Published in final edited form as:

Hepatology. 2008 June ; 47(6): 1974–1982. doi:10.1002/hep.22226.

Adoptive transfer of CD8⁺ T cells from dnTGFβRII mice induces autoimmune cholangitis into Rag1^{-/-} mice

Guo-Xiang Yang¹, Zhe-Xiong Lian¹, Ya-Hui Chuang¹, Yuki Moritoki¹, Ruth Y. Lan¹, Kanji Wakabayashi¹, Aftab A. Ansari², Richard A. Flavell³, William M. Ridgway⁴, Ross L. Coppel⁵, Koichi Tsuneyama⁶, Ian R. Mackay⁷, and M. Eric Gershwin¹

¹ Division of Rheumatology, University of California at Davis, CA 95616

² Department of Pathology, Emory University School of Medicine, Atlanta, GA 30322

³ Section of Immunobiology and Howard Hughes Medical Institute, Yale University school of medicine, New Haven, CT 06520

⁴ Division of Rheumatology and Immunology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261

⁵ Department of Microbiology, Monash University, Melbourne, Australia

⁶ Department of Pathology (I), University of Toyama School of Medicine, Toyama, Japan

⁷ Department of Biochemistry and Molecular Biology, Monash University, Clayton, Victoria, Australia

Abstract

We recently reported that mice with a T cell-restricted expression of a dominant negative (dn) form of the TGF-β receptor type II spontaneously develop autoimmune cholangitis that resembles human PBC, including antimitochondrial antibodies and extensive portal CD4⁺ and CD8⁺ lymphocytic infiltrates. Based upon this data, we performed a series of experiments to determine whether the pathology was secondary to direct dnTGFβRII disruption of liver and/or alternatively the appearance of autoreactive T cells. Firstly, using dnTGFβRIIRag1^{-/-} mice, we noted a normal hepatic and biliary structure. Hence we performed a rigorous series of adoptive transfer studies using Ly5.1⁺ unfractionated, CD4⁺ or CD8⁺ T cells from dnTGFβRII into B6/Rag^{-/-} (Ly 5.2) recipients. In unmanipulated dnTGFβRII mice, there was a marked increase in CD4⁺ and CD8⁺ T cell biliary infiltrates with AMA. Indeed B6/Rag^{-/-} recipients of dnTGFβRII unfractionated cells develop features of liver disease similar to PBC, suggesting that splenic loss of self tolerance alone is sufficient to cause disease in this model and therefore that there is no specific abnormality in the biliary targets required for appearance of disease. More importantly, adoptive transfer of CD8⁺ but not CD4⁺ T cells into B6/Rag^{-/-} mice led to liver histopathology remarkably similar to PBC, emphasizing a prominent role for CD8 T-cell mediated pathogenesis. In contrast, B6/Rag^{-/-} recipients of CD4⁺ T cells from dnTGFβRII mice predominantly developed inflammatory bowel disease associated with relatively higher levels of serum IFN-γ and TNF-α.

Conclusion—these data suggest that in this model of PBC autoreactive CD8⁺ cells destroy bile ducts.

*Correspondence to: M. Eric Gershwin, Division of Rheumatology, Allergy and Clinical Immunology, University of California at Davis School of Medicine, 451 Health Sciences Drive, Suite 6510, Davis, CA 95616 Phone: 530-752-2884; Fax: 530-752-4669; email: megershwin@ucdavis.edu; or Zhe-Xiong Lian, Division of Rheumatology, Allergy and Clinical Immunology, University of California at Davis School of Medicine, 451 Health Sciences Drive, Suite 6605A, Davis, CA 95616; Telephone: 530-752-3285; Fax: 530-754-6047; Email: zxlian@ucdavis.edu.

