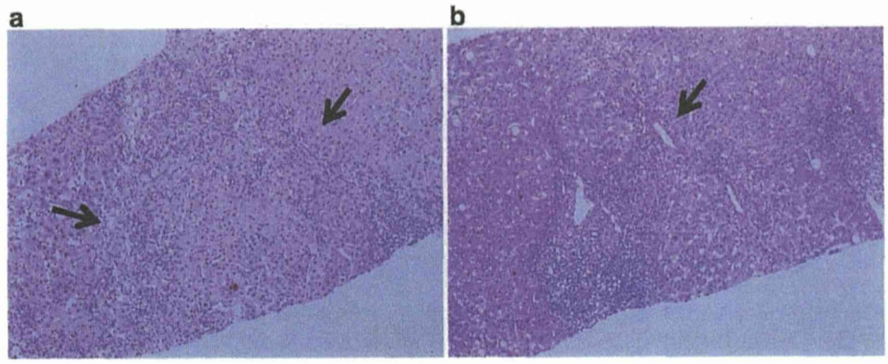

Fig. 4.4 Confluent necrosis. Bridging necrosis (**a**, *arrow*) and perivenular zonal necrosis (**b**) are observed in AIH cases

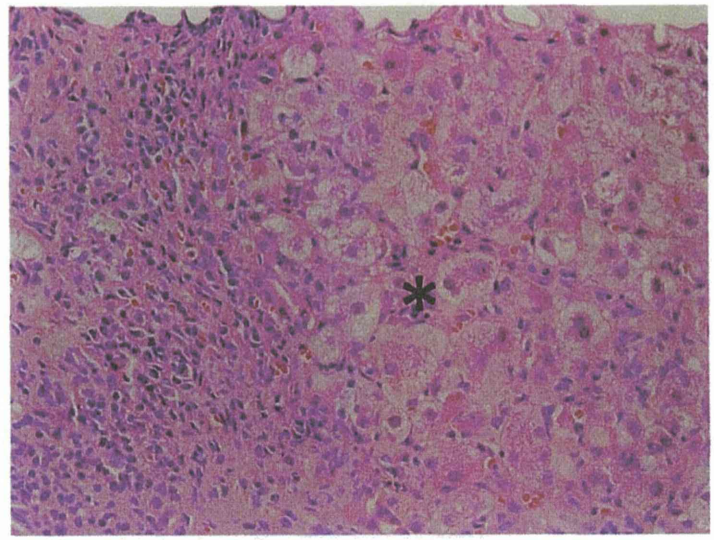

Fig. 4.5 Ballooning degeneration of hepatocytes (*) is observed in the periportal area in autoimmune hepatitis (AIH)

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

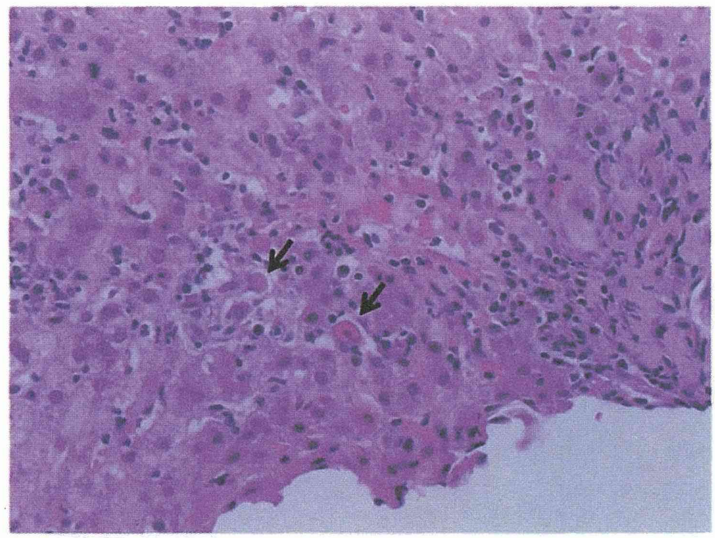

Fig. 4.6 The presence of acidophilic bodies indicating the apoptosis of hepatocytes (*arrows*) reflects the marked parenchymal hepatitis in autoimmune hepatitis (AIH)

