Chapter 3
Spleen and Liver

Takuji Torimura

Abstract In liver cirrhosis, the spleen is a source of nitric oxide which affects a hyperdynamic state typical of portal hypertension. It is generally accepted that pancytopenia results predominantly from the increased phagocytosis and destruction of hemocytes in splenic macrophages. In addition, liver fibrosis is amplified by migrated Th2 lymphocytes and transforming growth factor beta from the spleen. There is a possibility that increase of the spleen stiffness is the primary factor of idiopathic portal hypertension. Spleen stiffness is caused by bleeding, fibrosis, and calcareous deposits after increase in red pulp pressure due to venous congestion. In nonalcoholic steatohepatitis, macrophage activity in the spleen is upregulated. In addition, high levels of inflammatory cytokines are produced and T cell shows increased proliferation in the spleen. In autoimmune hepatitis model, $CD4^+$ T cells are differentiated into follicular helper T cells (T_{FH}) in the spleen. T_{FH} cells promoted hypergammaglobulinemia and antinuclear antibodies production. T_{FH} cells migrate from the spleen to the liver, triggering induction of autoimmune hepatitis in this model. IgM-positive B cells localize in the CD21-positive lymph follicle in the spleen of primary biliary cholangitis. These findings prove that the spleen influences on the pathogenesis and severity of several kinds of liver disease.

Keywords Hyperdynamic state • Immune cell • Hypersplenism • TGF beta-1 • Follicular helper T cells

3.1 Introduction

The spleen, in healthy adult humans, is approximately 12 cm in length and usually 100–200 g in weight. It consists of red pulp and white pulp. The spleen is the largest lymphoid organ in the human body. However, it was considered a neglected organ. Since 1952, when King and Schumacker reported overwhelming post-splenectomy infection [1], there has been a growing recognition of the importance of the spleen

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in the human body. Especially, in recent years, studies of spleen organization and structure, cell function, secretion, and innervations, a better understanding of the function has been gained. It has a fundamental role as the destruction of red blood cells, as the modulation of the immune system, and as the maintenance of peripheral tolerance via clearance of circulating apoptotic cells, the differentiation and activation of T and B cells, and production of antibodies in the white pulp [2, 3].

In the spleen, splenic macrophages and endothelial cells of the marginal sinus compose the blood-spleen barrier. The cells of the blood-spleen barrier can trap circulating infectious organisms and monocytes clear them from the blood stream and providing a selective environment for monocyte differentiation into macrophages and subsequent phagocytosis of the microorganisms. The interactions between the circulating lymphocytes and macrophages may regulate the entry of lymphocytes into the white pulp. The functions of the blood-spleen barrier in the spleen are to filter antigens, to keep the microenvironment of the white pulp stable, and to present antigen information to white pulp through the effects of the mechanical barrier which depends on the connection between cells and the phagocytosis of macrophages. The blood-spleen barrier in the spleen is relatively loose without the tight junction between cells. However, the blood-spleen barrier has more ability to stop and phagocytize more xenobiotic materials than other barriers, such as the blood-brain barrier and the blood-thymus barrier [4, 5].

Table 3.1 summarizes the functions of the spleen. As the spleen is strictly linked to liver, it influences on several kinds of liver disease, such as liver cirrhosis,

Table 3.1 Function of the spleen

Red pulp
Extramedullary hematopoiesis
Facilitating an environment wherein erythrocytes rid themselves of solid waste material
Blood filter for foreign material and damaged and senescent blood cells
Storage site for iron, erythrocytes, platelets, plasmablasts, and plasma cells
Rapid release of antigen-specific antibodies into the circulation produced by red pulp plasma cells
Defense against bacteria using iron metabolism by its macrophages
White pulp
T-cell zone (peripheral lymphatic sheath) and B cell zone (follicles)
Storage site for B and T lymphocytes
Development of B and T lymphocytes upon antigenic challenge
Release of immunoglobulins upon antigenic challenge by B lymphocytes
Production of immune mediators involved in clearance of bacteria
Marginal zone
Phagocytosis of circulating microorganisms and immune complexes by marginal zone macrophages
Development of marginal zone B lymphocytes upon T1-2 antigenic challenge
Blood trafficking of B and T lymphocytes
Release of immunoglobulins upon antigenic challenge by splenic B lymphocytes

