. In the 11 patients treated with UDCA alone, the biochemical response in terms of AIH features (ALT $<2\times$ ULN and IgG <16 g/L) was observed in only three patients, whereas the other eight were nonresponders, with increased fibrosis found in four. Overall, fibrosis progressed more frequently in non-cirrhotic patients under UDCA monotherapy (4/8) than under combined therapy (0/6).

In the histopathological analysis of liver biopsies obtained before and after four years of therapy with UDCA from patients with PBC, the severity of lymphocytic piecemeal necrosis and lobular inflammation at entry was significantly associated with the progression of fibrosis [38]. Other reports also support an effect of interface hepatitis and serum AST levels on PBC progression [42, 43]. These findings suggest that patients with PBC having biochemical and histological features of AIH are more likely to exhibit rapid progression of fibrosis than those without and that these patients may require a combination of UDCA and corticosteroids.

With the caveat that this strategy is not evidence based, recent EASL guidelines recommend combined therapy with UDCA and corticosteroids in patients with PBC-AIH overlap syndrome [17]. Corticosteroids improved serum liver parameters and histological features, but markedly worsened bone mineral density in patients with PBC, thereby prohibiting its long-term use [44]. One study reported the use of prednisolone at an initial dose of 0.5 mg/kg/day, which was then progressively tapered once ALT levels showed a response [25]. As an alternative approach, UDCA can be the initial therapy and corticosteroids added if an adequate biochemical response has not been achieved within three months [17]. Another study reported that in PBC-AIH overlap syndrome, AIH-like features are dominant in liver histology, and the simplified IAIHG scoring system could predict patients who needed corticosteroids with a higher specificity [10]. The clinical assessment of and therapeutic approaches for patients with PBC-AIH overlap syndrome are presented in Fig. 20.1.

20.1.5 Prognosis

Patients with PBC-AIH overlap syndrome reportedly have a higher risk of developing symptomatic portal hypertension and have worse outcomes compared to those with PBC alone [11]. Furthermore, the effects of combination therapy with