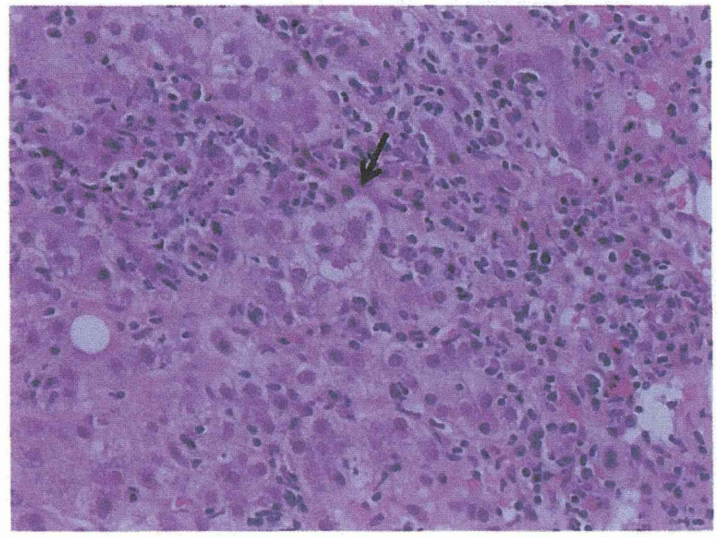

Fig. 4.7 Hepatocyte rosette formation (*arrow*) around a severe interface hepatitis area

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.

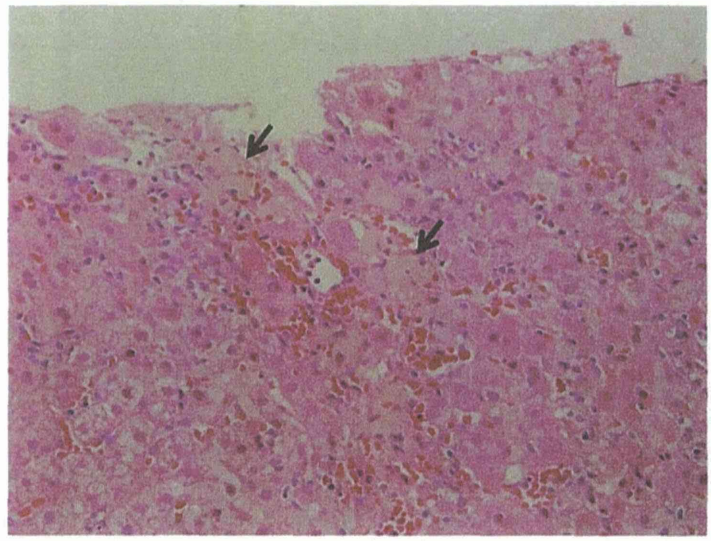

Fig. 4.8 Pigmented macrophages (*arrows*) are scattered in a necrotic area of autoimmune hepatitis (AIH)

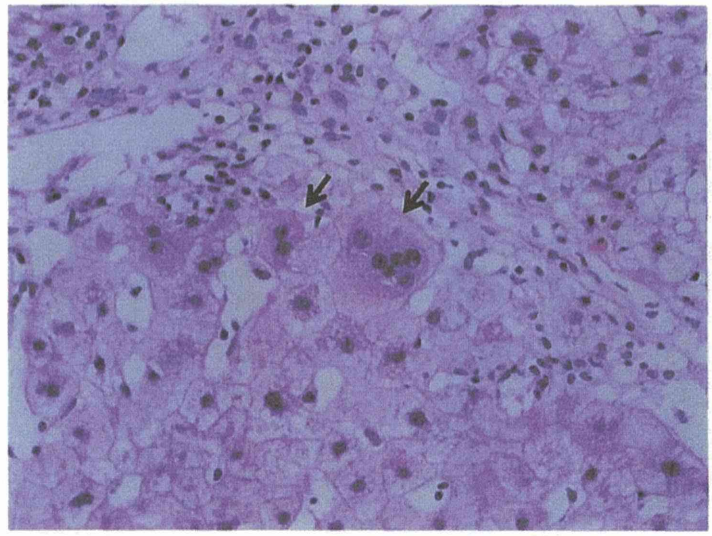

Fig. 4.9 Giant syncytial multinucleated hepatocytes (*arrows*) are present in some autoimmune hepatitis (AIH) cases

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.

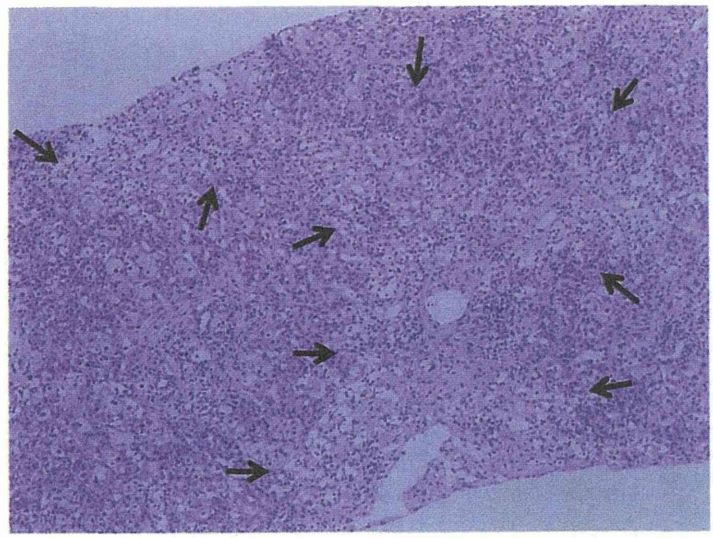

Fig. 4.10 Broad collapse of hepatocytes (*arrows*) in acute exacerbation of autoimmune hepatitis (AIH)