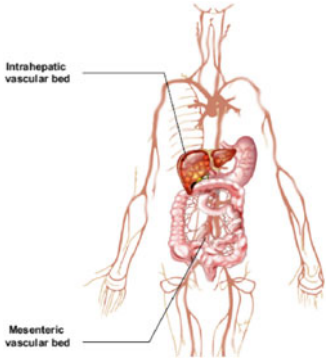
idiopathic portal hypertension, nonalcoholic steatohepatitis, autoimmune hepatitis, and primary biliary cholangitis. In this chapter, I would like to review the participants of the spleen in liver diseases.

3.2 Liver Cirrhosis

Portal hypertension, pancytopenia, liver fibrosis, and regeneration and insulin resistance are common symptoms of liver cirrhosis. I will describe the participation of the spleen to such symptom [6, 7].

In patients with liver cirrhosis, increase of splenic blood flow is a common finding. There is a good correlation between the spleen volume and portal flow volume [8]. Kayacetin et al. [9] reported that splenic vein congestion was significantly increased in the Child-Pugh class C compared with the Child-Pugh class A. In addition, liver cirrhosis patients with esophageal varices bleeding as well as ascites have greater splenic blood flow volume, high splenic vein congestion, and high portal vein congestion.

In liver cirrhosis, a number of vasoactive substances have been implicated as potential mediators of portal hypertension. Especially, nitric oxide (NO), angiotensin II (Ang II), and endothelins (ETs) have received the greatest attention [10]. NO is a potent vasodilator, and there are 3 isoforms of NO synthase (NOS): inducible NO (iNOS), endothelial NOS (eNOS), and neuronal NOS [11, 12]. ETs are a family of 3 related peptides, ETs1, 2, 3. ETs act as potent vasoconstrictors [13]. The respective roles of NOS and ET-1 have been well studied in cirrhotic liver [14, 15]. The mechanical obstruction is assumed to be caused by distortion and compression of the hepatic vasculature by increased fibrosis and nodule formation [16]. In addition, intrahepatic vascular bed is typified by an increase in resistance to blood flow in cirrhotic liver. Liver injury causes a reduction in the production of vasodilators by sinusoidal endothelial cells such as NO; concomitantly, there is an increase in the synthesis of vasoconstrictors such as ET1 and Ang II by other cells in the sinusoids. In mesenteric vasculature, increased portal blood flow from the splanchnic circulation augments portal pressure and thereby contributes to the maintenance and exacerbation of portal hypertension. In cirrhotic condition, the expression of ET-1 and Ang II is reduced in splanchnic circulation. On the contrary, eNOS phosphorylation and NO production are increased. Arterial vasodilatation in the splanchnic circulation plays a critical role in increasing the blood flow to the portal vein. Increased eNOS-derived NO plays a principal role in arterial vasodilatation [17] (Fig. 3.1). In liver cirrhosis, the spleen is also a source of NO and secreted into portal blood flow affects a hyperdynamic state typical of patients with liver cirrhosis. A recent study indicates the possibility of the involvement of renin-angiotensin (Ang) system [18]. Ang II is a vasoconstrictor generated by Ang-converting enzyme (ACE) and is further cleaved by ACE2 to Ang-(1-7). Ang-(1-7) is a vasodilator, which binds to the G-protein coupled receptor Mas (MasR) and leads to eNOS activation and NO production in endothelial cells



Molecular biology	Physiology
Intrahepatic vascular bed	
Increased expression of vasoconstrictors(ET-1, Ang II)	Increased resistance Increased pressure
Reduced eNOS phosphorylation, activity and NO production	Reduced portal blood flow
Splanchnic vascular bed	
Reduced expression of vasoconstrictors(ET-1, Ang II)	Arterial vasodilatation
Increased eNOS phosphorylation, activity and NO production	Increased portal blood flow