Keywords

dnTGF β RII mice; Adoptive transfer; Primary biliary cirrhosis; Anti-mitochondrial antibody

Primary biliary cirrhosis (PBC) is a progressive autoimmune cholangiolitis with destruction of intrahepatic bile ducts, cholestasis and progressive development of fibrosis, cirrhosis, and eventual liver failure (1–3). Serologically PBC is characterized by the presence of anti-mitochondrial autoantibodies (AMAs) to autoantigens of the family of 2-oxo-acid dehydrogenase complexes located in the inner mitochondrial membrane (4,5), including the pyruvate dehydrogenase E2 complex (PDC-E2), E3-binding protein (E3-BP), ketoglutaric acid dehydrogenase E2 complex (OGDC-E2), and branched-chain 2-oxo-acid dehydrogenase E2 complex (BCKD-E2) (6). AMA is a key diagnostic marker in PBC, and can precede disease by several years (7). There are also autoreactive T cells that target mitochondrial autoantigens (8–10). However the precise nature of the autoimmune effector mechanisms of biliary ductular damage remains unclear, partly due to the inherent difficulty in studying primary cellular events during the early, asymptomatic phase of PBC and the general limitations of human-based research (11–15).

We recently reported that mice with a T cell-restricted expression of a dominant negative (dn) form of the TGF- β receptor type II (dnTGF β RII) spontaneously developed periductular aggregates in the liver with loss of self tolerance to mitochondrial proteins leading to autoimmune-mediated liver pathology that closely resembles the histopathological features of human PBC, included development of significant levels of autoantibodies with specificity against PDC-E2, BCOADC-E2, and OGDC-E2 and extensive CD4⁺ and CD8⁺ lymphocytic infiltrates in portal tracts, associated with biliary destruction (16). We reasoned that the pathology that develops in these mice could be due either to a structural or functional change induced in liver tissue due to disruption of the TGF β II pathway, or alternatively to a breakdown of self tolerance. Such thoughts prompted us to carry out studies to distinguish between these possibilities. We performed adoptive transfer studies in which unfractionated or highly enriched populations of either CD4⁺ or CD8⁺ T cells from dnTGF β RII mice were adoptively transferred into recombinate-deficient (Rag1^{-/-}) mice that lack a diversified B and T cell receptor repertoire. Results from these studies demonstrated that whereas the CD8⁺ T cell recipient mice demonstrated a significant expansion of donor T cells and portal tract infiltrates, the CD4⁺ T cell population expanded but did not home to, or aggregate within the portal tracts. These data imply that in this murine model of PBC, the CD8⁺ T cell is the pathogenic effector pathway.

Materials and Methods

Mice

dnTGF β RII mice (17) were bred onto the congenic C57BL/6-Ly5.1-Pep3b mice (B6 Ly5.1, bought from The Jackson Laboratory, Bar Harbor, ME) at the University of California animal facility (Davis, CA). Female dnTGF β RII (Ly5.1) mice and control littermates were used at 4 to 22 weeks of ages. dnTGF β RII and wild-type littermates were fed sterile rodent Helicobacter Medicated Dosing System (three-drug combination) diets (Bio-Serv, Frenchtown, NJ) and maintained in individually ventilated cages under specific pathogen-free condition. B6/Rag1^{-/-} mice (Ly5.2) were obtained from Jackson Laboratory. dnTGF β RIIRag1^{-/-} mice were generated by breeding dnTGF β RII mice with B6/Rag1^{-/-} mice. All studies were performed with approval from the University of California Animal Care and Use Committee.

Isolation and flow cytometric analysis of liver T cells

dnTGF β RII and control B6 mice were sacrificed at serial ages and liver lymphoid cells isolated as described previously (18). Briefly, PBS-perfused livers were passed through a 100- μ m nylon cell strainer (BD Bioscience, Bedford, MA). Lymphocytes were separated from hepatocytes by centrifugation with Histopaque-1.077 (Sigma-Aldrich, St. Louis, MO). Single cell suspensions were washed and viability confirmed using trypan blue exclusion. For flow cytometry, FcR were blocked by incubation with the 2.4G2 mAb (eBioscience, San Diego, CA) and cells stained with fluorochrome-conjugated Abs specific for CD3, CD4, CD8, NK1.1 and CD19 (eBioscience) at 4°C in PBS/0.2% BSA for 30 min. NK1.1 and CD19 Abs were used to exclude NKT cells and B cells, respectively. Stained cells were subjected to multiple-color flow analyses using a FACScan flow cytometer updated by Cytex Development (Fremont, CA) to allow for 5-color analysis.