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

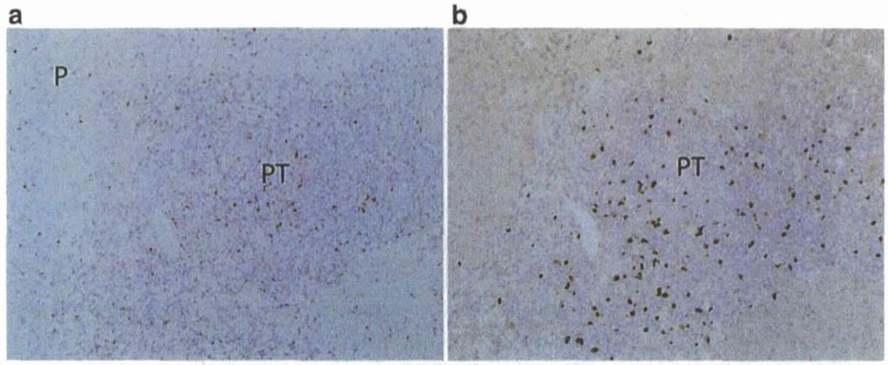

Fig. 4.11 Immunohistochemistry for T-bet (**a**) and Foxp3 (**b**). Many T-bet-positive Th1-type T cells are observed in portal tracts (PT) and parenchyma (P) (**a**). Foxp3-positive Treg cells are scattered in PTs (**b**)

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

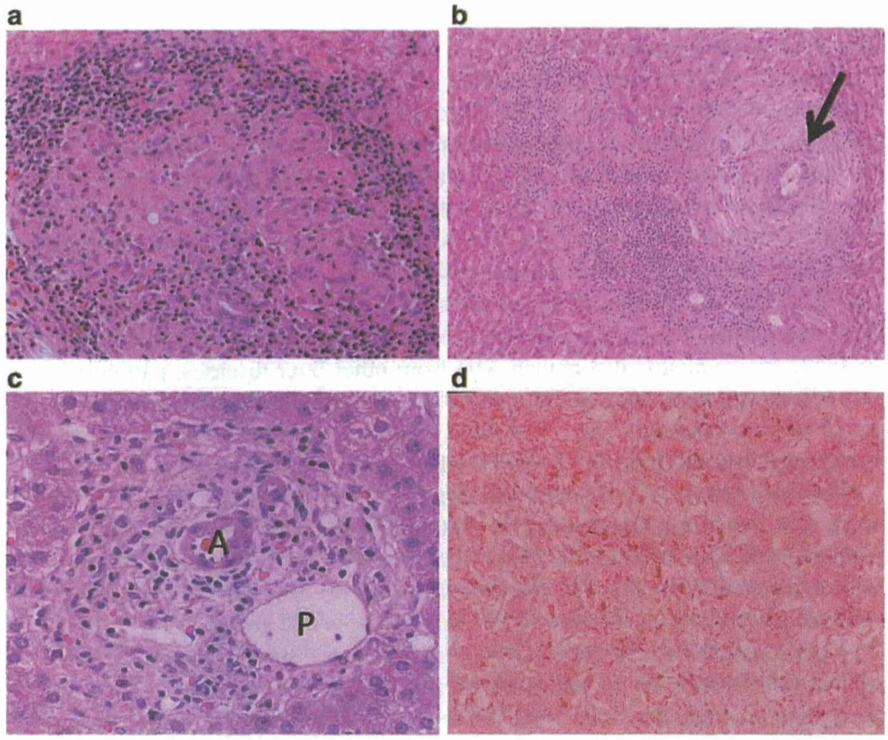

Fig. 4.12 Biliary changes raised by modified criteria (1999) of the autoimmune hepatitis (AIH) scoring system. (**a**) Granulomatous cholangitis in primary biliary cirrhosis. (**b**) Severe concentric periductal fibrosis in primary sclerosing cholangitis. (**c**): Ductopenia. The portal vein (P) and 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