Fig. 3.1 Prominent vascular beds and their pathophysiology. *ET-1* endothelin-1, *Ang II* angiotensin II, *NO* nitric oxide, *eNOS* endothelial nitric oxide synthase, *VEGF* vascular endothelial growth factor, *PDGF* platelet-derived growth factor, *PlGF* placental growth factor (Image from Published Paper Iwakiri et al. 2014)

[19, 20]. Besides NO, other vasodilators, such as CO, prostacyclin, adrenomedullin, endocannabinoids, and endothelium-derived hyperpolarizing factor, also mediate arterial vasodilatation. These multiple factors seem to be involved in the excessive vasodilatation, observed in the splanchnic circulation.

3.2.1 Pancytopenia

Abnormalities in hematological parameters are common in patients with liver cirrhosis. The pathogenesis of abnormal hematological indices in liver cirrhosis is multifactorial such as portal hypertension, alterations of bone marrow stimulating factors, viral- and toxin-induced bone marrow suppression, and consumption loss (Table 3.2).

Splenic sequestration and destruction of platelets, white blood cells, and red blood cells in the portal hypertension-induced enlarged spleen is defined as hypersplenism. Liangpunsakul et al. [21] reported that 33 % patients with chronic liver disease were associated with severe hypersplenism. Although a number of factors contribute to hematological indices in patients with liver cirrhosis, portal hypertension and alterations in bone marrow hormones appear to be the strongest

Table 3.2 Causes of pancytopenia in liver cirrhosis

Portal hypertension-induced splenic sequestration
Alteration in thrombopoietin
Bone marrow suppression mediated by hepatitis B virus, hepatitis C virus, and alcohol
Consumptive coagulopathy (low-grade DIC, acquired intravascular coagulation, and fibrinolysis)
Increased blood loss
Alterations in granulocyte-colony stimulating factor and granulocyte macrophage-colony stimulating factor
Alterations in erythropoietin

contributions. It is generally accepted that pancytopenia results predominantly from the increased phagocytosis and destruction of hemocytes in splenic macrophages [22]. The rate of phagocytosis and the index of phagocytosis of splenic macrophages are notably in negative correlation with the count of leukocyte and platelet in peripheral blood. Regarding thrombocytopenia, the mechanisms include decreased production, splenic sequestration, endothelial dysfunction, and autoimmune destruction. Tana et al. [23] reported that the peripheral platelet count in patients with chronic hepatitis C was associated with liver fibrosis, thrombopoietin level, immature platelet fraction, and spleen size. Other factors that may influence the platelet count include platelet-associated antibodies and antiplatelet immunoglobulins. However, previous reports described that the presence of antiplatelet antibodies did not affect the platelet count [24].

Regarding the relationship between thrombocytopenia and liver fibrosis, Kondo et al. [25] clarified that patients with liver cirrhosis had a more extensive platelet area in the liver compared with those with normal liver. In cirrhotic liver, most platelets were present in the sinusoidal space of the periportal area with inflammation, where hepatic stellate cells expressing platelet-derived growth factor (PDGF) receptor-beta were frequently observed. Hepatic stellate cell is a key mediator of liver fibrosis. PDGF is the basic mediator involved in platelet granules. PDGF stimulates the proliferation of hepatic stellate cells [26]. So, they speculate that the accumulation of platelets in the liver may be involved in thrombocytopenia and liver fibrosis through the activation of hepatic stellate cells.

Recently, Li et al. [27] investigated the expression of microRNA of splenic macrophages in hypersplenism due to portal hypertension in hepatitis B virus-related liver cirrhosis. In this study, they identified 99 microRNA differences in expression between splenic macrophages associated with hypersplenism and those from the normal spleen. In those microRNAs, has-miR-615-3p was significantly upregulated in hypersplenism. As dysregulation of microRNA levels would affect the translation of multiple protein-coding genes, they speculated that has-miR-615-3p might regulate the activation of splenic macrophages and involved in the pathogenesis of hypersplenism due to portal hypertension.