Adoptive cell transfer

Spleen cells were collected from 10 to 12 weeks old dnTGF β RII (Ly5.1) or B6 Ly5.1 control mice and used for transfer. For T cell adoptive transfer, the CD4⁺ or CD8⁺ T cells were purified by positive selection with CD4 or CD8 microbeads (Miltenyi Biotec, Auburn, CA). 8–10-week-old female B6/Rag1^{-/-} (Ly5.2) mice were used as recipients. Body weight and survival of recipients were measured at serial time points post-transplantation. The numbers of donor-derived cells (Ly5.1⁺Ly5.2⁻) in liver and spleen were calculated based on flow cytometric analysis of stained cells with fluorochrome-conjugated Abs specific for Ly5.1 or Ly5.2 (BioLegend, San Diego, CA) (19). The tissue sections from a variety of organs were prepared for routine hematoxylin (DakoCytomation, Glostrup, Denmark) and eosin (American Master Tech Scientific, Lodi, CA) (H & E) staining.

Immunohistology

The liver was removed and immediately fixed with a 10% solution of formalin overnight at room temperature (RT). After deparaffinization, sections were incubated in a Decloaking Chamber (Biocare Medical, Concord, CA; set point (SP) 1 123°C for 2 min, SP2 85°C for 10 s, SP limit 10°C), soaked in 3% H₂O₂ methanol solution for 5 min, then 15 min in 1x Universal blocking solution (BioGenex, San Ramon, CA) and 20 min in 10% goat serum to prevent non-specific staining. Rat mAbs against mouse CD4 (1/200 dilution) and CD8 (1/200 dilution) were applied for 1 h at RT in a moist chamber. After three washes with 0.1% Tween 20 in PBS (PBST) for 5 min, HRP-conjugated polyclonal rabbit anti-rat Ig (DakoCytomation, 1/100) was applied as secondary Ab for 1 h at RT in a moist chamber. After three washes with PBST, the sections were developed with 3,3'-diaminobenzidine (DAB; DakoCytomation) and counterstained with Mayer's hematoxylin (DakoCytomation).

ELISA for AMA

Serum anti-PDC-E2 (AMA) was measured by an ELISA with purified recombinant PDC-E2 (20). Briefly, PDC-E2 at 10 μ g/mL in carbonate buffer (pH 9.6) was coated onto 96-well ELISA plates at 4°C overnight, washed 5 times with PBS containing 0.05% Tween-20 (FisherBiotech, Fair Lawn, NJ) (PBS-T), and blocked with 3% skim milk in PBS for 30 minutes. 100 μ L of the diluted sera to be tested (1:100) was added for 1 hour at RT and plates re-washed. 100 μ L of horseradish peroxidase (HRP)-conjugated anti-mouse immunoglobulin M, G or A (IgM, IgG, IgA) (1:2,000) (Zymed, San Francisco, CA) was added to each well for 1 hour at RT, the microtiter wells were thence re-washed. Immunoreactivity was detected by measuring optical density (OD) at 450 nm after exposure for 15 minutes to 100 μ L of ABTS peroxidase substrate (KPL, Gaithersburg, MD) (20).

Measurement of serum cytokines

Serum samples were collected from recipient B6/Rag1^{-/-} mice at serial time points after T cell transfer and stored at -70°C until assayed. Concentrations of IFN- γ and TNF- α were measured simultaneously with a mouse inflammatory cytometric bead array (CBA) kit (BD Biosciences); Samples were analyzed on a FACScan flow cytometer (BD Immunocytometry Systems) using cytometric bead array software (BD Biosciences).

Statistical analysis

An unpaired, two-tailed Student's t-test was used for comparison of weight, cell number, and frequencies of cell surface markers, and cytokine production in sera. Differences were considered statistically significant when $p < 0.05$.

