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Th17 promotes acute rejection following liver transplantation in rats*

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Abstract: T help cell 17 (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
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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

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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 allocorneal 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 (Demetrius *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

retrieval in 10 mmol/L sodium citrate buffer (pH 6.0). The sections were blocked with 5% (v/v) bovine serum albumin (BSA) diluted in phosphate buffer saline (PBS) for 30 min, and then incubated with each primary antibody in a moist chamber at 4 °C overnight. The following antibodies were used in this study: IL-17 and transforming growth factor-β (TGF-β) (1:50 and 1:100 (v/v), respectively; Santa Cruz Biotechnology, CA, USA), IL-6 and IL-8 (1:100 (v/v), respectively; Boster Biological Technology, Wuhan, China), and myeloperoxidase (MPO) (1:100 (v/v); Lab Vision, Fremont, CA, USA). After washing three times with PBS for 5 min each, horseradish peroxidase (HRP) polymer-linked secondary antibody was added and incubated for 60 min at room temperature. The sections were then visualized with diaminobenzadine (DAB) and counterstained with hematoxylin. For immunofluorescence double staining of IL-17 and CD4, cryostat sections of liver tissues were frozen in optimal cutting temperature (OCT) compound, fixed in cold acetone for 10 min, and incubated with rabbit anti-rat IL-17 antibody (1:50 (v/v); Santa Cruz Biotechnology) and mouse anti-rat CD4 antibody (1:100 (v/v); eBioscience, San Diego, USA) in a moist chamber at 4 °C overnight. Then liver sections were stained with Alexa Fluor 488-labeled goat anti-mouse immunoglobulin G (IgG) antibody and Alexa Fluor 555-labeled goat anti-rabbit IgG antibody (1:400 and 1:200 (v/v), respectively; Invitrogen, Carlsbad, USA).

2.6 Flow cytometry

Liver tissues were diced into 2-mm cubes and digested with 0.05% (w/v) type IV collagenase (Gibco, BRL, USA), followed by isopycnic sedimentation in 40% Percoll gradient (Sigma, St. Louis, MO, USA). T lymphocytes were isolated from liver grafts and further purified by sequential passage over nylon wool columns. Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized blood by Ficoll-Hypaque (Pharmacia, Piscataway, NJ, USA) density gradient centrifugation. Cells were resuspended in 100 μl of RPMI-1640 medium with 10% (v/v) goat serum (Sigma). Single-cell suspensions (1×10^6 ml^{-1}) of T lymphocytes or PBMCs were restimulated with phorbol myristate acetate (PMA, 50 ng/ml; Sigma-Aldrich) and ionomycin (1 μg/ml; Sigma-Aldrich) for 5 h at 37 °C. Cells were first stained with CD4 antigen using polyethylene (PE)-labeled CD4 antibody (BD Pharmingen, San Diego, CA, USA), fixed, and permeabilized using Fix & Perm reagents (Caltag Laboratories, Burlingame, CA, USA) according to the manufacturer's instructions. Then cells were incubated with rabbit anti-rat IL-17 antibody (Santa Cruz Biotechnology) for 60 min at room temperature. After washing in PBS, cells were incubated with fluorescein isothiocyanate (FITC)-conjugated mouse anti-rabbit antibody (Invitrogen) for 30 min at 4 °C. Then cells were washed in PBS and collected for flow cytometry (FC500, Beckerman-Coulter) analysis. $CD4^+$ lymphocytes were gated. Quadrants were set according to the staining of mouse IgG_1 isotype-matched controls. The data were analyzed using the CellQuest software (Coulter, Becton Dickinson, USA).

2.7 Enzyme-linked immunosorbent assay (ELISA)

The levels of cytokines in serum and liver homogenate were measured by ELISA assay using a commercially available TGF-β (BioSource, Camarillo, USA), IL-17, and IL-6 ELISA kit (R&D Systems, Minneapolis, USA) in accordance with the manufacturer's instructions.

2.8 Real-time quantitative reverse transcription polymerase chain reaction (RT-qPCR)

Total RNA was extracted from each frozen sample using TRIzol® reagent (Invitrogen) according to a standard protocol. The isolated total messenger RNA (mRNA) was converted into complementary DNA (cDNA) using Moloney murine leukemia virus (M-MLV) reverse transcriptase (Promega, Madison, USA) for RT-PCR. Real-time PCR primers were designed on the basis of the reported cDNA sequences. The sequences for primers are as follows: β-actin, forward 5′-TGACCCAGATGTTTGAGAC CTT-3′ and reverse 5′-CGGAGTCCATCACAATG CCAGT-3′; IL-17, forward 5′-ACTACCTCAACC GTTCCACTTCA-3′ and reverse 5′-CTTCAGGACC AGGATCTCTTGCT-3′; IL-21, forward 5′-AGAGG ACCCTTGTCTGTC-3′ and reverse 5′-GTGTTTGA TGGCTTGAGT-3′; IL-23, forward 5′-GATCCGCC AGGTGTT-3′ and reverse 5′-GGCGAGGCATCT GTTGAGTG-3′. Real-time PCR was performed using an ABI 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) and an SYBR®