3.2.2 Liver Fibrosis and Regeneration

Clinical observations have revealed that splenectomy not only increases the platelet count but also improves liver function [28, 29]. Some basic studies have shown that splenectomy suppresses the progression of liver fibrosis. For instance, platelets are able to inhibit the development of liver fibrosis, and splenectomy exerts an antifibrotic effect via thrombocytosis [30, 31]. The loss of spleen-derived transforming growth factor beta (TGF beta)-1 mediates the inhibitory effect of splenectomy on liver fibrosis. TGF beta-1 induces the phenotypic transition of hepatic stellate cells to proliferating myofibroblast-like cells that increase the production of extracellular matrix components and reduce the degradation of extracellular matrix proteins. Decreases in portal flow and portal pressure are other possible mechanisms of the antifibrotic effect of splenectomy.

The spleen also plays important roles in the immune system. The immune system can affect the progression of liver fibrosis because strain-specific differences in murine hepatic fibrosis are mediated by divergent T helper cytokine response [32]. In addition, immunosuppressants differently modulate hepatic fibrosis in mice and humans [33, 34]. These findings suggest that the spleen modulates hepatic fibrosis through its immunological functions. Previous studies have revealed that Th1 cytokines have a suppressive effect on liver fibrosis. In particular, interferon-r is a potent inhibitor of the activation of hepatic stellate cells [35, 36]. In contrast, Th2 cytokines such as IL-4 and IL-13 promote activation of hepatic stellate cells and progression of liver fibrosis [37, 38]. In mouse liver fibrosis model, $CD4^+$ cells in the spleen migrated to the liver in response to liver injury. As Th2 lymphocytes are the main constituent of the immune environment in the spleen, most of the spleen-derived $CD4^+$ cells in the fibrotic liver were Th2 cells. These findings as well as previous reports indicate that Th2-predominant splenic T lymphocytes migrate to the injured liver and promote liver fibrosis by modifying the Th1/Th2 balance in the liver toward Th2 dominance. Splenectomy decreases the absolute number of Th2 lymphocytes in the liver and reverses this Th1/Th2 imbalance, resulting in inhibition of liver fibrosis [39]. In human samples, Nomura et al. [40] reported that splenectomy significantly decreased liver fibrosis and the $CD4^+/CD8^+$, suggesting that splenectomy modified Th1/Th2 balanced to Th2 dominant and improved liver fibrosis in human.

Experimental studies have reported that splenomegaly inhibits liver regeneration in liver cirrhosis [41, 42] and splenectomy improves liver regeneration [43]. After a partial splenectomy in cirrhotic patients with splenomegaly, the remnant liver tends to regenerate to a small volume than in patients without splenomegaly [44]. However, the exact underlying mechanism for this effect has been elusive thus far. Two general mechanisms have been put forward. First, it has been proposed that the cytokines or growth factors released from the spleen influence liver regeneration by way of the portal circulation. The spleen promotes liver regeneration via preferential upregulation of heme oxygenase 1 and downregulation of tumor necrosis factor alpha [45]. In addition, spleen-derived TGF beta-1 has been reported to play a