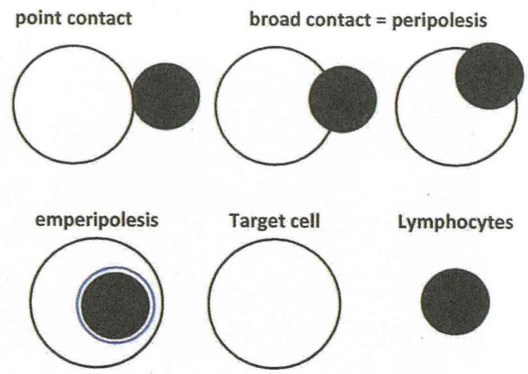

Fig. 4.13 Immunological contact pattern between target cells and lymphocytes. Emperipolesis is a unique feature of penetration of lymphocytes into hepatocytes, and the tightest pattern of target cells (hepatocytes) and effector cells (lymphocytes)

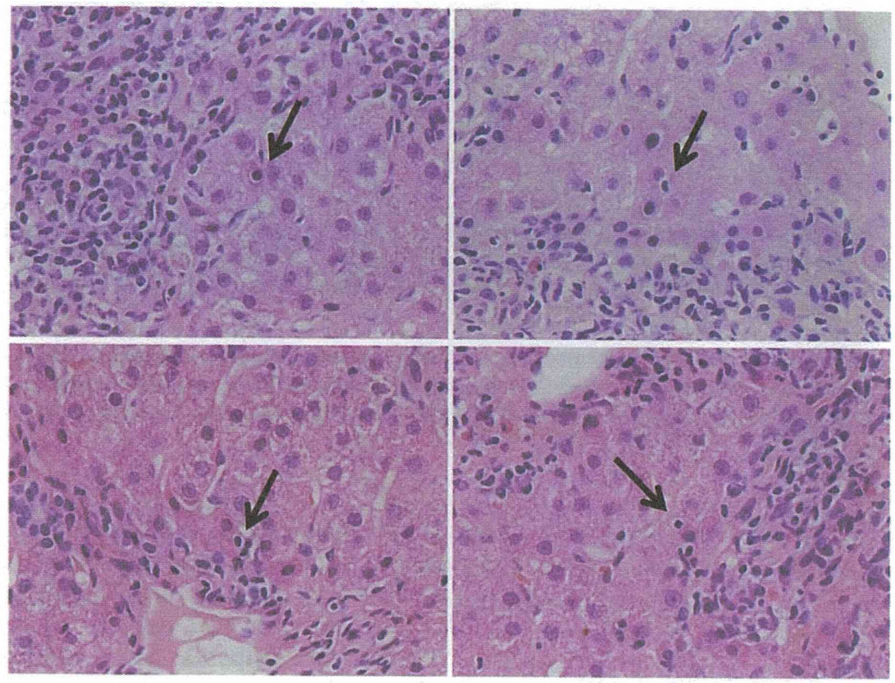

Fig. 4.14 Emperipolesis in autoimmune hepatitis (AIH). Emperipolesis is observed around the interface area. Although *arrows* indicate emperipolesis, all *arrows* are possibly hard to evaluate as emperipolesis

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]

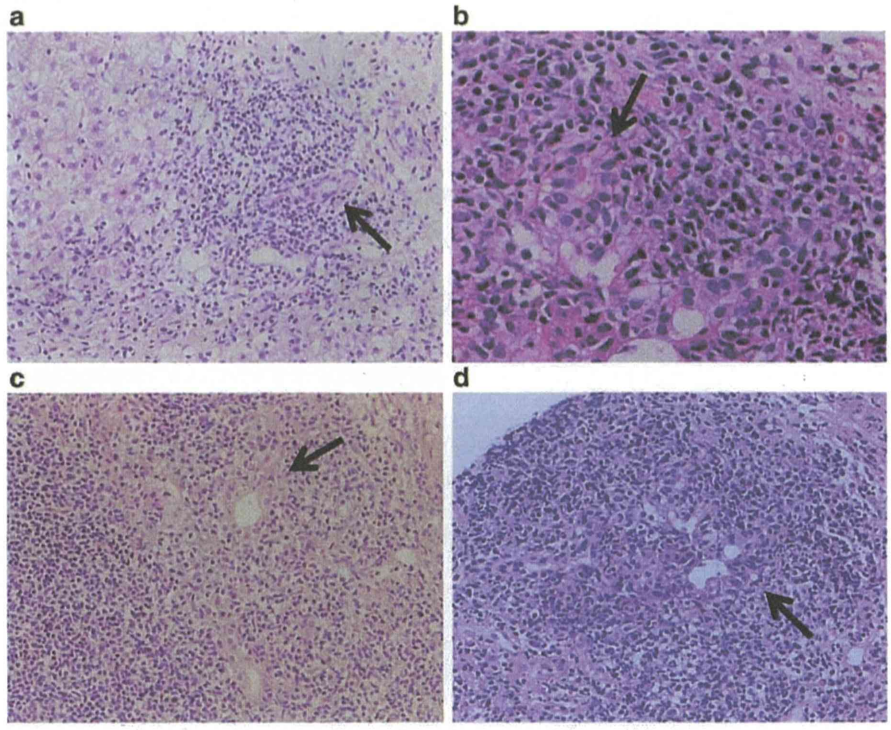

Fig. 4.15 Bile duct damage (hepatitic bile duct injury) in autoimmune hepatitis (AIH). *Arrows* denote the damaged interlobular bile ducts at various degrees (**a–d**). In particular, bile ducts in **c** and **d** show destructive changes resembling chronic nonsuppurative destructive cholangitis (CNSDC)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

