Chapter I

General introduction

1. Overview of autoimmune-related hepatic lesions induced by graft-versus-host reaction (GVHR).

Primary biliary cirrhosis (PBC) is a chronic cholestatic liver disease characterized by progressively destroying intrahepatic bile ducts with portal inflammation and ultimately resulting in cirrhosis. The diagnostic characteristic feature of PBC is a chronic nonsuppurative destructive cholangitis (CNSDC) [1]. The serological hallmark is the presence of antimiochondrial antibodies (AMA) [2, 3]. Serum alkaline phosphatase (ALP) and immunoglobulin (Ig) M are usually increased, and the elevation of serum transaminase levels and hypercholesterolemia is frequently shown. In advanced cases, an increased serum bilirubin level is also observed. Several other autoimmune diseases, most commonly Sjögren's syndrome (SjS), occur in patients with PBC [4]. Aberrant expression of major histocompatibility complex (MHC) class II antigen on biliary epithelial cells and increased expression of MHC class I antigen on hepatocytes and biliary epitherial cells are recognized in patients of PBC [5, 6]. The cytokine profile in the pathogenesis of PBC has not been clarified. PBC is still a disease of unknown etiology; however, these findings indicate that the autoimmune process might be implicated in the pathogenesis of PBC. Several mechanisms have been proposed regarding the immune mediated bile duct damage in PBC, including the possible roll of T cell-mediated cytotoxicity [7] and intracellular interaction between the IgA class of AMA and mitochondrial autoantigens [8].

It has been revealed that the chronic graft-versus-host reaction (cGVHR) in cases such as bone marrow transplants is analogous to PBC. The similarities are as follows; damage to the epithelium of intrahepatic bile ducts, association of SjS, a high expression of MHC in bile duct epithelial cells (an aberrant expression of HLA class II antigen and an increased expression of HLA class I) and production of AMA [9]. Based on these findings, murine GVHR in an F1 hybrid disease system has been utilized as an experimental model to analyze the pathogenesis of PBC.

Saitoh *et al.* demonstrated that histological findings resembling those of PBC and SjS were detected in MHC class II disparate (bm12 x B6) F1 mice injected with B6 T cells [10-12]. Moreover, the production of AMA [13] and the aberrant expression of MHC class II antigen on biliary epitherial cells were

recognized in this murine GVHR model [14]. Three important immunopathogenesis mechanisms have been reported by analyzing MHC class II-disparate GVHR. 1) Donor cluster of differentiation (CD) 4^+ T cells recognize MHC class II disparity [14] and retain their alloreactivity towards MHC class II antigens for a long time [15]. 2) Recipient-derived T cells invade at the site of the hepatic lesions, suggesting that the tolerance of self-reactive T cells is abrogated by GVHR [16]. 3) Recipient mice produce autoantibodies of a recipient origin [17]. Based on these autoimmune-related mechanisms, we have analyzed the relationship between the formation of hepatic lesions and the cytokine profile by using murine GVHR in B6 T cells-injected MHC class II disparate (bm12 x B6) F1 mice [18,19]. In our previous study, early production of interferon (IFN)-γ and delayed production of interleukin (IL)-10 were observed, whereas there was no up-regulation for IL-2 and IL-4 from liver-infiltrating $CD4^+$ T cells. It is suggested that IFN-γ may be related to the pathogenesis of GVHR hepatic lesions [19].

The similarities between PBC and the murine GVHR model are mentioned above, however, the discrepancies are as follows: 1) The progressive destructive changes of bile ducts and piecemeal necrosis are not observed in this murine GVHR model. Moreover, there is no up-regulation of ALP and serum transaminase levels in this model. Namely, some of typical findings of PBC are not observed in this murine GVHR model. Therefore, this model should be analyzed as a model of some pathological situation of autoimmune-related liver diseases. 2) This GVHR system does not progress into fibrosis or cirrhosis, that is, it is self-limited. Therefore, this model is insufficient for the analysis of the progression mechanism of the autoimmune-related liver diseases. We therefore focused on the T-cell-activate function of concanavalin A (Con A). Concanavalin A is a lectin and a T-lymphocyte mitogen *in vitro* that leads to the production of cytokines and lymphocyte proliferation [20]. Moreover, it has been demonstrated that Con A induced immune-mediated hepatic lesions in mice [21]. Recently, natural killer T (NKT) cells are suggested to be a cellular source for the induction of hepatocyte injury in Con A hepatitis [22,23]. Therefore, we are interested in analyzing whether Con A has the possibility to deteriorate hepatic lesions undergoing murine GVHR.

The ability of endogenous and exogenous IL-10 to prevent the autoimmune diseases has been shown in several animal models [24, 25]. Therefore, we

hypothesized that the production of IL-10 by liver-infiltrating $CD4^+$ T cells might suppress the progression of the hepatic lesions induced by GVHR. However, IL-10 was suggested to have an immunostimulating effect on $CD8^+$ T cells [26]. The role of delayed production of IL-10 in this GVHR model has not been clarified up to now.

2. The aim of the present research

The aim of the present studies is as follows:

1. To produce a murine model which shows a progressed autoimmune-related liver disease by using GVHR and Con A, and to elucidate the participation of the cytokines of liver-infiltrating $CD4^+$ T cells.

2. To clarify the role of IL-10 in the autoimmune-related hepatic lesions induced by GVHR, and to elucidate the change of the cytokine profile in the liver under the neutralization of IL-10.