Results

CD4⁺ and CD8⁺ T cell expansion in liver of dnTGF β RII mice

We first analyzed the characteristics of T-cell liver infiltrates in the liver of dnTGF β RII mice compared with B6 littermates. Total CD3⁺ T cell counts in liver were significantly increased at 8 weeks of age with maximal levels occurring at week 12 (Fig. 1A). Although both CD4⁺ and CD8⁺ T cell populations were expanded in the liver of dnTGF β RII mice, the ratio of CD8/CD4 was significantly higher in dnTGF β RII mice from 12 weeks of age (Fig. 1A). The cellular infiltration in the liver histologically also peaked at 12 weeks (data not shown). Hence infiltration of T cells, and an increase in the ratio of CD8/CD4, appears to be key components in dnTGF β RII liver disease. Since immunohistology demonstrated both CD4⁺ and CD8⁺ T cell infiltrates in affected portal tracts (Fig. 1B), we performed adoptive transfer studies to identify the role of each of these cell lineages in bile duct cell damage.

Transfer of dnTGF β RII splenocytes results in disease in Rag1^{-/-} recipient mice

A standard adoptive transfer protocol was used to determine whether PBC-like changes seen in the liver of dnTGF β RII mice could be transferred. Thus, 1×10^7 splenocytes from dnTGF β RII (Ly5.1) or control B6 Ly5.1 mice were intravenously (i.v.) transferred into B6/Rag1^{-/-} (Ly5.2) mice. As shown in Fig. 2A, the recipients began to lose body weight 4 weeks after transfer and developed diarrhea (as do dnTGF β RII mice that are not raised under SPF conditions) (17), which was associated with a significant increase in lymphocyte counts in the liver of recipients of dnTGF β RII cells as compared with recipients of control B6 Ly5.1 mice (Fig. 2B). The majority of cells infiltrating the liver were donor-derived Ly5.1⁺ CD3⁺ T cells (data not shown). On histological assessment, there was a marked lymphoid cell infiltration that surrounded the portal tracts and central veins in the liver, accompanied by inflammatory infiltrates in colon and lungs (Fig. 3). Because spontaneous production of AMAs was detected in sera of dnTGF β RII mice (16), we also measured autoantibody levels to PDC-E2 in the sera of these spleen-cell recipient mice. Sera AMA of the dnTGF β RII spleen cell recipients were positive 4 weeks after transfer (Fig. 2C). Thus a PBC-like disease could be transferred into B6/Rag1^{-/-} recipients by adoptive transfer of unfractionated dnTGF β RII splenocytes.

We next backcrossed the dnTGF β RII mice to B6/Rag1^{-/-} mice that lack a repertoire of mature T and B cells. dnTGF β RIIRag1^{-/-} mice were studied at 20 weeks of age, and tissues examined histologically (Fig. 4). No lymphocyte infiltration or inflammation was evident in the liver, intestine or lung. The normal liver histology, similar to that of B6/Rag1^{-/-} mice, supports our view that autoreactive T cells cause bile duct damage in the dnTGF β RII mice, i.e. there is no intrinsic liver defect.

Transfer of CD4⁺ and CD8⁺ cells into Rag1^{-/-} recipients

To determine which of the T cell lineages generated PBC-like liver disease in mice, 1×10^6 CD4⁺ or CD8⁺ T cells from the spleens of dnTGFβRII (Ly5.1) mice were injected i.v. into B6/Rag1^{-/-} (Ly5.2) mice. Mice that were recipients of CD4⁺ T cells displayed wasting starting at 4 weeks after transfer (Fig. 5A), similar to that of mice receiving unfractionated spleen cells from dnTGFβRII mice (Fig. 2). These CD4⁺ T cell recipient mice had a 100% mortality by 16 weeks following cell transfer, whereas mice that received CD8⁺ T cells survived more than 30 weeks (Fig. 5B). To compare the differences in the cellular infiltrates between mice that received CD4⁺ or CD8⁺ T cells, a nested group of recipient mice were killed at 8 weeks. As shown in Fig. 5A, the body weight of recipients of CD4⁺ T cells fell significantly, and these animals became ill at this time. However these effects on the animals were not due to demonstrable liver pathology. Rather, flow cytometric analysis demonstrated that the proportion and absolute number of intrahepatic T lymphocytes was significantly higher in recipients of CD8⁺ T cells compared to that in CD4⁺ T cell recipients (Fig. 5C, and data not shown). Recipients of CD4⁺ T cells develop more severe intestinal disease and die without permitting longer term observations. Recipient lungs appear similar in both CD4⁺ and CD8⁺ T cell groups.