Green PCR Master Mix (Applied Biosystems) in a final volume of 10 μl with the following thermal cycling conditions: 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Each primer pair was validated by performing electrophoresis and melting temperature analysis of the PCR product to ensure a correct size of PCR product and absence of primer dimers. β-actin was used as an internal control. Samples were assayed in triplicate. One isograft liver tissue sample was used to calibrate each PCR reaction. Relative amount of each cytokine mRNA in each sample was calculated as the ratio of cytokine/β-actin. Measurement of the relative amount of amplified product was performed using the comparative threshold cycle method as described in the manufacturer's manual.

2.9 Statistical analysis

Statistical analysis was performed using the SPSS version 11.6 for Windows (SPSS, Chicago, IL, USA). Data were presented as mean±standard deviation (SD). Distributions of continuous variables were tested for normality by using the Shapiro-Wilk test. The Student's *t*-test was used to evaluate statistical significance of differences between groups. Correlation analyses were conducted to calculate the relationship between IL-17 and IL-6 and TGF-β, and between IL-17 and IL-21 and IL-23. $P<0.05$ was considered statistically significant.

3 Results

3.1 Acute rejection in allograft group after OLT

H&E staining results showed serious acute rejection in the allograft group, which was characterized by severe infiltration of leucocytes into hepatic lobules, hepatocyte necrosis, bile duct damage, and subendothelial inflammation, while no histological rejection occurred in the isograft group (Fig. 1). RAIs at the 5th day (5.50±1.05) and 10th day (8.67±0.52) postoperatively in the allograft group were significantly higher than those in the isograft group (1.00±0.63 and 1.17±0.75, respectively, $P<0.01$). The values of serum AST, ALT, and TB in the allograft group were markedly increased at the 5th and 10th days after OLT compared with those in the isograft group ($P<0.01$), as shown in Table 1.

3.2 Increased Th17/IL-17 levels in liver allograft and peripheral blood

Immunohistochemical staining results demonstrated that abundant IL-17-positive cells infiltrated the liver allografts, while few IL-17-positive cells were observed in the isograft group (Fig. 2). Flow cytometric analysis revealed that the ratios of intragraft and peripheral Th17 in the allograft group were relatively higher than those in the isograft group both at 5th and 10th days postoperatively ($P<0.01$) (Fig. 3). The levels of IL-17 detected by ELISA assay in liver homogenate and serum were significantly higher in the allograft group compared with the isograft group both at the 5th and 10th days postoperatively ($P<0.01$) (Fig. 4). RT-qPCR analysis also showed higher mRNA levels of IL-17 in the allograft group ($P<0.01$) (Fig. 5). Correlation analysis indicated that RAIs were related to the number of the intragraft Th17 ($r=0.861$, $P<0.01$).

3.3 Correlation of IL-6, TGF-β, IL-21, and IL-23 with Th17 differentiation

Similarly, positive staining for IL-6 and TGF-β was observed in allografts by immunohistology (Fig. 2). ELISA assays indicated that IL-6 concentrations in liver homogenate and serum in the allograft group were higher than those in the isograft group at the 5th and 10th days postoperatively ($P<0.01$). In the allograft group, TGF-β level was increased in liver homogenate but decreased in serum ($P<0.01$) (Fig. 4). Simultaneously, mRNA levels of IL-21 and IL-23 increased at the 5th postoperative day, and further ascended at the 10th postoperative day (Fig. 5). Significant correlation relationships were observed between IL-17 and IL-6 and TGF-β ($r=0.957$, $P<0.01$; $r=0.869$, $P<0.01$), and between IL-17 and IL-21 and IL-23 ($r=0.668$, $P<0.01$; $r=0.583$, $P<0.01$).

3.4 Increased expression of IL-8 and MPO in liver allografts

Few IL-8 and MPO-positive cells were detected in the isografts. However, in the allografts at the 5th postoperative day, some abnormal expression of IL-8 and MPO antigens was observed in infiltrating mononuclear cells and increased progressively to the 10th postoperative day (Fig. 2).

Table 1 RAI and live function after rat OLT

Group	*n*	RAI	ALT (U/L)	AST (U/L)	TB (μmol/L)
Allograft					
Day 5	6	5.50±1.05*	743.67±316.54*	1807.67±656.96*	26.00±13.97*
Day 10	6	8.67±0.52*	568.67±176.02*	1301.67±418.23*	278.67±71.94*
Isograft					
Day 5	6	1.00±0.63	47.67±13.11	146.00±79.06	4.00±0.00
Day 10	6	1.17±0.75	99.67±34.26	236.33±150.84	3.67±0.82

Data are expressed as mean±SD. * Statistically significant difference (P<0.01) between the allograft and isograft groups. RAI: rejection activity index; ALT: alanine aminotransferase; AST: aspartate aminotransferase; TB: total bilirubin