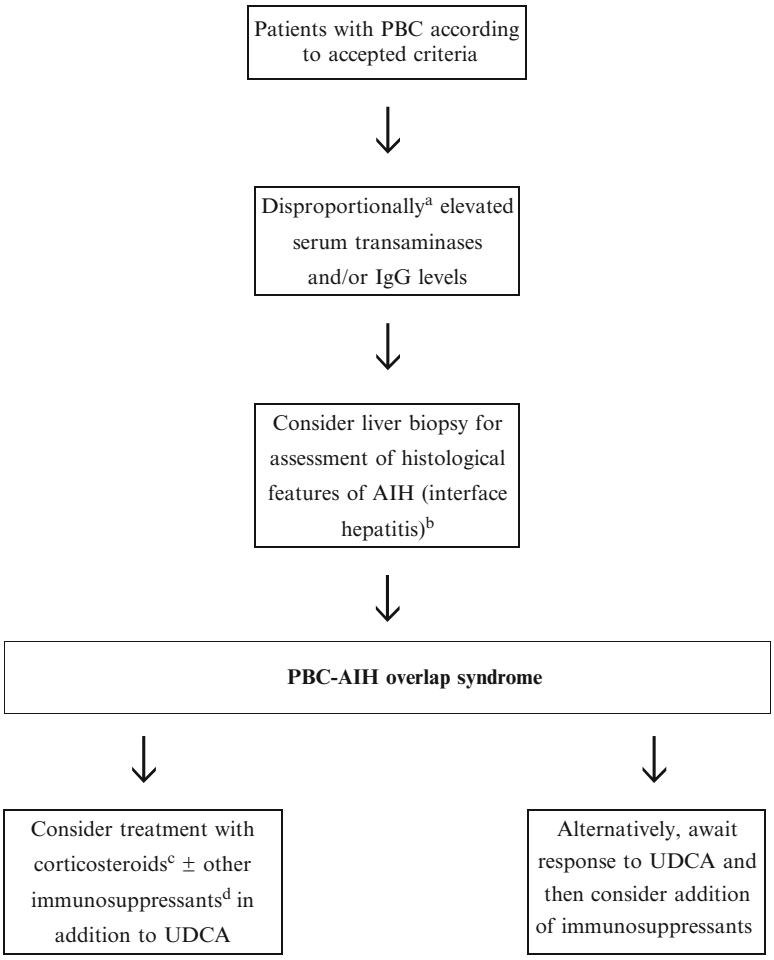

Fig. 20.1 Clinical assessment and therapeutic strategies for PBC-AIH overlap syndrome based on recommendations in the EASL guidelines and Boberg et al. [17, 28]. [a]AST ≥5× ULN and IgG ≥2× ULN (previously suggested as criteria for AIH). [b]Grading of necroinflammatory activity (HA: hepatitis activity) was performed as previously reported [34]. [c]The simplified IAIHG scoring system can predict patients who need corticosteroids with higher specificity [10]. [d]Immunosuppressive treatment is not evidence based. Initial treatment is given according to AIH guidelines

UDCA and immunosuppressants on long-term outcomes of patients with PBC-AIH overlap syndrome have not been evaluated. One study reported that three patients with PBC-AIH overlap syndrome treated with either UDCA or immunosuppressants developed cirrhosis, varices, GI bleeding, ascites, and encephalopathy or died of liver-related causes during the follow-up period, while the remaining 13 patients treated with both UDCA and immunosuppressants responded well, with normal ALT and ALP levels [13]. In another study, among 28 patients with PBC-AIH overlap syndrome, responders showed excellent prognosis, while those who could

not be treated with corticosteroids and nonresponders to corticosteroid therapy had a poor prognosis [16]. A high ALP level, ASMA negativity, and gp210 positivity were identified as risk factors for no response to corticosteroid therapy [16]. Therefore, distinguishing between patients with PBC-AIH overlap syndrome and those with PBC alone is important, as each population will likely require different therapeutic strategies, and long-term outcomes may differ [11].

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Chapter 21
Liver Transplantation for Primary Biliary Cirrhosis

Takuya Genda and Takafumi Ichida

Abstract Primary biliary cirrhosis (PBC) is one of the most common indications for adult liver transplantation in both Western countries and Japan. Recently, the number of liver transplantations for PBC has shown a decreasing trend in Europe and the United States, likely due to advances in medical therapies using ursodeoxycholic acid. However, liver transplantation remains the sole life-saving treatment method for PBC that has progressed to end-stage cirrhosis. Additionally, liver transplantation is occasionally indicated due to declines in the quality of life arising from severe cutaneous pruritus or chronic fatigue in PBC patients. The appropriate timing of the transplantation is calculated using several different prognosis prediction models. The results of liver transplantations for PBC have been excellent compared with those for other diseases, with all studies reporting 5-year survival rates higher than 70 %. Moreover, differences have not been observed between the results of transplants from living donors and those from deceased donors. PBC can recur in the graft liver after transplantation, but this phenomenon is poorly understood, including its frequency, risk factors, and long-term prognosis.

Keywords Deceased donor • Living donor • Prediction model • Prognosis • Recurrence

21.1 Introduction

Primary biliary cirrhosis (PBC) is an autoimmune liver disease characterized by progressive intrahepatic cholestasis resulting from destruction of the interlobular and septal bile ducts. PBC mainly affects middle-aged women and progresses

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slowly to decompensated cirrhosis, with severe jaundice, ascites, and variceal hemorrhage. The resultant liver damage occurs at different rates and with varying severity in afflicted individuals. Patients progressing to liver failure are now recognized to represent only the severest part of the disease's wider clinical spectrum. Currently, patients are more likely than in the past to be asymptomatic at the time of diagnosis because of routine biochemical screening and the use of antimitochondrial antibodies (AMAs). The 10-year survival rate for asymptomatic PBC patients is high (50–70 %), whereas the median survival period for symptomatic PBC patients is reportedly 5–8 years [1, 2]. The main causes of death are liver failure and rupture of esophageal varices due to portal hypertension [3]; histologically advanced cases may be complicated by hepatocellular carcinoma [4].