critical role in inhibiting the growth of hepatocytes [46]. TGF beta-1 induces liver fibrosis. However, it also acts directly on hepatocytes by inhibiting cell proliferation and inducing apoptosis [47]. TGF beta-1 plays an essential role in the normal quiescent liver by inducing a tonic effect to hepatocytes to keep them in the G0 phase [48]. Although TGF beta-1 is produced principally in the hepatic stellate cells in the liver, it is also produced by the macrophages in the spleen and secreted into the portal vein in a liver injury model (Fig. 3.2). Morinaga et al. reported that splenectomy significantly improved liver regeneration with reduction of plasma TGF beta-1 in cirrhotic model. Second, the hemodynamic alterations induced by a splenectomy are proposed to provide a favorable environment for liver regeneration. The splenectomy-induced increase in hepatic arterial flow with increased oxygen supply is also suggested to induce liver regeneration [49]. It is noteworthy that other spleen-derived factors such as hepatocyte growth factor (HGF) activator-inhibitor (HAI) and ET-1 also impair liver regeneration in cirrhotic liver. Furthermore, Lee et al. [21] reported that splenectomy-induced upregulation of hepatocyte growth factor (HGF) and downregulation of TGF beta-1 in the injured liver. TGF beta-receptor II was more highly expressed in the injured liver, and c-Met, a HGF receptor, was more highly expressed in the injured liver with splenectomy. TGF beta-1 binds to its receptor, TGF beta-receptor II, at the end of the regeneration phase, and it induces the production of extracellular matrix in the liver. Newly synthesized extracellular matrix, especially glycosaminoglycans, would be capable of inhibiting urokinase-induced HGF activation by binding to HGF itself. This set of events would bring hepatocytes back into a stage of quiescence, surrounded by HGF and TGF beta-1. Splenectomy increased HGF level in the portal vein and also upregulated the expression of HGF in the liver. HGF in the liver is mainly produced in the hepatic stellate cells [50] and is known to be essential to initiate the process of liver regeneration. Activated HGF binds to c-Met and stimulates hepatocytes DNA synthesis via endocrine or paracrine mechanism.

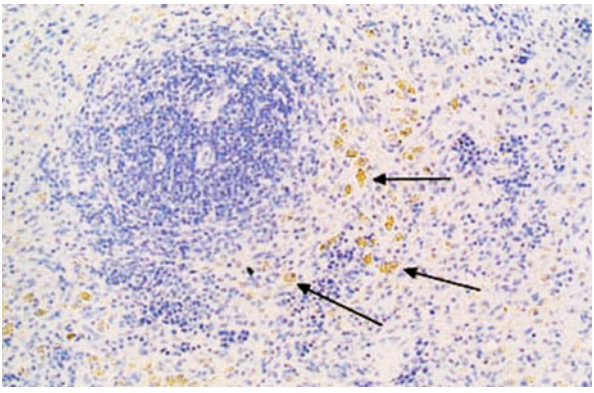


Fig. 3.2 Localization of TGF beta1-positive cells in the spleen. TGF beta1 was produced by macrophages in the red pulp (*arrows*). TGF beta1, transforming growth factor-beta1 (Image from Published Paper Akahoshi et al. 2002)

3.2.3 Insulin Resistance

Shimizu et al. [51] clarified that partial splenectomy reversed insulin resistance in patients with liver cirrhosis. The mechanism by which partial splenectomy reverses insulin resistance primarily involves hemodynamic improvements in the intestinal venous flow into the liver. In case of portal hypertension, the intestinal venous flow to the liver is disturbed due to the overflow of splenic venous return. The decrease in the splenic venous flow achieved with partial splenectomy may contribute to the increase in the intestinal venous flow relative to the total portal venous flow.

3.3 Idiopathic Portal Hypertension

Idiopathic portal hypertension is a disease that is characterized by splenomegaly and portal hypertension without liver cirrhosis. Occlusion of peripheral portal veins in the liver and increased splenic blood flow as a result of splenomegaly have been reported to be the causes that give rise to portal hypertension. The presence of fibrotic portal tracts and thin fibrous septa in the absence of cirrhosis are pathological criteria for making a diagnosis of idiopathic portal hypertension. Clinically, the median value of liver stiffness in patients with idiopathic portal hypertension is lower than that in patients with liver cirrhosis. To the contrary, spleen stiffness is higher in patients with idiopathic portal hypertension [52]. Histologically, splenomegaly in idiopathic portal hypertension was attributed to an increase in venous sinuses in the red pulp [53]. In addition, the lymph follicles and the reticular fibers and collagen fibers around the splenic cords grow thicker in a diffuse manner, and narrowing of cord width and narrowing of the sinus lumens is seen. Moreover, the rod-shaped cells became deformed, fall into disarray, and diversify, and the slits between the rod-shaped cells enlarge [54, 55]. The basal lamina of the sinuses also thickens. Therefore, the splenic tissue hyperplasia characterized by fibrogenesis enlargement and passive spleen congestion are likely to cause the increase of spleen tissue stiffness. The spleen is also known to undergo fibrosis in liver cirrhosis, primarily, in the red pulp. The appearance of venous sinus hyperplasia in patients with liver cirrhosis is not so marked as in patients with idiopathic portal hypertension, and the rod-shaped cells in the venous sinuses do not diversify as much. The degree of splenic congestion and fibrosis in patients with liver cirrhosis might be smaller than that in patients with idiopathic portal hypertension. Regarding the pathological difference of the spleen between idiopathic portal hypertension and liver cirrhosis, Sato et al. [44] reported that there is increase of Gamna-Gandy nodules, which developed in the lymphocyte sheaths around arteries and the splenic trabeculae, in idiopathic portal hypertension. Gamna-Gandy nodules are formed as a result of bleeding, fibrosis, and calcareous deposits after an increase in red pulp pressure due to venous congestion [56]. Gamna-Gandy nodules seem to be involved