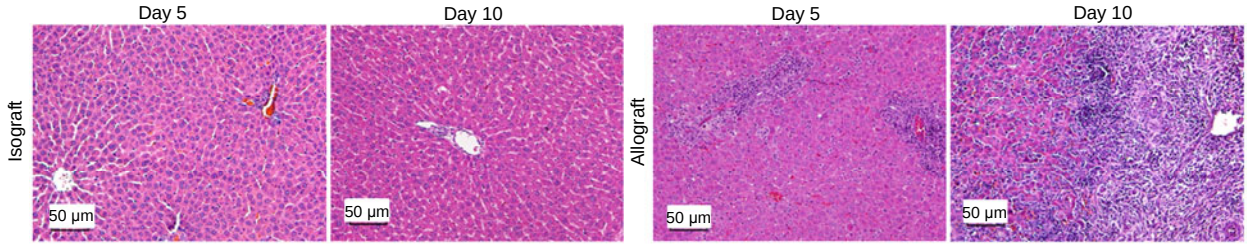

Fig. 1 H&E staining of liver grafts

H&E staining showed infiltration of leucocytes into hepatic lobules, hepatocyte necrosis, bile duct damage, and subendothelial inflamation in the allograft group, especially with more intensive rejection reactions at the 10th postoperative day. But no changes were observed in the isograft group

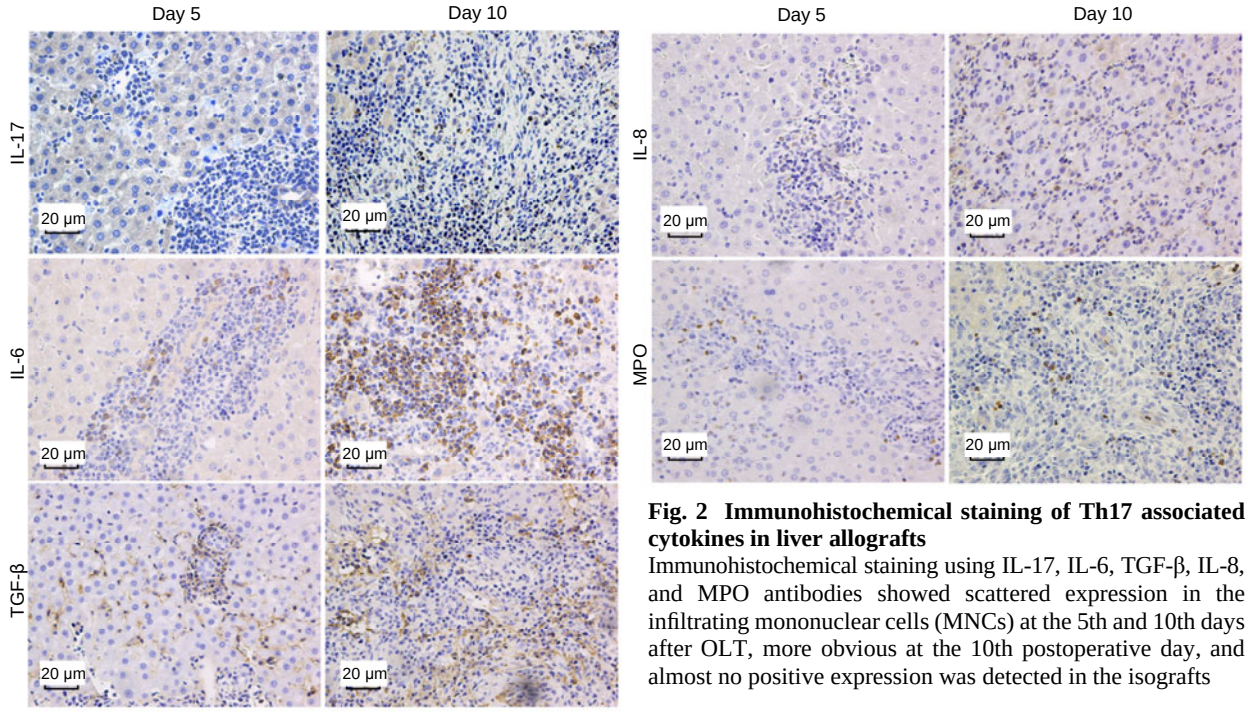

Fig. 2 Immunohistochemical staining of Th17 associated cytokines in liver allografts

Immunohistochemical staining using IL-17, IL-6, TGF-β, IL-8, and MPO antibodies showed scattered expression in the infiltrating mononuclear cells (MNCs) at the 5th and 10th days after OLT, more obvious at the 10th postoperative day, and almost no positive expression was detected in the isografts