Although the underlying pathogenesis of PBC remains unclear and a complete cure has not yet been developed, ursodeoxycholic acid (UDCA) has been shown, in several randomized controlled trials, to suppress PBC progression and is currently the first-line treatment used in clinical practice. Continuous administration of UDCA has been shown to improve the biochemical examination results of PBC patients, as well as inhibit histological progression and improve the survival rate [5–7]. However, UDCA administration does not improve the prognosis of every PBC patient. The survival rates of patients where UDCA administration was initiated for histologically advanced disease and in patients exhibiting only slight improvements in biochemical examination results were not very different from the survival rate in untreated patients [7, 8]. Thus, medical therapies are not currently available to effectively improve the prognosis of PBC patients with end-stage cirrhosis; liver transplantation remains the only life-saving treatment option. Here, we outline the current status of liver transplantation for PBC.

21.2 Trends in Liver Transplantation for PBC

Since the first human liver transplantation by Starzl and colleagues in 1963, many advances in surgical techniques and perioperative care, including immunosuppression regimens, have been made, and liver transplantation has become a widely acceptable therapy for end-stage liver disease. End-stage cirrhosis, due to PBC, became a major disease indication for transplantation immediately after liver transplants became widely accepted in clinical practice. Iwatsuki et al. reported that 16.5 % of 1,000 liver consecutive transplantations, from 1980 to 1987, were performed in patients with PBC, making this the second most frequent disease indication among adults [9]. According to the European Liver Transplant Registry (ELTR), of the 39,196 total liver transplantations performed across Europe from 1988 to 2001, 2,969 (8 %) were performed for PBC, followed by alcoholic cirrhosis, hepatitis C virus-related cirrhosis, and acute liver failure [10]. At the end of December 2009, according to the latest statistical

report from the Organ Procurement and Transplant Network (OPTN), 7.9 % of American adults waiting for deceased donor liver transplantation had cholestatic disease, including PBC [11].

These reports also suggest that the proportion of PBC cases, among all transplant indications, is showing a downward trend compared with former reports. An increasing number of patients undergoing transplantation for other indications, such as hepatitis B virus-related cirrhosis and hepatocellular carcinoma, may partly contribute to this trend. However, although the total number of liver transplantations continues to increase, both the proportion and absolute number of PBC liver transplantations performed are declining. In the United States, the number of liver transplantations increased, on average, by 249 transplants per year from 1995 to 2006. However, the number of transplants performed because of PBC decreased by an average of 5.4 transplants per year, during the same period [12]. A single center in the United Kingdom also reported that 35 % of all transplants were performed for PBC patients in 1990, whereas the number had declined to 21 % in 1998 [13]. The proportion of PBC-related transplants in the Netherlands has also decreased from 11.7 % to 4.5 % of all transplants during the decade from 1998 to 2008 [14]. Interestingly, over this time, the proportion of cases of primary sclerosing cholangitis, another intrahepatic cholestatic disease, has remained largely unchanged. These observations clearly indicate a decline in the number of PBC-related liver transplantations. The reason for the trend towards decreased rates of PBC transplants has not been fully clarified. Considering that the incidence and prevalence of PBC continues to demonstrate a steady increase [15], the protective effect of UDCA against disease progression may improve the natural history of PBC, decreasing the need for liver transplantation.

The world's first adult-to-adult living donor liver transplantation was performed in a patient with PBC, in Japan [16], indicating that PBC is also an important indication for liver transplantation in Japan. Because of the severe shortage of deceased donor livers, the application of deceased donor liver transplantation, in Japan, had lagged behind that in other areas of the world; therefore, the abovementioned success led to a marked increase in the number of living donor liver transplantations being performed in Japan. By the end of 2011, a total of 139 deceased donor liver transplantations had been performed, compared with 6,503 living donor liver transplantations, which means that 97.9 % of the liver transplants in Japan were from living donors. Of the 4,056 initial adult living donor liver transplantations performed by the end of 2011, 567 (14.0 %) were performed in PBC patients, showing that PBC is one of the main indications for liver transplantation in Japan (Fig. 21.1) [17]. The Japanese Organ Transplant Network (JOT) Registry also showed that PBC is the second most common indication for patients waiting for deceased donor liver transplantation (Fig. 21.2) [18].