Since both CD4⁺ and CD8⁺ T cells can produce inflammatory cytokines, we measured the production of inflammatory cytokines in serum of recipients. Both IFN-γ and TNF-α production were significantly higher in mice that were recipients of CD4⁺ T cells than in mice that were recipients of CD8⁺ T cells (Fig. 6), indicating that dnTGFβRII CD4⁺ T cells were more potent in inducing systemic inflammation following adoptive transfer.

Activated CD8⁺ T cells induce PBC-like changes in the liver

The liver histology of human PBC is characterized by CD4⁺ and CD8⁺ T cell infiltrates, associated with selective small bile duct destruction. We addressed the role of T cells in induction of liver disease by analysis of the cellular infiltrates in the liver of Rag1^{-/-} mice that were recipients of CD4⁺ or CD8⁺ T cells from dnTGFβRII mice. Immunohistological staining of the T cell infiltrates, shown in Fig. 7, indicates that recipients of CD8⁺ T cells had more severe inflammatory cell infiltrates in liver than did recipients of CD4⁺ T cells (Fig. 7A and B). CD4⁺ T cell infiltrates primarily surrounded central veins and hepatic lobules, and focal necrosis and granulomas were found within the intra-lobular area (Fig. 7E). In contrast, recipients of CD8⁺ T cells demonstrate that most CD8⁺ T cell infiltrates surrounded portal tracts but not central veins (Fig. 7F). Although CD4⁺ T cell infiltrates were evident in some portal tract areas in the CD4⁺ T cells recipient mice, small biliary ducts were not their primary targets (Fig. 7C). In contrast, moderate to severe lymphoid cell infiltrates were detected within the portal tracts 8 weeks after CD8⁺ T cell transfer. The small bile ducts in portal tracts that entrapped the infiltrating CD8⁺ T cells were often seen to be damaged and undergoing destruction (Fig. 7B). In some portal tracts, bile ducts had disappeared and significant granuloma had developed (Fig. 7D). Interestingly, histological examination of the colon and lungs demonstrated substantial lymphocytic infiltrates in recipients of CD4⁺ T cells. In contrast, recipients of CD8⁺ T cells had only mild lymphoid cell infiltrates in these organs (Fig. 8).

Discussion

We have previously demonstrated that dnTGFβRII mice deprived of regulatory TGF-β signaling restricted to T cells develop liver lesions similar to those of human PBC (16), indicative of T cells being the likely effectors of autoimmune cholangitis. Here we have transferred splenic lymphocytes isolated from dnTGFβRII mice into Rag1^{-/-} mice; the recipient mice rapidly develop a liver pathology similar to that seen in the dnTGFβRII mice.

Further, in $\text{dnTGF}\beta\text{RIIRag1}^{-/-}$ mice that lack mature T and B cells, there was no evidence of liver pathology indicating the need for immunocompetent T or B cells, or both, for the PBC-like phenotype seen in $\text{dnTGF}\beta\text{RII}$ mice. Finally, after adoptive transfer of CD4^+ and CD8^+ T cells from $\text{dnTGF}\beta\text{RII}$ mice, both cell types proliferated in the liver of recipients but only the CD8^+ T cell population underwent significant expansion and was the major constituent of the portal tract infiltrates. Histologically the bile duct epithelial cells appeared to be the target of the transferred CD8^+ T cells. Accordingly, and based on these observations, we suggest that impairment of the $\text{TGF-}\beta$ signaling pathway in the CD8^+ cytotoxic T cell population is essential for the development of autoimmune biliary epithelial cell damage in this model. In contrast, autoreactive CD4^+ T cells in this model are primarily responsible, via the production of pro-inflammatory cytokines, for systemic inflammation. In our cell transfer experiments, we addressed the role of CD8^+ T cells by studying the histopathology of recipient lung, intestine and liver and, as described herein, the biliary changes were specific for CD8^+ T cells.