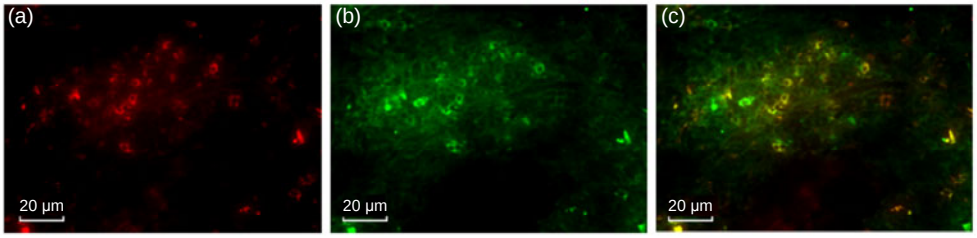

Fig. 6 Immunofluorescence double staining of IL-17 and CD4 in liver allografts at the 10th day postoperatively

(a) Positive staining for IL-17 (red); (b) Positive staining for CD4 (green); (c) Merge of (a) and (b)

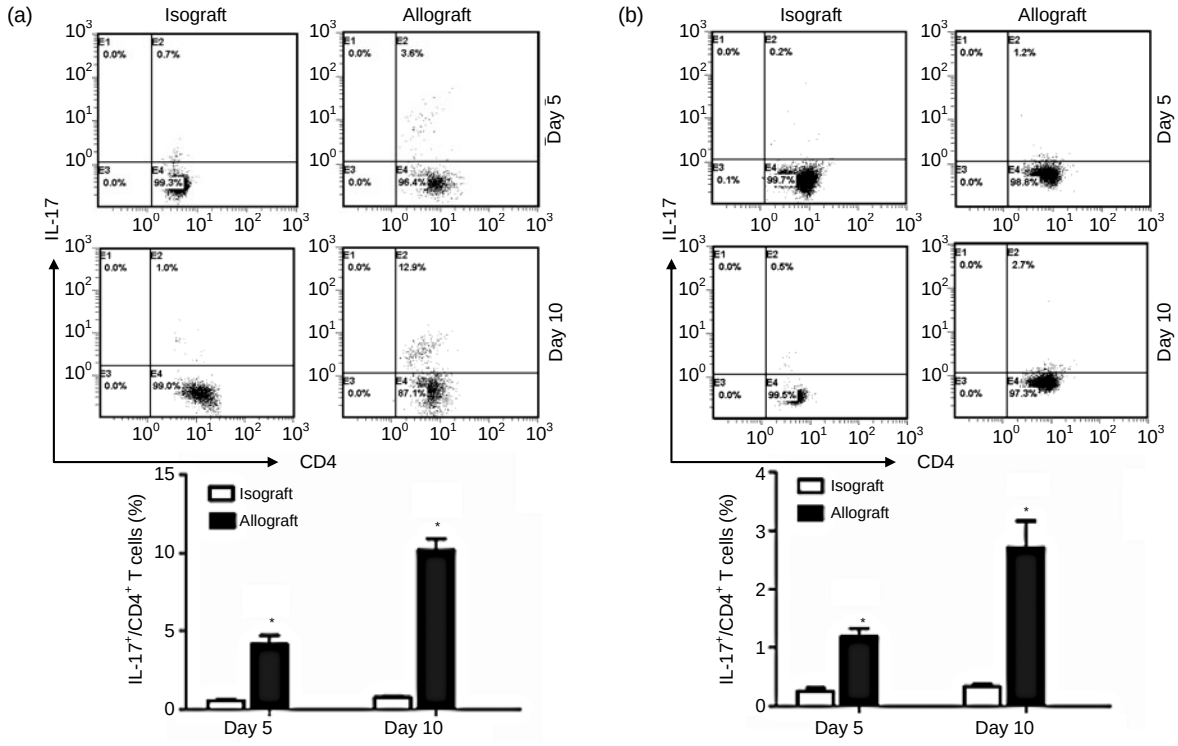

Fig. 3 Flow cytometry analysis of ratios of intragraft and peripheral Th17 cells

* Statistically significant difference (P<0.01) between the allograft group and the isograft group at the 5th and 10th days postoperatively [(4.13±1.38)% vs. (0.55±0.16)% and (10.20±1.79)% vs. (0.77±0.21)%, respectively, in liver lymphocytes (a); (1.18±0.34)% vs. (0.25±0.15)% and (2.72±1.09)% vs. (0.33±0.08)%, respectively, in PBMCs (b)]