The JOT showed that median wait-list survival of PBC patients was nearly 1 year and was poorest among all indications (Fig. 21.3) [18]. To date, there has been little evidence regarding the PBC transplantation trend in Japan, and the emergence of a decreasing need for liver transplantation in Japanese PBC patients

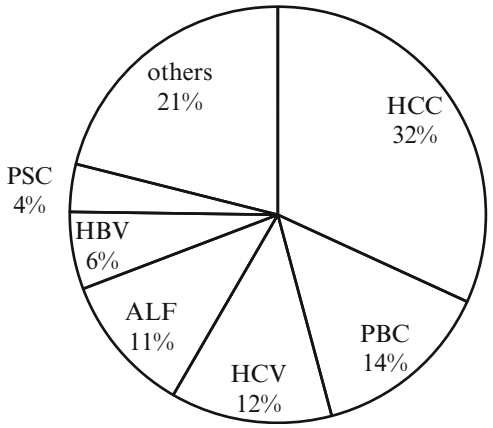

Fig. 21.1 Etiology of liver disease in adult patients undergoing living donor liver transplantation, in Japan, from 1989 to 2011 (the Japanese Liver Transplant Society). *ALF* acute liver failure, *HBV* hepatitis B virus, *HCC* hepatocellular carcinoma, *HCV* hepatitis C virus, *PBC* primary biliary cirrhosis, *PSC* primary sclerosing cholangitis

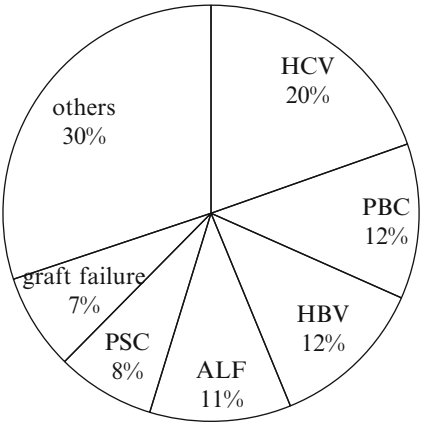

Fig. 21.2 Etiology of liver disease in adult patients registered in the Japan Organ Transplant Network as candidates for deceased donor liver transplantation, 1997–2011 (The Assessment Committee of Indication for Transplantation). *ALF* acute liver failure, *HBV* hepatitis B virus, *HCV* hepatitis C virus, *PBC* primary biliary cirrhosis, *PSC* primary sclerosing cholangitis

is unclear. Although the relative incidence of PBC transplantation appears to be higher in Japan than in Europe or the United States, when the data are examined in detail, cases of transplantation due to alcoholic cirrhosis, which are common in the West, are almost nonexistent in Japan. Therefore, the relative incidence of PBC transplantation, in Japan, is believed to be higher than those in Europe or the United States.

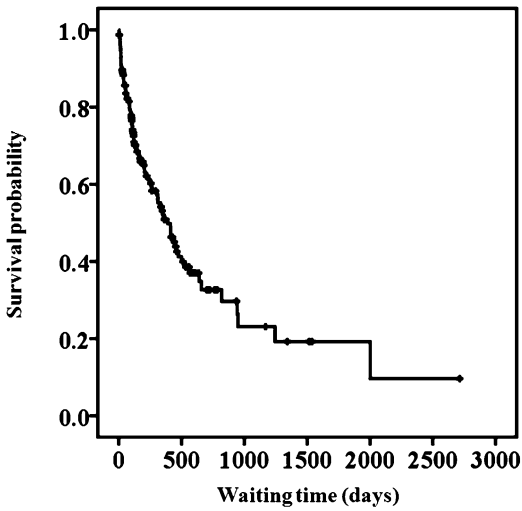

Fig. 21.3 Kaplan–Meier curves showing the cumulative wait-list survival probability of patients with primary biliary cirrhosis registered in the Japan Organ Transplant Network as candidates for deceased donor liver transplantation, 1997–2011 ($n = 156$, The Assessment Committee of Indication for Transplantation)

