Th17 promotes acute rejection following liver transplantation in rats*

Xiao-jun XIE, Yu-fu YE, Lin ZHOU, Hai-yang XIE, Guo-ping JIANG,
Xiao-wen FENG, Yong HE, Qin-fen XIE, Shu-sen ZHENG†‡

(Key Laboratory of Combined Multi-organ Transplantation of Ministry of Public Health, Key Laboratory of Organ Transplantation of Zhejiang Province, Division of Hepatobiliary and Pancreatic Surgery, Department of Surgery, the First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou 310003, China)

†E-mail: shusenzheng@zju.edu.cn

Abstract:  Th17, recently identified as a new subset of CD4+ T cells, has been implicated in autoimmune diseases, tumor immunity, and transplant rejection. To investigate the role of Th17 in acute hepatic rejection, a rat model of allogeneic liver transplantation (Dark Agouti (DA) to Brown Norway (BN)) was established and isogeneic liver transplantation (BN to BN) was used as controls in the study. The expression of Th17-related cytokines in the liver and peripheral blood was determined by immunohistochemistry, flow cytometry, enzyme-linked immunosorbent assay (ELISA), or real-time quantitative reverse transcription polymerase chain reaction (RT-qPCR). Strong expression of interleukin-17A (IL-17A), IL-6, transforming growth factor-β (TGF-β), IL-8, and myeloperoxidase (MPO) was observed in liver allografts. The ratios of Th17 to CD4+ lymphocytes in the liver and peripheral blood were dramatically increased in the allograft group compared with the control (P<0.01). Secreted IL-17 and IL-6 in liver homogenate and serum were significantly elevated in the allograft group, while secreted TGF-β was increased in liver homogenate and decreased in serum compared with the control (P<0.01). The messenger RNA (mRNA) levels of IL-17, IL-21, and IL-23 were enhanced in the allografts compared with the control (P<0.01). Correlation analysis showed significant correlations between IL-17 and IL-6 and TGF-β and between IL-17 and IL-21 and IL-23. The present study demonstrates that Th17 plays a role in promoting rat liver allograft rejection.

Key words:  Th17, Liver transplantation, Rejection, Transplant immunology


1 Introduction

Orthotopic liver transplantation (OLT) is currently accepted as a viable therapeutic option for various end-stage liver diseases. Though liver enjoys immune privilege compared to other organs, the incidence of acute rejection after OLT was still more than 30% (http://www.ustransplant.org/, accessed on Sept. 11, 2008), ultimately leading to chronic graft dysfunction and decreased graft survival.

CD4+ T lymphocytes have been implicated in playing critical roles in allograft rejection by secreting various cytokines and providing help for other effector cells (Xiang et al., 2008). Traditionally, CD4+ T helper (Th) cells are thought to differentiate into Th1 and Th2 cell subsets. Th1 cells are characterized by the production of interferon-γ (IFN-γ) and inducing cell-mediated immunity against intracellular pathogens, whereas Th2 cells produce interleukin-4 (IL-4) and stimulate humoral immunity against parasitic helminthes (Reiner, 2007). A Th1 response is associated with transplant rejection, while a Th2 response may contribute to tolerance and stable graft survival (Wadia and Tambur, 2008). Expression of IFN-γ was found to be elevated in heart and kidney transplants of recipients during...
rejection (Saiura et al., 2001; Obata et al., 2005). However, others reported that the rejection was aggravated in the heart and kidney implants when the IFN-γ gene was knocked out (Halloran et al., 2001; Miura et al., 2003). These studies indicated that rejection might not be activated by Th1 alone.

Recently, a newly identified CD4+ T cell subset, Th17, distinct from Th1 or Th2, is characterized by the production of interleukin-17A (IL-17A), which participates in orchestrating a specific kind of inflammatory response (Miura et al., 2003). A mounting body of evidence demonstrated that Th17 plays an important role in allograft rejection, previously thought to be Th1 function. Elevated IL-17 levels have been associated with renal and lung graft rejection in humans (Loong et al., 2002; Vanaudenaerde et al., 2008). Study in acute rat renal allograft rejection model has also identified an elevation of IL-17 protein as early as 2 d after transplantation (Hsieh et al., 2001). Th17 expression was markedly increased in inflamed transplants and draining lymph nodes at the early stage of allogeneic rejection in mouse (Chen H. et al., 2009). In a mouse heart transplantation model, antagonism of the IL-17 pathway via administration of an IL-17 inhibitor can reduce intragraft production of inflammatory cytokines and prolong graft survival (Yuan et al., 2008; 2009). The roles of Th17 and Th17-related cytokines in liver transplantation are poorly studied. At present, Fábrega et al. (2009) reported an obvious elevation of serum levels of IL-17 and IL-23 in patients with acute rejection after liver transplantation. However, the exact mechanism of Th17 pathway in acute rejection after liver transplantation remains unclear. Thus, the present study established a rat model of acute liver rejection and investigated the role and mechanisms of Th17 in acute hepatic rejection.

2 Materials and methods

2.1 Animals

Inbred male Dark Agouti (DA) and Brown Norway (BN) rats (8 to 12-week-old, 200 to 250 g in weight) were purchased from Beijing Vital River Company, China. The rats were housed in cages in a temperature- and light-controlled environment. Animals were allowed free access to tap water and food. The project was approved by the China Association of Laboratory Animal Care and the Institutional Animal Care Committee.

2.2 Orthotopic liver transplantation model

DA to BN liver allograft recipients were selected because they showed severe rejection with an average survival time of 10 to 15 d (Kamada, 1988). Liver transplantations from DA to BN allografts (n=12) and from BN to BN isografts as controls (n=12) were performed according to Kamada and Calne (1979). Briefly, rats underwent anesthesia and systemic heparinization. After perfusion in situ with cold Ringer’s lactate solution through the abdominal aorta, the donor liver was transplanted orthotopically into the recipient rat without hepatic artery reconstruction. The anastomosis of suprahepatic vena cava was sutured. The portal vein and intrahepatic vena cava were connected using cuff technique. Bile duct was cannulated with a polyethylene tube. After operation, food and water were available ad libitum and no further treatment was given. Recipients that died within 5 d after transplantation were considered technical failures and excluded from the study. Six recipient rats of each group were sacrificed on the 5th and 10th days postoperatively. Peripheral blood and liver graft tissues were obtained for further study.

2.3 Histological examination

Tissues from sacrificed animals were fixed in 10% (v/v) neutral buffered formalin and embedded in paraffin. The 4-μm sections were stained with hematoxylin and eosin (H&E) for histological examination. Acute allograft rejection was scored with rejection activity index (RAI) according to the Banff 97 working classification of hepatic allograft pathology (Demetris et al., 1997) by a single-blinded pathologist.

2.4 Liver function

Serum liver function markers, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and total bilirubin (TB), were examined by an automatic chemical analyzer (Hitachi 7600-100, Tokyo, Japan).

2.5 Immunostaining

Paraffin-embedded slides were deparaffinized and rehydrated, followed by microwave antigen