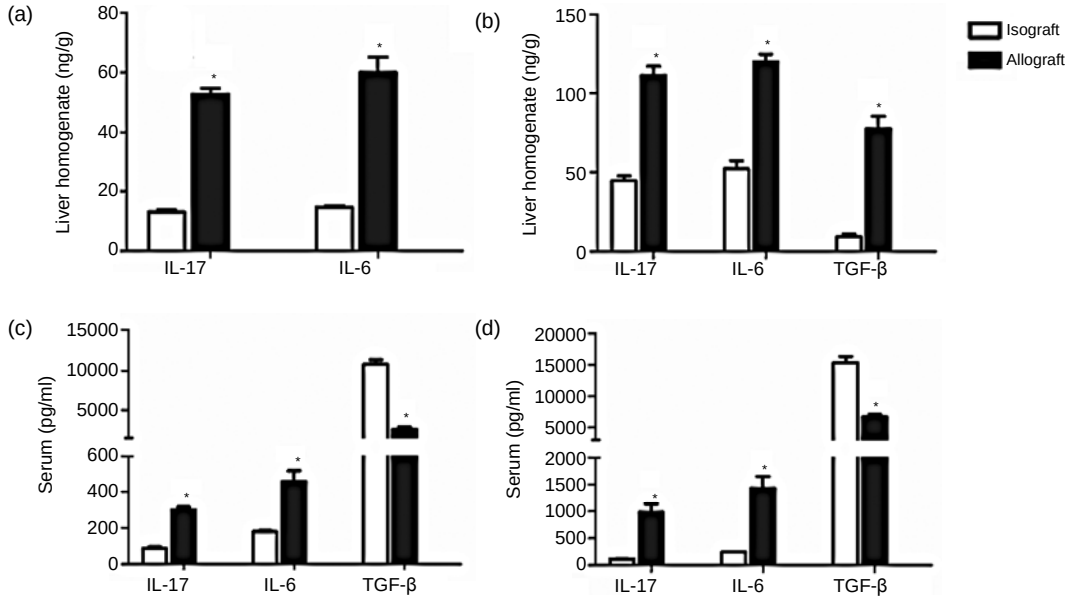

Fig. 4 ELISA assays of IL-17, IL-6, and TGF-β in liver homogenate and serum

(a, b) Liver homogenate at the 5th and 10th days, respectively; (c, d) Serum at the 5th and 10th days, respectively. IL-17 and IL-6 levels of the allograft group were higher than those in the control in both liver homogenate and serum after OLT (P<0.01). TGF-β level was increased in liver homogenate but decreased in serum in the allograft group compared with the control. * Statistically significant difference at P<0.01

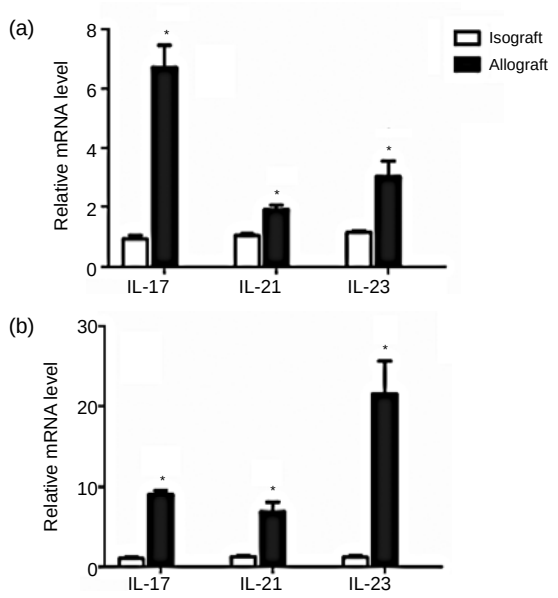

Fig. 5 mRNA levels of IL-17, IL-21, and IL-23 in grafts at the 5th (a) and 10th (b) days

The mRNA concentrations of IL-17, IL-21, and IL-23 were increased significantly in the allograft group compared with the isograft group at the 5th day postoperatively, and the differences between two groups were more obvious at the 10th day postoperatively. * Statistically significant difference at $P<0.01$

4 Discussion

Currently, the role of Th17 in transplant rejection is emerging as a hot topic of research. Numerous animal experiments and clinical trials have focused on the effect of Th17 in the kidney, lung, heart, and cornea after transplantation. In this study, we provide evidence that Th17 participates in the allogeneic liver rejection.

In Fábrega *et al*. (2009)'s report, serum levels of IL-17 were elevated in patients with acute hepatic rejection, which is consistent with our results that serum levels of IL-17 were increased in the rats with acute rejection after liver transplantation. Furthermore, we found that IL-17 level increased in liver homogenate in allografts, which might confirm that production of secreted IL-17 was enhanced not only systematically but also locally. Also, the ratios of Th17 in the allograft group were relatively higher than those in the isograft group, with a significantly higher level in the liver than in peripheral blood. These results are correlated with the degree of rejection, and further confirm that Th17 plays a key role in pathophysiology of acute hepatic rejection.

IL-17 is a distinctive cytokine of Th17 cells, and was found to be derived mainly from $CD4^+$ T cells, as well as expressed by $CD8^+$ T cells, γδ T cells, eosinophils, neutrophils, and monocytes (Weaver *et al*., 2007). In this study, double staining of IL-17 and CD4 antigens showed that IL-17 expression was primarily restricted to $CD4^+$ T cells (Fig. 6, in p. 823). As we have known, $CD4^+$ T cells can initiate the rejection reaction, so how Th17 takes effect in acute liver allograft rejection is in question.