21.3 PBC Transplantation Indications and Timing

Patients with PBC-induced cirrhosis are indicated for transplantation when they are complicated with intractable ascites, hepatic encephalopathy repeated variceal hemorrhages, or hepatocellular carcinoma (within the Milan criteria, Table 21.1) [19]. These indications are similar to those for patients with cirrhosis due to other chronic liver diseases. Moreover, liver transplantation is occasionally indicated when a PBC patient experiences a marked decline in quality of life (QOL) due to severe cutaneous pruritus or chronic fatigue, both of which are characteristic symptoms of cholestatic liver disease [20]. PBC patients have been reported to feel that their overall QOL is worse than that of the control population, possibly due to chronic fatigue [21], but a recommended therapy is not currently available. Pruritus is a more specific symptom of PBC than fatigue, but is less common than it was previously because patients with PBC are often asymptomatic at diagnosis. Several drugs, such as cholestyramine, rifampicin, sertraline, ondansetron, and naltrexone, have been used to ameliorate pruritus in PBC patients. However, liver transplantation remains the last therapy for treating intractable pruritus, but it might be proposed even for patients with otherwise well-preserved liver function.

An important issue in the clinical management of patients with PBC is the optimal timing of transplantation. Generally, liver transplantation is performed when the posttransplantation survival is estimated to be longer than the estimated survival in the absence of transplantation. The timing of transplantation is too early when the prognosis following transplantation is worse than without transplantation and is too late when the patient becomes too sick for transplantation or dies before transplantation. Christensen et al. demonstrated that the predicted gain in survival after transplantation becomes increasingly positive when the 6-month probability

Table 21.1 The Milan criteria

1 Nodule ≤5 cm
2–3 Nodules: each ≤3 cm
Without intrahepatic portal or hepatic vein involvement

Table 21.2 Child–Turcotte–Pugh (CTP) score

Points	1	2	3
Encephalopathy	None	1 and 2	3 and 4
Ascites	Absent	Slight	Moderate
Bilirubin (mg/dL)	1–2	2–3	>3
Albumin (g/dL)	>3.5	2.8–3.5	<2.8
INR	<1.7	1.7–2.3	>2.3
For PBC: bilirubin	1–4	4–10	>10

INR international normalized ratio, *PBC* primary biliary cirrhosis

Table 21.3 Prognosis model used for primary biliary cirrhosis (PBC) patients

1. Model for end-stage liver disease (MELD) score
   $9.57 \times \ln(\text{creatinine mg/dL}) + 3.78 \times \ln(\text{bilirubin mg/dL}) + 11.2 \times \ln(\text{international normalized ratio [INR]}) + 6.43$
   Laboratory values <1.0 were set to 1.0, the maximum serum creatinine was set to 4.0 mg/dL
2. The Mayo natural history model for PBC
   $R = 0.039\ (\text{age}) + 0.871\ \ln(\text{bilirubin}) - 2.53\ \ln(\text{albumin}) + 2.38\ \ln(\text{prothrombin time}) + 0.859\ (\text{edema}^*)$
   *0, no edema without diuretic therapy; 0.5, edema without diuretic therapy or edema resolved with diuretic therapy; 1, edema with diuretic therapy
   (http://www.mayoclinic.org/gi-rst/mayomodel1.html)
3. The updated Mayo natural history model for PBC
   $R = 0.051\ (\text{age}) + 1.209\ \ln(\text{bilirubin}) - 3.304\ \ln(\text{albumin}) + 2.754\ \ln(\text{prothrombin time}) + 0.675\ (\text{edema}^*)$
   (http://www.mayoclinic.org/gi-rst/mayomodel2.html)
4. The Japanese Liver Transplantation Society model
   $\lambda = -4.33 + 1.2739\ \ln(\text{total bilirubin}) + 4.4880\ \ln(\text{AST/ALT})$
   $\text{6-month mortality}\ (\%) = 1/(1 + e - \lambda) \times 100$

AST aspartate aminotransferase, *ALT* alanine aminotransferase

of survival without transplantation falls below 85 %; this occurs, on average, about 8 months before the patient is expected to die if transplantation does not occur [22].