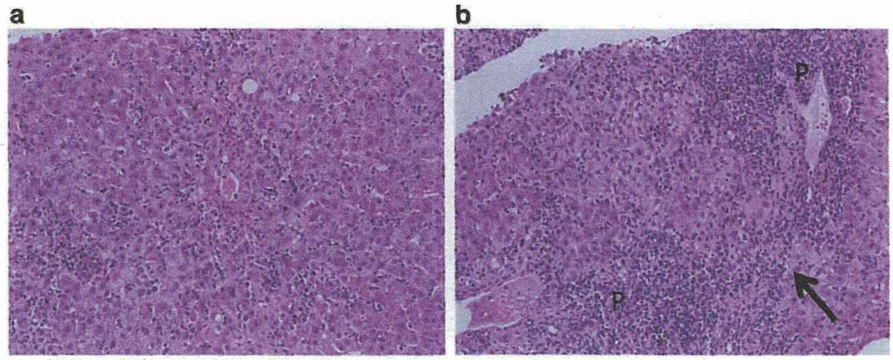

Fig. 4.16 Acute exacerbation of autoimmune hepatitis (AIH). (**a**) Many focal necroses are diffusely seen in parenchyma. (**b**) Bridging necrosis (*arrow*) is observed between enlarged portal tracts with inflammation (P)

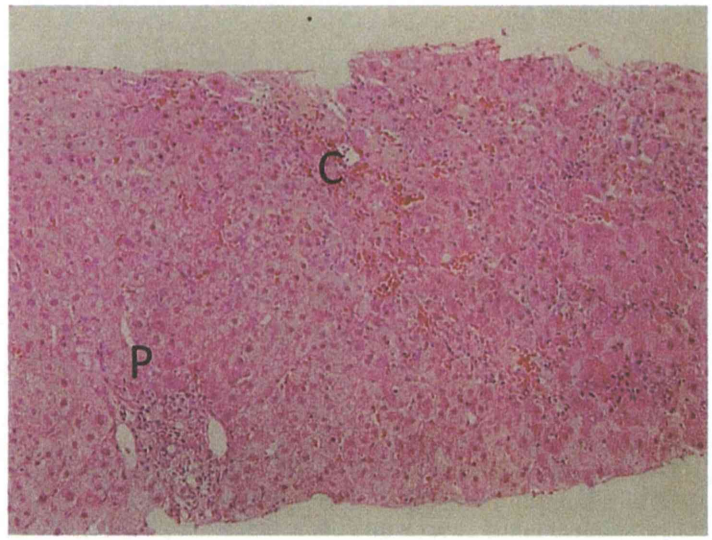

Fig. 4.17 Acute-onset autoimmune hepatitis (AIH) case. In parenchyma, many focal necroses and pigmented macrophages are scattered and accumulate around the central vein (C). Portal tracts (P) are almost preserved