Although the mechanism by which Th17 promotes allograft rejection in the liver remains to be determined, it is generally believed that Th17 could induce tissue damage by stimulating inflammatory cells to release proinflammatory cytokines, such as IL-6, tumor necrosis factor-α (TNF-α), acute phase proteins, and neutrophil-mobilizing cytokines like IL-8 (Kolls and Linden, 2004). Our results show that IL-6 levels were increased in both liver tissue and serum during rejection. It is well known that IL-6 plays an important role in the regulation of inflammatory and immunological responses (Kishimoto, 1989). Kita *et al*. (1994) and Warlé *et al*. (2003) reported that IL-6 expression was markedly elevated during liver allograft rejection. The present study displayed a close correlation between IL-6 and IL-17. Vanaudenaerde *et al*. (2008) and Chen L. *et al*. (2009) also found that both IL-6 and IL-17 levels were increased at the same time in lung and cardiac allograft rejection. It was postulated that IL-17 potentially promoted liver allograft rejection reaction by inducing the expression of IL-6. Furthermore, previous study has reported that IL-17 induced IL-8 production by immune cells and epithelial cells, which regulate neutrophils migration in vitro (Prause *et al*., 2003). In vivo, IL-17 selectively recruits neutrophils via the release of IL-8. This effect can be inhibited by IL-17 neutralization (Laan *et al*., 1999; Hoshino *et al*., 2000). In the present study, the immunohistochemical analysis revealed that the expression of IL-8 and neutrophil marker MPO was increased in implants when rejection occurred, indicating that IL-8 initiates an alternative pathway to promote Th17 to take effect in rejection.

Cytokines encountered by naive $CD4^+$ T cells govern helper T cell differentiation. IL-12 and IL-4 drive naive $CD4^+$ cells toward Th1 and Th2 cells,

respectively (Xiang *et al*., 2008), whereas IL-6 plus TGF-β stimulates their differentiation into Th17 cells (Bettelli *et al*., 2006; Mangan *et al*., 2006). IL-6 is both a downstream cytokine of IL-17 pathway and a differentiation factor for Th17. By inducing IL-6 production, IL-17 activates a positive feedback loop that promotes differentiation of naive $CD4^+$ T cells into Th17. Different from IL-6, TGF-β had been reckoned as an immunosuppressive cytokine that causes naive T cells to develop into suppressor regulatory T cells (Chen *et al*., 2003; Li *et al*., 2006). Our data demonstrated a higher serum level of TGF-β in the isograft group than that in the allograft group. However, the expression of TGF-β in liver allograft was increased, which is in line with the IL-17. Theoretically, TGF-β level should be decreased in liver graft and serum because TGF-β produced by Treg cells is reduced during rejection (Demirkiran *et al*., 2006). However, in the present study, we observed that TGF-β level was increased in liver graft. It was considered that a large number of liver Kupffer cells, stellate cells, and liver sinusoidal endothelial cells secrete large amounts of TGF-β in the pathophysiological conditions (Malik *et al*., 2002). Besides, we found that IL-21 and IL-23 levels were also increased in acute liver rejection. Studies showed that IL-23 maintained the proliferation and survival of Th17 (Stritesky *et al*., 2008), and IL-21 was a specific cytokine secreted by Th17 and in turn influenced the differentiation of Th17 in an autocrine manner (Nurieva *et al*., 2007). These elevated cytokines and chemokines during liver transplant rejection provide a favorable immune microenvironment for Th17 cell differentiation.

In summary, our data clearly demonstrate that Th17 participates in rejection following liver transplantation in rats. IL-17 promotes the secretion of IL-6 and IL-8 and thus likely causes the aggregation of neutrophils in liver tissue, leading to transplant rejection. Meanwhile, IL-6, TGF-β, IL-21, and IL-23 probably assist the maturity of Th17, and the differentiation of Th17 may be favored by hepatic microenvironment. Further studies need to be done by using an IL-17 inhibitor or IL-17 knockout animal to prove the direct involvement of Th17 in acute liver rejection.

References

Bettelli, E., Carrier, Y., Gao, W., Korn, T., Strom, T.B., Oukka, M., Weiner, H.L., Kuchroo, V.K., 2006. Reciprocal developmental pathways for the generation of pathogenic effector Th17 and regulatory T cells. *Nature*, **441**(7090): 235-238. [doi:10.1038/nature04753]

Chen, H., Wang, W., Xie, H., Xu, X., Wu, J., Jiang, Z., Zhang, M., Zhou, L., Zheng, S., 2009. A pathogenic role of IL-17 at the early stage of corneal allograft rejection. *Transpl. Immunol.*, **21**(3):155-161. [doi:10.1016/j.trim.2009.03.006]

Chen, L., Ahmed, E., Wang, T., Wang, Y., Ochando, J., Chong, A.S., Alegre, M.L., 2009. TLR signals promote IL-6/IL-17-dependent transplant rejection. *J. Immunol.*, **182**(10): 6217-6225. [doi:10.4049/jimmunol.0803842]