The Child–Turcotte–Pugh (CTP) score was initially proposed as a means of evaluating hepatic functional reserve in candidates for portacaval shunt surgery (Table 21.2) [23, 24]. The model for end-stage liver disease (MELD) score was established to predict the prognosis of patients receiving transjugular intrahepatic portosystemic shunts (Table 21.3) [25]. Later, both of these scoring systems were recognized as useful and versatile tools for predicting patient prognoses in a wide variety of chronic liver diseases. In cases where the CTP score exceeds 7, or when the MELD score exceeds 15, the survival rate is believed to be higher when a transplant is performed than when a transplant is not performed, and a liver

transplantation is recommended [26]. In addition, the MELD score has been found to be superior to the CTP score in predicting mortality in patients with end-stage liver disease and are waiting for liver transplantation [27–29]. Thus, the MELD score has been used as a liver allocation measure in the United Network for Organ Sharing (UNOS) liver allocation system [30]. Unfortunately, using the MELD score as a current liver allocation measure might decrease the chance of transplantation for PBC patients with severe cutaneous pruritus or chronic fatigue, but who have well-preserved liver function.

Because of the similarities in the natural disease courses among PBC patients, several PBC-specific prognosis prediction models have been created. To define factors used to create a prognosis prediction formula, the Mayo Clinic (Rochester, MN, USA) used data from 312 untreated PBC cases that were observed for a median of 5.5 years. The Mayo Clinic natural history model is calculated using patient age, serum bilirubin levels, albumin levels, prothrombin time, presence of edema, and use of diuretic agents (Table 21.3) [31]. The risk score obtained from the Mayo model can be used to calculate the predicted survival rate up to 7 years later. The utility of this model was confirmed in another 106 cases from the Mayo Clinic and 176 cases from other institutions [32]. Unlike previously published prognosis prediction models [33, 34], the Mayo model is clinically advantageous because its variables do not include histological evaluations, making it widely used. Moreover, this model can predict the prognosis for the natural disease course in PBC patients but also their posttransplant course. A pretransplantation Mayo risk score of >7.8 indicates an increase in the posttransplant risk of death and a significant increase in the length of the intensive care unit stay, hospitalization duration, and transfusion amount. Thus, transplants are best performed before the Mayo risk score exceeds 7.8 [35, 36]. The Mayo risk score is also superior to the CTP score for predicting liver transplantation outcomes in PBC patients [36].

A chronological examination of the risk scores obtained using the Mayo model showed that the scores increase at an annualized rate of 0.23 if the patient is at least 2 years away from death; however, within 2 years before death, the annualized rate of change in the score rises rapidly to 1.4. This suggests that the Mayo model overevaluates survival rates for patients at high risk of dying in the short term. Therefore, Murtaugh et al. created an updated Mayo model using data from chronological observations of patients (Table 21.3) [37]. This updated model is better at predicting survival within 2 years of death than was the original model and is recommended for predicting short-term prognoses.

The Japanese Liver Transplantation Society analyzed 141 cases of symptomatic PBC to create a unique prognosis prediction formula that uses two variables—namely, serum bilirubin level and the aspartate aminotransferase-to-alanine aminotransferase ratio (Table 21.3) [38]. This model can estimate 6-month mortality and is currently used by the JOT registry. For the JOT registry, the demographic, clinical, and laboratory data, including CTP score, MELD score, and Japanese Liver Transplantation Society model-estimated mortality, of all PBC candidates are reviewed. Using these data, each candidate is assigned a priority according to

the clinical judgment of the Assessment Committee of Indication for Transplantation. However, PBC patients are reported to be at a high risk of wait-list mortality in the current Japanese allocation system, and a MELD-based allocation might reduce that risk [18].