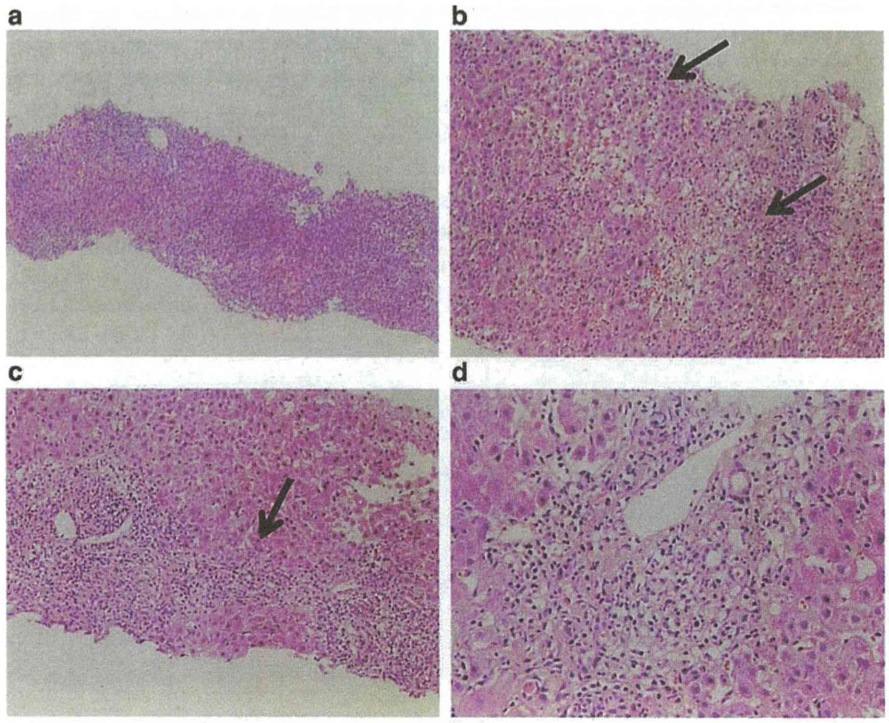

Fig. 4.18 Acute-onset autoimmune hepatitis (AIH) case without preceding liver dysfunction. (**a**) Lower magnification. A diffuse inflammatory change is observed. (**b**) Perivenular zonal necrosis is observed (*arrows*). (**c**) Bridging necrosis is seen between portal tracts. (**d**) Although mild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

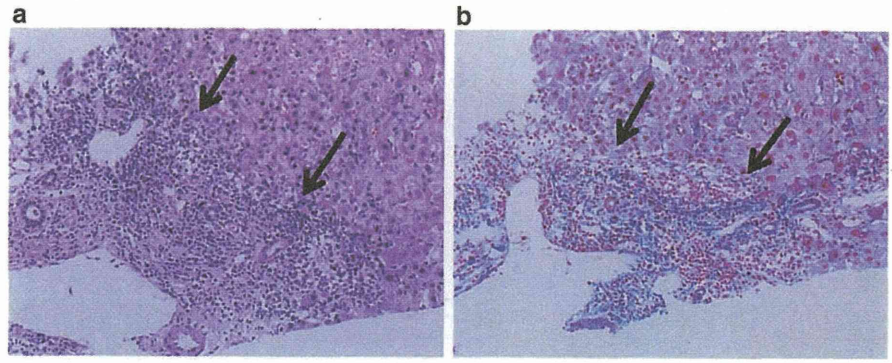

Fig. 4.19 Acute-onset autoimmune hepatitis (AIH) case without preceding liver dysfunction and drug intake. Antinuclear antibody is positive (1280×). In addition to diffuse lobular hepatitis, periportal zonal necrosis is observed (*arrows*, **a**). Azan–Mallory staining indicates an absence of fibrosis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

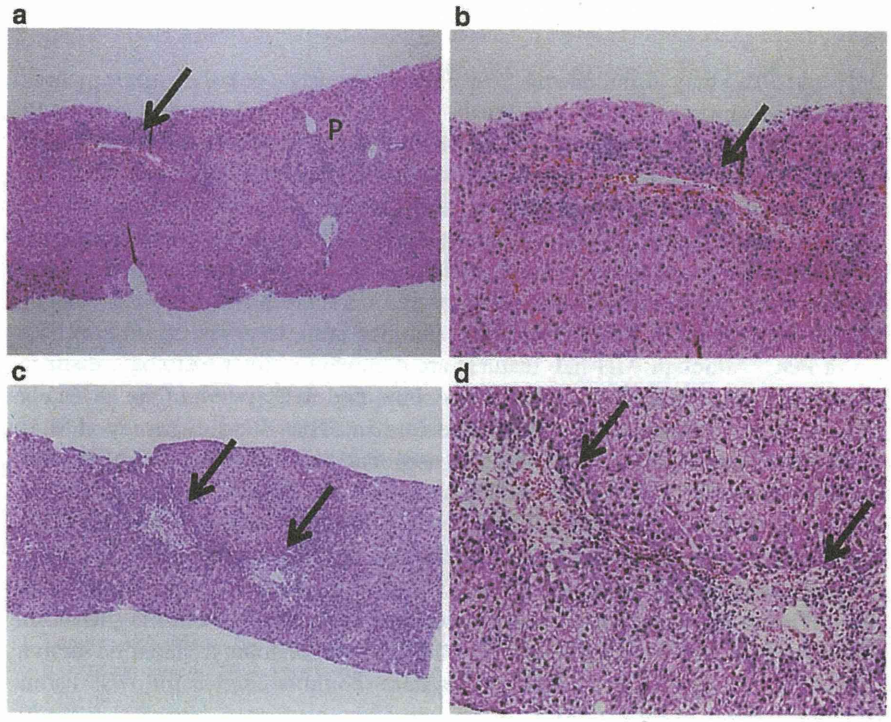

Fig. 4.20 Two cases of acute-onset autoimmune hepatitis (AIH) showing zone 3 necrosis. **a** and **b**: Perivenular zonal necrosis with hemorrhage is observed but portal inflammation is minimal (P). **c** and **d**: Perivenular zonal necrosis resembling hepatocellular necrosis of drug-induced liver injury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