Chen, W.J., Jin, W.W., Hardegen, N., Lei, K.J., Li, L., Marinos, N., McGrady, G., Wahl, S.M., 2003. Conversion of peripheral $CD4^+CD25^-$ naive T cells to $CD4^+CD25^+$ regulatory T cells by TGF-β induction of transcription factor *Foxp3*. *J. Exp. Med.*, **198**(12):1875-1886. [doi:10.1084/jem.20030152]

Demetrius, A.J., Batts, K.P., Dhillon, A.P., Ferrell, L., Fung, J., Geller, S.A., Hart, J., Hayry, P., Hofmann, W.J., Hubscher, S., *et al*., 1997. Banff schema for grading liver allograft rejection: an international consensus document. *Hepatology*, **25**(3):658-663. [doi:10.1002/hep.510250328]

Demirkiran, A., Kok, A., Kwekkeboom, J., Kusters, J.G., Metselaar, H.J., Tilanus, H.W., van der Laan, L.J., 2006. Low circulating regulatory T-cell levels after acute rejection in liver transplantation. *Liver Transpl.*, **12**(2): 277-284. [doi:10.1002/lt.20612]

Fábrega, E., Lopez-Hoyos, M., San-Segundo, D., Casafont, F., Pons-Romero, F., 2009. Changes in the serum levels of interleukin-17/interleukin-23 during acute rejection in liver transplantation. *Liver Transpl.*, **15**(6):629-633. [doi:10.1002/lt.21724]

Halloran, P.F., Miller, L.W., Urmson, J., Ramassar, V., Zhu, L.F., Kneteman, N.M., Solez, K., Afrouzian, M., 2001. IFN-γ alters the pathology of graft rejection: protection from early necrosis. *J. Immunol.*, **166**(12):7072-7081.

Hoshino, H., Laan, M., Sjostrand, M., Lotvall, J., Skoogh, B.E., Linden, A., 2000. Increased elastase and myeloperoxidase activity associated with neutrophil recruitment by IL-17 in airways in vivo. *J. Allergy Clin. Immunol.*, **105**(1):143-149. [doi:10.1016/S0091-6749(00)90189-1]

Hsieh, H.G., Loong, C.C., Lui, W.Y., Chen, A., Lin, C.Y., 2001. IL-17 expression as a possible predictive parameter for subclinical renal allograft rejection. *Transpl. Int.*, **14**(5):287-298. [doi:10.1111/j.1432-2277.2001.tb00062.x]

Kamada, N., 1988. Experimental Liver Transplantation. CRC Press, Boca Raton, FL, p.55-66.

Kamada, N., Calne, R.Y., 1979. Orthotopic liver transplantation in the rat. Technique using cuff for portal vein anastomosis and biliary drainage. *Transplantation*, **28**(1): 47-50. [doi:10.1097/00007890-197907000-00011]

Kishimoto, T., 1989. The biology of interleukin-6. *Blood*, **74**(1):1-10.

Kita, Y., Iwaki, Y., Demetris, A.J., Starzl, T.E., 1994. Evaluation of sequential serum interleukin-6 levels in liver allograft recipients. *Transplantation*, **57**(7):1037-1041. [doi:10.1097/00007890-199404150-00009]

Kolls, J.K., Linden, A., 2004. Interleukin-17 family members and inflammation. *Immunity*, **21**(4):467-476. [doi:10.1016/j.immuni.2004.08.018]

Laan, M., Cui, Z.H., Hoshino, H., Lötvall, J., Sjöstrand, M., Gruenert, D.C., Skoogh, B.E., Lindén, A., 1999. Neutrophil recruitment by human IL-17 via C-X-C chemokine release in the airways. *J. Immunol.*, **162**(4):2347-2352.

Li, M.O., Wan, Y.Y., Sanjabi, S., Robertson, A.K., Flavell, R.A., 2006. Transforming growth factor-beta regulation of immune responses. *Annu. Rev. Immunol.*, **24**(1):99-146. [doi:10.1146/annurev.immunol.24.021605.090737]

Loong, C.C., Hsieh, H.G., Lui, W.Y., Chen, A., Lin, C.Y., 2002. Evidence for the early involvement of interleukin 17 in human and experimental renal allograft rejection. *J. Pathol.*, **197**(3):322-332. [doi:10.1002/path.1117]

Malik, R., Selden, C., Hodgson, H., 2002. The role of non-parenchymal cells in liver growth. *Semin. Cell Dev. Biol.*, **13**(6):425-431. [doi:10.1016/S1084952102001301]

Mangan, P.R., Harrington, L.E., O′Quinn, D.B., Helms, W.S., Bullard, D.C., Elson, C.O., Hatton, R.D., Wahl, S.M., Schoeb, T.R., Weaver, C.T., 2006. Transforming growth factor-beta induces development of the T(H)17 lineage. *Nature*, **441**(7090):231-234. [doi:10.1038/nature04754]

Miura, M., El-Sawy, T., Fairchild, R.L., 2003. Neutrophils mediate parenchymal tissue necrosis and accelerate the rejection of complete major histocompatibility complex-disparate cardiac allografts in the absence of interferon-gamma. *Am. J. Pathol.*, **162**(2):509-519.