21.4 PBC Transplantation Results

In the 1980s, groups from King's College Hospital (United Kingdom) and University of Pittsburgh (United States) showed that the actual posttransplantation survival rate was clearly higher than the survival rate predicted using a prognosis prediction model. This finding established liver transplantation as an effective treatment for PBC patients with end-stage cirrhosis [39, 40]. In the early 1980s, the survival rate following PBC-related transplantation was 76 % at 1 year and 75 % at 2 years; by the 1990s, improvements in perioperative management helped improve those rates to 93 % and 90 %, respectively [35]. The ELTR statistics for Europe, up to 2001, showed 1-, 5-, and 10-year posttransplantation survival rates for PBC patients of 83 %, 77 %, and 69 %, respectively [10]. This is clearly better than the 5-year posttransplantation survival rates for viral cirrhosis (72 %), acute liver failure (59 %), and hepatocellular carcinoma (58 %). PBC is associated with the best transplantation outcomes, alongside metabolic diseases and other disorders. The OPTN statistics from 1997 to 2004 also showed very good results for transplantation in patients with cholestatic disease, which includes PBC, with a 1-year survival rate of 89.8 % and a 5-year survival rate of 79.7 % [41]. Using the UNOS database, an examination of transplantation results, by disease, indicated that PBC transplantations yielded the best results with a 5-year survival of 80 % [42]. Furthermore, interesting results were demonstrated through an analysis of PBC deaths, by age. In the 1980s, PBC deaths exhibited bimodal peaks among women—one peak for women in their late 50s and another for women in their 70s. However, the younger peak had disappeared by the 1990s, with the number of deaths increasing only with age, as also observed for healthy people [43]. The increasing use of liver transplantation and improvements in results are believed to be responsible for the decreased mortality among younger PBC patients.

In Japan, liver transplants for PBC are reported to have survival rates of 76.5 % and 55.6 % at 5 and 10 years, respectively [17], with a single institution reporting 3- and 5-year survival rates of 88 % and 80 %, respectively [44]. Although Japan differs from Western countries in that more than 98 % of liver transplants are obtained from living donors as opposed to deceased donors, these results show that the results of the liver transplants for PBC from living donors do not differ from the results of transplants from deceased donors.

The symptoms of cutaneous pruritus and fatigue are observed more frequently in PBC and other cholestatic liver diseases than in other disorders. Liver transplantation is occasionally indicated when these symptoms lead to a lowered QOL. Gross

et al. examined the results of liver transplantations occurring as a result of QOL issues in 157 patients with PBC or primary sclerosing cholangitis [45]. They reported that before transplantation, 51 % of the patients experienced fatigue, insomnia, or cutaneous pruritus that was difficult to endure; however, 1 year after transplantation, this proportion had decreased to 25 %. Nevertheless, a relatively large number of patients continued to experience posttransplantation chronic fatigue. However, the proportion of patients who reported that they felt "able to lead a nearly normal life" increased from 29 % before transplantation to 61 % after transplantation, demonstrating that liver transplantation not only influences survival prognosis but can also improve declining QOL caused by disease-associated, severe cutaneous pruritus and fatigue. Unfortunately, the preoperative factors enabling clinicians to predict the ability of a transplant to improve QOL have not been determined.

Among PBC patients, the incidences of acute and chronic rejection were reported to be as high as 56 % and 9.3 %, respectively [46–48]. One study demonstrated that although both acute and chronic rejections were less frequent in PBC patients than in patients with autoimmune hepatitis, they tended to be more common than in patients undergoing transplantation for other indications [47]. The major causes of posttransplantation death in PBC patients include sepsis and multiorgan failure within 6 months of transplantation, and sepsis, malignant tumors, kidney failure, and chronic rejection in later stages [13].

21.5 Posttransplantation Recurrence of PBC

The recurrence of PBC in allografts, posttransplantation, was first described in 1982 [49]. However, many aspects remain unclear about the recurrence of PBC in grafted livers. As such, the diagnostic criteria for recurrence that have been reported are not uniform. One diagnostic criterion, based on the preoperative state, which is currently in general use, utilizes three items—namely (1) an increase in alkaline phosphate (ALP) levels as a biochemical finding associated with cholestasis, (2) the presence of AMAs, and (3) chronic nonsuppurative destructive cholangitis as a histological finding [20]. However, increased serum ALP levels are observed in a variety of posttransplantation pathologies, including chronic rejection, cytomegalovirus infection, and drug-induced cholestasis. AMAs, particularly M2 antibodies, have also been reported to be present after transplantation, even without histological recurrence [50]. Even after disappearing, AMAs may later become detectable again. Thus, the typical clinical presentation of PBC is not fully reliable in the posttransplant setting, and clinicians must rely mainly on histological examinations to accurately diagnose posttransplantation recurrence.