in increase of splenic stiffness. There is a strong possibility that portal hypertension due to narrowing of portal vein branches in the liver is not the only cause of idiopathic portal hypertension, and specific increase of spleen stiffness is the material primary factor.

3.4 Non-alcoholic Steatohepatitis

Non-alcoholic fatty liver disease (NAFLD), the most common cause of steatosis, is associated with obesity, mainly visceral, and insulin resistance. In the presence of more severe risk factors, simple hepatic steatosis may be complicated by liver inflammation named non-alcoholic steatohepatitis (NASH). NASH can lead to perisinusoidal fibrosis. Fat-laden hepatocytes are swollen, and in steatohepatitis, further swelling occurs due to ballooning of hepatocytes to cause sinusoidal distortion. It reduces intrasinusoidal volume and microvascular blood flow. Involvement of other cell types, such as sinusoidal endothelial cells, Kupffer cells, and hepatic stellate cells, and recruitment of inflammatory cells as well as platelets lead to dysregulation of microvascular blood flow. In animal models, the net effect of such changes is a marked reduction of sinusoidal space by approximately 50 % and a decrease in the number of normally perfused sinusoids. Such microvascular damage could accentuate further liver injury and disease progression in NASH.

NASH is a progressive liver disease characterized by Kupffer cell dysfunction which contributes to its pathogenesis. It is noteworthy that the reticular-endothelial system also plays a key role in the spleen. Colloid scintigraphy is a good method of reflecting Kupffer cell activity. Duman et al. [57] Reported that Colloid shift to the spleen was observed in patients with NASH comparing with simple steatosis. In a nutritional model of NASH in rats fed a methionine-and choline-deficient diet, the liver/spleen uptake ratio was significantly decreased in rats after 8 weeks of a methionine-and choline-deficient diet in comparison with control diet-fed rats [58]. In addition, the patients with NASH demonstrated higher IL-6 blood levels, spleen longitudinal diameter values, and VEGF concentrations than those of healthy subjects [59].

The relationship between NAFLD and immune functions is still under investigation. The spleen may have an important role. In the splenic lymphocytes of obese rats, the expression of glucose transporter 1 (GLUT-1) was lower compared to lean rats. The decreased expression of GLUT-1 in obese rats was associated with a decreased uptake of glucose into immune cells [60]. Miyake et al. [61] reported that in NAFLD mice immunized with hepatitis B vaccine containing hepatitis B surface antigen (HBsAg) and hepatitis B core antigen (HBcAg), levels of anti-HBs and proliferation activity of HBsAg and HBcAg-specific lymphocytes were significantly lower compared to controls. In addition, higher levels of inflammatory cytokines were produced and T cells showed increased proliferation rate in splenic cells of NAFLD than control. Splenic dendritic cells processing and presenting antigen activities were significantly decreased in NAFLD mice.

3.5 Autoimmune Hepatitis

Autoimmune hepatitis is characterized by mononuclear cell infiltration in the liver and elevated levels of gamma globulins as well as by the production of a variety of characteristic autoantibodies, including antinuclear antibodies. Liver-infiltrating T cells are considered the primary disease mediators of inflammatory liver damage, and circulating autoantibodies are diagnostic hallmarks. However, clinical manifestations are varied in patients with autoimmune hepatitis, ranging from non-symptomatic mild chronic hepatitis to fulminant hepatic failure.