Nurieva, R., Yang, X.O., Martinez, G., Zhang, Y., Panopoulos, A.D., Ma, L., Schluns, K., Tian, Q., Watowich, S.S., Jetten, A.M., *et al*., 2007. Essential autocrine regulation by IL-21 in the generation of inflammatory T cells. *Nature*, **448**(7152):480-483. [doi:10.1038/nature05969]

Obata, F., Yoshida, K., Ohkubo, M., Ikeda, Y., Taoka, Y., Takeuchi, Y., Shinohara, N., Endo, T., Baba, S., 2005. Contribution of $CD4^+$ and $CD8^+$ T cells and interferon-γ to the progress of chronic rejection of kidney allografts: the Th1 response mediates both acute and chronic rejection. *Transpl. Immunol.*, **14**(1):21-25. [doi:10.1016/j.trim.2004.11.003]

Prause, O., Laan, M., Lotvall, J., Linden, A., 2003. Pharmacological modulation of interleukin-17-induced GCP-2-, GRO-α- and interleukin-8 release in human bronchial epithelial cells. *Eur. J. Pharmacol.*, **462**(1-3):193-198. [doi:10.1016/S0014-2999(03)01341-4]

Reiner, S.L., 2007. Development in motion: helper T cells at work. *Cell*, **129**(1):33-36. [doi:10.1016/j.cell.2007.03.019]

Saiura, A., Mataki, C., Murakami, T., Umetani, M., Wada, Y., Kohro, T., Aburatani, H., Harihara, Y., Hamakubo, T., Yamaguchi, T., *et al*., 2001. A comparison of gene expression in murine cardiac allografts and isografts by means DNA microarray analysis. *Transplantation*, **72**(2):320-329. [doi:10.1097/00007890-200107270-00027]

Stritesky, G.L., Yeh, N., Kaplan, M.H., 2008. IL-23 promotes maintenance but not commitment to the Th17 lineage. *J. Immunol.*, **181**(9):5948-5955.

Vanaudenaerde, B.M., de Vleeschauwer, S.I., Vos, R., Meyts, I., Bullens, D.M., Reynders, V., Wuyts, W.A., van Raemdonck, D.E., Dupont, L.J., Verleden, G.M., 2008. The role of the IL23/IL17 axis in bronchiolitis obliterans syndrome after lung transplantation. *Am. J. Transplant.*, **8**(9):1911-1920. [doi:10.1111/j.1600-6143.2008.02321.x]

Wadia, P.P., Tambur, A.R., 2008. Yin and yan of cytokine regulation in solid organ graft rejection and tolerance. *Clin. Lab. Med.*, **28**(3):469-479. [doi:10.1016/j.cll.2008.08.001]

Warlé, M.C., Metselaar, H.J., Hop, W.C., Gyssens, I.C., Kap, M., Kwekkeboom, J., de Rave, S., Zondervan, P.E., IJzermans, J.N., Tilanus, H.W., *et al*., 2003. Early differentiation between rejection and infection in liver transplant patients by serum and biliary cytokine patterns. *Transplantation*, **75**(1):146-151. [doi:10.1097/00007890-200301150-00026]

Weaver, C.T., Hatton, R.D., Mangan, P.R., Harrington, L.E., 2007. IL-17 family cytokines and the expanding diversity of effector T cell lineages. *Annu. Rev. Immunol.*, **25**(1):821-852. [doi:10.1146/annurev.immunol.25.022106.141557]

Xiang, Z., Ma, L.L., Manicassamy, S., Ganesh, B.B., Williams, P., Chari, R., Chong, A., Yin, D.P., 2008. $CD4^+$ T cells are sufficient to elicit allograft rejection and major histocompatibility complex class I molecule is required to induce recurrent autoimmune diabetes after pancreas transplantation in mice. *Transplantation*, **85**(8):1205-1211. [doi:10.1097/TP.0b013e31816b70bf]

Yuan, X., Paez-Cortez, J., Schmitt-Knosalla, I., D′Addio, F., Mfarrej, B., Donnarumma, M., Habicht, A., Clarkson, M.R., Iacomini, J., Glimcher, L.H., *et al*., 2008. A novel role of CD4 Th17 cells in mediating cardiac allograft rejection and vasculopathy. *J. Exp. Med.*, **205**(13):3133-3144. [doi:10.1084/jem.20081937]

Yuan, X., Ansari, M.J., D'Addio, F., Paez-Cortez, J., Schmitt, I., Donnarumma, M., Boenisch, O., Zhao, X., Popoola, J., Clarkson, M.R., *et al*., 2009. Targeting Tim-1 to overcome resistance to transplantation tolerance mediated by CD8 T17 cells. *PNAS*, **106**(26):10734-10739. [doi:10.1073/pnas.0812538106]