The main reports regarding posttransplantation PBC recurrence, in both deceased and living donor allografts, are described in Table 21.4 [13, 46, 48, 51–58]. The frequency of recurrence is roughly 10–30 %, with the period

Table 21.4 Reports regarding posttransplantation primary biliary cirrhosis (PBC) recurrence

Author	Year	*N*	Observation period (median, months)	Liver biopsy	Recurrence rate (%)	Time for recurrence (months)	References
Liermann Garcia	2001	400	56	Protocol	17	36	[13]
Sanchez	2003	156	72	Protocol	11	50	[51]
Sylvestre	2003	100	44	Protocol	17	56	[52]
Neuberger	2004	485	79	Protocol	23		[53]
Jacob	2006	100	118	Protocol	14	61	[48]
Charatcharoenwitthaya	2007	154		Protocol	34		[46]
Morioka	2007	50	29	Ad hoc	18	36	[54]
Yamagiwa	2007	221			10	36	[55]
Montano-Loza	2010	108	83	Ad hoc	26	70	[56]
Manousou	2010	103	108	Protocol	26	44	[57]
Kaneko	2011	81	74	Ad hoc	1	61	[58]

before recurrence varying from 3 to 6 years. As histological PBC recurrence is not always accompanied by clinical symptoms or abnormal examination values [13], the incidence of PBC recurrence is believed to differ depending on whether liver biopsies were performed upon the appearance of symptoms (ad hoc) or whether protocol-driven liver biopsies were conducted. Because biochemical analyses may remain normal for several years after histological diagnosis of recurrence [48], PBC recurrence may be missed and its frequency may be underestimated unless protocol-driven biopsies are performed. The incidence of PBC recurrence may appear also to be higher in more recent reports because of longer follow-up durations and the increasing awareness of PBC recurrence as a clinical entity.

Factors reported to be related to recurrence include the age [13, 48] and sex of the recipient [46], type of immunosuppressant used [46, 48, 53], and the human leukocyte antigen suitability of the donor and recipient [54]; however, the association of these factors with PBC recurrence remains controversial and none have been confirmed to be associated with recurrence. Regarding the type of immunosuppressant, several reports demonstrated that PBC recurrence was not only more frequent, but may also occur earlier in patients receiving tacrolimus than in those receiving cyclosporine. A systematic review showed a higher tendency of recurrence in patients using tacrolimus than in those using cyclosporine (30 % vs. 23 %), but the difference was not statistically significant [59]. For living donor allografts, most donors are blood relatives who have close genetic similarity to the recipients. Therefore, when considering the genetic background of PBC, the similarity of genetic factors between the donor and recipient in living donor transplantation may affect the incidence of PBC recurrence. However, current evidence suggests that there is no difference in the incidence of PBC recurrence between living and deceased donor transplantation.

Because of the well-documented efficacy of UDCA for PBC, it is widely used for the PBC recurrence, without standardized guidelines [13, 46, 51]. Several studies reported improvements in the biochemical examination results from recurrent PBC patients undergoing UDCA treatment [46, 60], but the effects on histological progression and prognosis remain unclear. The appropriate dose of UDCA in the posttransplant setting has also not been established.

At present, recurrence in the graft liver is believed to have little effect on patient and graft prognosis. Garcia et al. reported that among 68 PBC patients who had posttransplantation recurrence, only two experienced disease progression to cirrhosis or required an additional transplantation [13]. In a Birmingham (United Kingdom) report involving patients undergoing liver transplantation due to PBC, only 5.4 % of all graft losses were attributable to recurrent disease; the highest risk of graft loss due to disease recurrence was shown in patients undergoing transplantation for hepatitis C-related cirrhosis, primary sclerosing cholangitis, or autoimmune hepatitis [61]. However, the effect of longer posttransplantation observation periods on recurrent PBC remains unclear.

21.6 Conclusion

Since the start of the application of UDCA in clinical practice, the number of PBC patients who reach end-stage cirrhosis has decreased. However, although some cases are resistant to UDCA therapy, transplantation remains the only life-extending option for such patients when their disease progresses to cirrhosis. Although the outcomes of liver transplantations for PBC—an indication for liver transplantation from an early stage after the introduction of the procedure—have been clarified, new issues continue to appear, such as the pathological mechanism, treatment, and prognosis of posttransplantation PBC recurrence.

Conflicts of Interest The authors of this paper report no conflicts of interest.

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