Previous studies in humans implicate both CD4^+ and CD8^+ T cell reactivities in the destruction of biliary epithelial cells (2,6,21,22). Hitherto the elucidation of early events in the induction of periductular inflammation and autoimmunity in PBC has been hampered by the lack of suitable animal models (23–25). Herein, using our unique model (16), we found that populations of both CD4^+ and CD8^+ T cells were expanded in the liver, but with a CD8/CD4 ratio biased in favor of CD8. This finding is interesting, in view of previous reports of an increase in CD8^+ precursors of CTLs in the blood of patients in early compared with advanced stages of PBC and a 10–15 fold increase of autoantigen-specific CTLs within the liver compared with peripheral blood (8). The increased number of CD8^+ CTLs in $\text{dnTGF}\beta\text{RII}$ mice is in agreement with findings in PBC. In addition, adoptively transferred CD4^+ T cells induced severe inflammation in intestine and lungs whereas adoptively transferred CD8^+ T cells induced inflammation predominantly in periductular regions of the liver which was histologically similar to what is observed in human PBC. Hence adoptive transfer of CD8^+ T cells to $\text{Rag1}^{-/-}$ mice not only provides an informative model for human PBC but also has implications for understanding $\text{TGF-}\beta$ signaling and autoimmunity in general, and as well for developing novel immunotherapies to prevent target organ damage (26,27). We should emphasize the ready detection of granulomas within the portal tracts in mice that received adoptive transfer of CD8^+ T cells. However, at least within the time period of 16 weeks studied herein, we did not find evidence of fibrosis. Future studies are focused on longer term survival with additional serial histopathology in order to identify the natural history and progression of disease. We should also note that the biliary system of the mouse is significantly different from humans and the distinction between small and large bile ducts within the mouse are not as clear. At this point, we cannot clearly determine whether the biliary duct lesions in these mice are specific for small versus large bile ducts.

Our hypothesis is that autoreactive $\text{dnTGF}\beta\text{RII}$ CD8^+ T cells have their preferred target cells in the liver. Thus, histologically, transferred CD4^+ T cells induced lymphoid cell infiltration that was mild in liver but more severe in gut and lungs. Moreover, in regard to the liver location, CD4^+ T cells were noted predominantly around the central vein and within the liver lobules (Fig. 7); the bile ducts were relatively spared. In contrast, CD8^+ T cells infiltrated the portal tract and specifically targeted bile duct cells (Fig. 7). Comparably, in another autoimmune setting, that of diabetic insulinitis of NOD mice, autoaggressive CD8^+ T cells destroy the insulin-secreting β cells of pancreatic islets (21,28,29). In contrast, autoaggressive CD4^+ T cells are the effectors of inflammatory bowel disease (30,31). Furthermore, for $\text{TGF}\beta 1^{-/-}$ mice on an MHC class I-deficient background ($\beta 2$ -microglobulin-null mice), survival is much longer than for $\text{TGF}\beta 1^{-/-}$ mice on a MHC class II-deficient background (32). Thus abnormal expression and presentation of self-antigens by MHC class II molecules to autoimmune CD4^+ T cells may be more potent effectors of

systemic inflammation and autoimmunity, likely by release proinflammatory cytokines, and CD8⁺ T cells may be the more potent effectors of tissue-specific cytotoxicity (33,34).

Previous work has demonstrated that while the levels of invariant NKT (iNKT) cells in the peripheral blood of PBC patients is lower than those of healthy individuals, the frequency of iNKT cells in PBC liver is significantly higher than blood. In addition, the frequency of iNKT cells in the liver is significantly higher in PBC than controls (35,36). These results imply that in addition to CD4 and CD8 T cells, iNKT cells may play a contributory role in the liver injury of PBC. To confirm this and using our dnTGFβRII murine model, we recently noted that iNKT cells exacerbate liver injury in the early stages of murine PBC by secreting IFN-γ, which lead to NK cells, and subsequently T and B lymphocyte activation and recruitment in liver (37). Future directions in this model include multiple areas but, based upon these observations, will use CD8⁺ knockout mice as another means of studying the pathways of immune injury. Such studies will also allow us to address the mechanisms of CD8⁺ T cell cytotoxicity and the relationships with Fas/Fas ligand, the perforin/Granzyme B pathway, and interactions with other cell populations, including the role of innate immunity. Finally, we should emphasize that the use of these animals allows studies of antigen specific CD8 T cell frequency studies as well as their homing characteristics, research possibilities that are not available with human tissue. Clearly there are multiple similarities but also differences in this murine model of autoimmune cholangitis and further work will allow us to define the extent to which these data can be extrapolated to humans for potential therapeutic intervention.