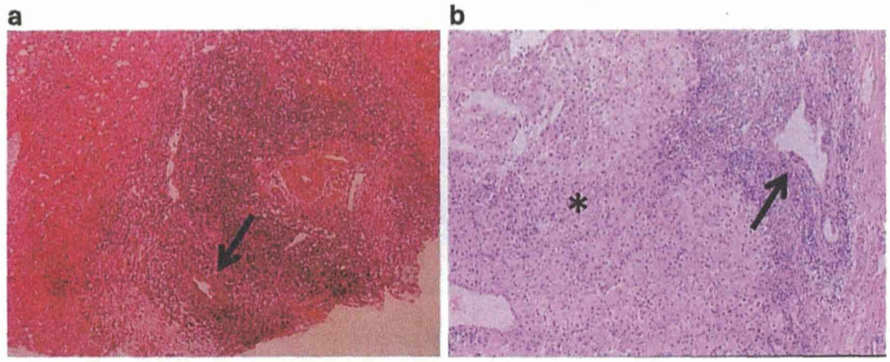

Fig. 4.21 Two cases of primary biliary cirrhosis–autoimmune hepatitis (PBC–AIH) overlap syndrome (hepatitic form of PBC). In addition to chronic nonsuppurative destruction cholangitis (CNSDC) (*arrows*), marked portal inflammation is observed. In the case on the right (**b**), interface hepatitis and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

Efficacy and tolerability of Entecavir for hepatitis B virus infection after hematopoietic stem cell transplantation

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Abstract

Introduction: Hepatitis B virus (HBV) flare is a serious problem following hematopoietic stem cell transplantation (HSCT), and the mortality rate is high if severe hepatitis occurs.

Case description: Although Entecavir (ETV) is a standard antiviral drug for HBV infection, the efficacy and safety of ETV therapy in HSCT are still unclear.

Discussion and Evaluation: To examine the efficacy and tolerability of ETV treatment in HSCT, we retrospectively identified 5 patients who received ETV for treatment of HBsAg carrier among patients undergoing HSCT in our institute. We reviewed their clinical information such as clinical course of serum HBV DNA levels, administration period and dose of ETV, and adverse events. There were no episodes of HBV flare or reactivation after HSCT in all patients during the observation period, as a 10-fold rise in HBV DNA levels or positive conversion of HBsAg were not observed.

Conclusion: ETV monotherapy is effective and safe for HBsAg carrier patients following HSCT.

Keywords: Hepatitis B virus; Hematopoietic stem cell transplantation; Entecavir

Introduction

Hepatitis B virus (HBV) flare and reactivation after hematopoietic stem cell transplantation (HSCT) is a life-threatening complication in patients with HBV infection (Liang et al. 1999). HBV related hepatitis is generally observed in hepatitis B surface antigen (HBsAg)-positive and/or HBV DNA-positive patients (Hui et al. 2005). Recently, however, HBV reactivation was reported in patients with even though resolved HBV infection that was indicated negative HBsAg and positive anti-hepatitis B core antibody (HBcAb) and/or HBsAb) at a lower rate (Knoll et al. 2004). It is well established that the frequency of HBV reactivation is higher in HSCT patients (Hammond et al. 2009). The underlying mechanism of HBV flare and reactivation following HSCT is likely to be related to impaired cellular immunity caused by prior chemotherapy and conditioning regimens. Furthermore, administration of immunosuppressive agents, including calcineurin inhibitors and steroids for graft-versus-host disease (GVHD), may exacerbate HBV replication (Xunrong et al. 2001).

HBV-infected patients at high risk of HBV flare and reactivation are recommended to receive preemptive antiviral therapy following HSCT. Lamivudine (LAM), an analogue of cytidine, is available for antiviral therapy in HSCT, (Tomblyn et al. 2009) and inhibits HBV reverse transcriptase, resulting in suppression of HBV replication. Several studies have reported the efficacy of LAM treatments in HSCT patients (Hsiao et al. 2006; Giaccone et al. 2010).

However, LAM treatment has the potential to induce development of drug-resistant mutations owing to the low genetic barrier. Since a small number of mutations are required for LAM resistance, the incidence of LAM-resistant HBV has recently been increasing, and LAM resistance is associated with a rebound in viral load and hepatitis. In fact, LAM treatments showed high resistance and recurrence rates in patients with chronic

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hepatitis B infection (CHB) undergoing liver transplantation (Perrillo et al. 2001; Mutimer et al. 2000; Lo et al. 2001). Similarly, the appearance of drug-resistant mutants and HBV DNA breakthrough within LAM treatments has been reported in HSCT patients (Hsiao et al. 2006). Thus, more effective agents with lower resistance rates are required in both liver transplantation and HSCT.