Aoki et al. [62] reported that in programmed cell death-1 (PD-1)-deficient mice underwent thymectomy 3 days after birth, immune dysregulation by a concurrent loss of $Foxp3^+$ regulatory T cells and PD-1-mediated signaling induced fatal autoimmune hepatitis resembling acute-onset autoimmune hepatitis. In this model, $CD4^+$ and $CD8^+$ T-cell infiltration from the spleen with massive lobular necrosis in the liver, hypergammaglobulinemia, and production of antinuclear antibodies were observed. The spleen is an induction site for autoimmune hepatitis in this model, and splenic $CD4^+$ T cells were differentiated into follicular helper T cells (T_{FH}) in the spleen. T_{FH} cells expressing inducible costimulatory (ICOS) and C-X-C chemokine receptor (CXCR) five comprise a newly defined effector T-cell subset that powerfully assists B cells in forming germinal centers [63]. Dysregulated T_{FH} cells promoted hypergammaglobulinemia and antinuclear antibody production in this model. T_{FH} cells migrated from the spleen to the liver through the C-C chemokine receptor 6/C-C chemokine ligand 20 axis, triggering induction of autoimmune hepatitis. Regarding the fetal progression of autoimmune hepatitis in the model, they also reported that dendritic cells (DC)-derived IL-18 induced the differentiation of Th1 and $CD8^+$ effector cells in the spleen and hepatic macrophages/Kupffer cells producing CXC ligand 9 (CXCL9) induced the migration of these cells to the liver. TNF-alpha directly induces maturation of DCs, TNF-alpha and IL-18 may directly induce inflammasome upregulation and skew toward IL-18 production. TNF-alpha induces cell death of hepatocytes and free DNA released from apoptotic hepatocytes activates Toll-like receptor 9, triggering a signal cascade to induce pro-IL-18 [64]. Therefore, TNF-alpha may induce apoptosis of hepatocytes, triggering canonical IL-18 production. However, IL-18 may act as an autocrine for skewing prolonged IL-18 secretion in DCs. They concluded that IL-18 induced differentiation of $CD4^+$ T into Th1 cells and $CD8^+$ cells into effector T cells, following CXCR3-CXCL9 axis-dependent migration of these cells to the liver which induce fatal progression of autoimmune hepatitis [65] (Fig. 3.3). In addition, they showed that although corticosteroid therapy was effective for autoimmune hepatitis in mice, it allows residual splenic dysregulated T_{FH} cells to remain after treatment, which appear to be responsible for relapse. Splenectomy overcomes this insufficiency, including prolonged remission of autoimmune hepatitis in mice [66]. Furthermore, they reported that in another mouse model which developed chronic autoimmune hepatitis with fibrosis, hypergammaglobulinemia, and antinuclear antibody production, neonatal splenectomy suppressed the onset of

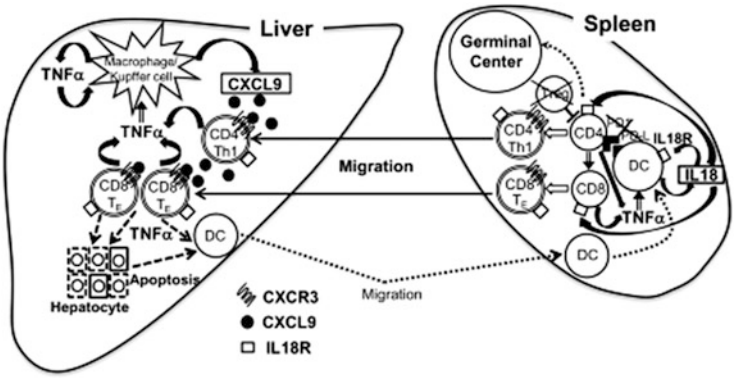


Fig. 3.3 Mechanistic links of cytokines and chemokines in the spleen and the liver in the progression of autoimmune hepatitis. *TN alpha* tumor necrosis factor alpha, *CXCL9* C-X-C motif ligand9, T_E effector T cell, *Th1* helper T cell, *DC* dendritic cell, *PD-1* programed cell death-1, *IL-18R* interleukin 18 receptor (Image from Published Paper Ikeda et al. 2014)

chronic autoimmune hepatitis. These findings indicate that the spleen is a very important organ for the induction and progression of autoimmune hepatitis.