Acknowledgments

Financial support provided by National Institutes of Health grants DK39588 and DK074768.

Abbreviations in this paper

PBC	primary biliary cirrhosis
AMA	anti-mitochondrial antibody
dnTGFβRII	dominant negative form of TGFβ receptor type II
Treg cell	regulatory T cell
PDC-E2	pyruvate dehydrogenase E2 complex
E3-BP	E3-binding protein
OGDC-E2	ketoglutaric acid dehydrogenase E2 complex
BCKD-E2	branched-chain 2-oxo-acid dehydrogenase E2 complex
SP	set point
RT	room temperature
OD	optical density
CBA	cytometric bead array
i.v	intravenously

References

1. Ueno Y, Moritoki Y, Shimosegawa T, Gershwin ME. Primary biliary cirrhosis: what we know and what we want to know about human PBC and spontaneous PBC mouse models. *J Gastroenterol.* 2007; 42:189–195. [PubMed: 17380276]

2. Kaplan MM, Gershwin ME. Primary biliary cirrhosis. *N Engl J Med.* 2005; 353:1261–1273. [PubMed: 16177252]
3. Chuang YH, Lian ZX, Cheng CM, Lan RY, Yang GX, Moritoki Y, Chiang BL, et al. Increased levels of chemokine receptor CXCR3 and chemokines IP-10 and MIG in patients with primary biliary cirrhosis and their first degree relatives. *J Autoimmun.* 2005; 25:126–132. [PubMed: 16243485]
4. Rieger R, Leung PS, Jeddeloh MR, Kurth MJ, Nantz MH, Lam KS, Barsky D, et al. Identification of 2-nonynoic acid, a cosmetic component, as a potential trigger of primary biliary cirrhosis. *J Autoimmun.* 2006; 27:7–16. [PubMed: 16876981]
5. Van de Water J, Fregeau D, Davis P, Ansari A, Danner D, Leung P, Coppel R, et al. Autoantibodies of primary biliary cirrhosis recognize dihydrolipoamide acetyltransferase and inhibit enzyme function. *J Immunol.* 1988; 141:2321–2324. [PubMed: 3049806]
6. Gershwin ME, Ansari AA, Mackay IR, Nakanuma Y, Nishio A, Rowley MJ, Coppel RL. Primary biliary cirrhosis: an orchestrated immune response against epithelial cells. *Immunol Rev.* 2000; 174:210–225. [PubMed: 10807518]
7. Ichiki Y, Shimoda S, Ishibashi H, Gershwin ME. Is primary biliary cirrhosis a model autoimmune disease? *Autoimmun Rev.* 2004; 3:331–336. [PubMed: 15246030]
8. Kita H, Matsumura S, He XS, Ansari AA, Lian ZX, Van de Water J, Coppel RL, et al. Quantitative and functional analysis of PDC-E2-specific autoreactive cytotoxic T lymphocytes in primary biliary cirrhosis. *J Clin Invest.* 2002; 109:1231–1240. [PubMed: 11994412]
9. Kita H, Lian ZX, Van de Water J, He XS, Matsumura S, Kaplan M, Luketic V, et al. Identification of HLA-A2-restricted CD8(+) cytotoxic T cell responses in primary biliary cirrhosis: T cell activation is augmented by immune complexes cross-presented by dendritic cells. *J Exp Med.* 2002; 195:113–123. [PubMed: 11781370]
10. Shimoda S, Nakamura M, Ishibashi H, Hayashida K, Niho Y. HLA DRB4 0101-restricted immunodominant T cell autoepitope of pyruvate dehydrogenase complex in primary biliary cirrhosis: evidence of molecular mimicry in human autoimmune diseases. *J Exp Med.* 1995; 181:1835–1845. [PubMed: 7536796]
11. Wang AP, Migita K, Ito M, Takii Y, Daikoku M, Yokoyama T, Komori A, et al. Hepatic expression of toll-like receptor 4 in primary biliary cirrhosis. *J Autoimmun.* 2005; 25:85–91. [PubMed: 16006099]
12. Padgett KA, Selmi C, Kenny TP, Leung PS, Balkwill DL, Ansari AA, Coppel RL, et al. Phylogenetic and immunological definition of four lipoylated proteins from *Novosphingobium aromaticivorans*, implications for primary biliary cirrhosis. *J Autoimmun.* 2005; 24:209–219. [PubMed: 15848043]
13. Zachou K, Rigopoulou EI, Tsikrikoni A, Alexandrakis MG, Passam F, Kyriakou DS, Stathakis NE, et al. Autoimmune hepatitis type 1 and primary biliary cirrhosis have distinct bone marrow cytokine production. *J Autoimmun.* 2005; 25:283–288. [PubMed: 16242912]
14. Rieger R, Gershwin ME. The X and why of xenobiotics in primary biliary cirrhosis. *J Autoimmun.* 2007; 28:76–84. [PubMed: 17360156]
15. Prince MI, James OF. The epidemiology of primary biliary cirrhosis. *Clin Liver Dis.* 2003; 7:795–819. [PubMed: 14594132]
16. Oertelt S, Lian ZX, Cheng CM, Chuang YH, Padgett KA, He XS, Ridgway WM, et al. Anti-mitochondrial antibodies and primary biliary cirrhosis in TGF-beta receptor II dominant-negative mice. *J Immunol.* 2006; 177:1655–1660. [PubMed: 16849474]
17. Gorelik L, Flavell RA. Abrogation of TGFbeta signaling in T cells leads to spontaneous T cell differentiation and autoimmune disease. *Immunity.* 2000; 12:171–181. [PubMed: 10714683]
18. Lian ZX, Okada T, He XS, Kita H, Liu YJ, Ansari AA, Kikuchi K, et al. Heterogeneity of dendritic cells in the mouse liver: identification and characterization of four distinct populations. *J Immunol.* 2003; 170:2323–2330. [PubMed: 12594254]
19. Yang GX, Lian ZX, Kikuchi K, Moritoki Y, Ansari AA, Liu YJ, Ikehara S, et al. Plasmacytoid dendritic cells of different origins have distinct characteristics and function: studies of lymphoid progenitors versus myeloid progenitors. *J Immunol.* 2005; 175:7281–7287. [PubMed: 16301633]