Entecavir (ETV), a cyclopentyl guanosine nucleoside analogue, has been approved for treatment of patients with CHB. ETV inhibits reverse transcriptase, DNA replication and transcription. Compared with LAM, ETV has greater antiviral potency and a higher genetic barrier to resistance (Lai et al. 2002). Several studies have shown the superiority of ETV treatments in liver transplantation (Xi et al. 2009; Fung et al. 2011), however, ETV treatment for patients undergoing HSCT has not been reported, and its role in HSCT remains unclear. Here, we describe HBsAg carrier patients administered ETV for treatment following HSCT, and consider its efficacy and tolerability.

Patients and methods

Patients

This study was approved by the local medical ethics committee of Tokyo Metropolitan Komagome Hospital. We retrospectively identified HBsAg carrier patients (serum HBsAg-positive and HBV-DNA positive) who received ETV for prophylaxis of HBV flare among patients undergoing HSCT between September 2006 and August 2011. We Laboratory data and clinical information were obtained from our institution's electronic medical records.

Hematopoietic stem cell transplantation methods

In our institution, myeloablative conditioning regimens were administered to allogeneic recipients aged <60 years, while elderly patients received fludarabine-based reduced-intensity regimens. GVHD prophylaxis usually comprised short-course methotrexate and cyclosporine A (CsA) or tacrolimus (FK506). For acute GVHD treatment, methylprednisolone (mPSL) 2 mg/kg i.v. in divided dose daily was administered. Antibacterial prophylaxis was provided by tosufloxacin. Steroid-resistant GVHD was treated with a steroid pulse (mPSL 1000 mg i.v. in divided dose daily for 3 days) or mycophenolate mofetil (MMF) 1000 mg twice daily p.o.. Antifungal prophylaxis consisted of fluconazole or itraconazole. Acyclovir or valacyclovir was administered for herpes simplex virus prophylaxis and ganciclovir or foscarnet was administered against cytomegalovirus reactivation.

Entecavir therapy

ETV 0.5 mg once daily p.o. was administered to patients with HBsAg-positive as primary treatments. The ETV dose was adjusted according to the kidney function. None of the patients received LAM or hepatitis B immunoglobulin at the time of transplantation or during the post-transplant period.

Hepatitis B virus assay

The levels of HBsAg, anti-HBc and anti-HBs were determined using commercially available chemiluminescence enzyme immunoassay kits (LUMIPULSE Presto HBsAg, LUMIPULSE Presto HBsAb-N, LUMIPULSE Presto HBcAb-III; Fujirebio, Tokyo, Japan). The cut-off index (COI) of the assay of HBsAg, anti-HBs and anti-HBc was 1.0, 10 mIU/mL and 1.0, respectively. The serum HBV DNA concentrations were quantified using the COBAS AmpliPrep/COBAS TaqMan HBV Test (Roche Diagnostics, Basel, Switzerland). The four major HBV genotypes (A–D) were determined by enzyme-linked immunosorbent assay with monoclonal antibodies directed against distinct epitopes on the preS2-region products using commercial kits (HBV GENOTYPE EIA; Institute of Immunology Co. Ltd., Tokyo, Japan) (Orito et al. 2001). HBV DNA sequences bearing the core promoter and precore or core regions were amplified by PCR with heminested primers. The PCR products were directly sequenced by the dideoxy chain termination method using a Big Dye Terminator (Applied Biosystems, Foster city, CA) and an ABI PRISM 3100-avant analyzer (Aritomi et al. 1998).

Case description

Patient profiles

Among the 376 patients who received HSCT in our hospital between September 2006 and August 2011, six HBsAg-positive and HBV-DNA positive patients were identified (Figure 1). Of those patients, five patients received ETV for prevention of HBV flare (Table 1). Four patients were inactive chronic hepatitis B (ICHB) (#1, #2, #4 and #5) and other patients were CHB (#3), respectively. Patient #5 has developed HBV reactivation during pre-transplantation therapy at previous treated hospital. Four patients underwent allogeneic HSCT, while one patient underwent autologous HSCT (#1). Within 4 allogeneic recipients, patient #4 received a graft from a matched related donor, while the other three patients received grafts from matched unrelated donors. All of donors were serum HBsAg negative. Myeloablative conditioning regimens were administered to three allogeneic recipients and a fludarabine-based non-myeloablative conditioning regimen was administered to the remaining allogeneic recipient. For GVHD prophylaxis, one patient (#2) received CsA and methotrexate and three patients received FK506 and methotrexate. At the time of transplantation, two patients had undetectable serum HBV DNA levels. Three patients were HBV genotype C and one patient was genotype B. In addition,