3.6 Primary Biliary Cholangitis

Primary biliary cholangitis is characterized by the presence of the most highly directed and specific autoantibody in human immunopathology: the antimitochondrial antibody and the presence of a high frequency of antigen-specific autoreactive CD4 and CD8 T cells [67, 68]. In autoimmune diseases, the germinal center may be a pathogenic hot spot for production of autoantibodies [69]. T_{FH} cells are located in germinal center where primarily drive B cells differentiate into memory B cells and form antibody-producing plasma cells [70]. Wang et al. [71] reported that Splenic T_{FH} cells ($CXCR5^+$ and $CD4^+$) increased in peripheral blood of primary biliary cholangitis than that in autoimmune hepatitis. Splenic T_{FH} cells were localized in germinal center-bearing B cell follicles in primary biliary cholangitis. Circulating ($CXCR5^+$ and $CD4^+$) T_{FH} cells persisting for a long time in blood. Upon subsequent antigenic stimulation, these memory cells may quickly form T_{FH} cells and promote germinal center responses. Uncontrolled generation of circulating T_{FH} cells may reflect germinal center dysregulation and play an important role in amplifying autoreactive B cells, promoting pathogenic autoantibody production, the onset of clinical symptoms, continued immune responsiveness, and eventually irreversible tissue damage [72]. In primary biliary cholangitis, there is a higher percentage of T_{FH} cells in antimitochondrial antibody-positive patients than antimitochondrial antibody negative patients suggesting that T_{FH} cells may be specifically involved in the production of autoantibodies [71]. In addition, they

also clarified that circulating $CXCR5^+$ and $CD4^+$ T_{FH} cells in patients with primary biliary cholangitis generated more IL-21 after stimulation with PMA/ionomycin and these cells induced naïve B cells to differentiate to plasmablasts in order to induce the production of immunoglobulin under the stimulation of IL-21 [71]. Levels of $CXCR5^+$ and $CD4^+$ T_{FH} cells were significantly lower in ursodeoxycholic acid responders than those in nonresponders. In patients with primary biliary cholangitis, circulating $CXCR5^+$ and $CD4^+$ T_{FH} cells were also decreased in number in responders to ursodeoxycholic acid treatment. In contrast, no significant changes in circulating $CXCR5^+$ and $CD4^+$ T_{FH} cells was observed in nonresponders to ursodeoxycholic acid treatment. These findings suggest that $CXCR5^+$ and $CD4^+$ T_{FH} cells in patients with primary biliary cholangitis interact with localized B cells to form lymphoid follicle-like structures that promote B cells to differentiate to plasmablasts, a key factor associated with the pathogenesis of primary biliary cholangitis.

Primary biliary cholangitis is usually accompanied by high IgM level in peripheral blood. The naïve B cells that are generated in the bone marrow enter the spleen through blood flow and subsequently enter the white pulp through the periarteriolar lymphoid sheath. These cells differentiated to the switched memory B cells that express IgG on cell surface and IgM memory B cells that actively produce high-affinity IgM [73, 74]. Circulating innate immune factors stimulate IgM memory B cells and increase IgM production in the spleen [75]. Kikuchi et al. [76] reported that accumulation of IgM-positive cells was observed in the CD21-positive lymph follicle in the spleen of primary biliary cholangitis patients. Toll-like receptor ligand CpG stimulated IgM memory B cells in the peripheral blood mononuclear cells of primary biliary cholangitis to produce an excessive amount of IgM [77]. From these findings, Kikuchi et al. speculate that circulating pathogen-associated molecular patterns stimulate proliferation of the IgM memory B cells and IgM overexpression in the spleen.

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