20. Moteiki S, Leung PS, Coppel RL, Dickson ER, Kaplan MM, Munoz S, Gershwin ME. Use of a designer triple expression hybrid clone for three different lipoyl domain for the detection of antimitochondrial autoantibodies. *Hepatology*. 1996; 24:97–103. [PubMed: 8707289]
21. Allina J, Hu B, Sullivan DM, Fiel MI, Thung SN, Bronk SF, Huebert RC, et al. T cell targeting and phagocytosis of apoptotic biliary epithelial cells in primary biliary cirrhosis. *J Autoimmun*. 2006; 27:232–241. [PubMed: 17222534]
22. Chuang YH, Lian ZX, Tsuneyama K, Chiang BL, Ansari AA, Coppel RL, Eric Gershwin M. Increased killing activity and decreased cytokine production in NK cells in patients with primary biliary cirrhosis. *J Autoimmun*. 2006; 26:232–240. [PubMed: 16730427]
23. Aoki CA, Roifman CM, Lian ZX, Bowlus CL, Norman GL, Shoenfeld Y, Mackay IR, et al. IL-2 receptor alpha deficiency and features of primary biliary cirrhosis. *J Autoimmun*. 2006; 27:50–53. [PubMed: 16904870]
24. Nakagome Y, Ueno Y, Kogure T, Fukushima K, Moritoki Y, Ridgway WM, Eric Gershwin M, et al. Autoimmune cholangitis in NOD. c3c4 mice is associated with cholangiocyte-specific Fas antigen deficiency. *J Autoimmun*. 2007; 29:20–29. [PubMed: 17482429]
25. Salunga TL, Cui ZG, Shimoda S, Zheng HC, Nomoto K, Kondo T, Takano Y, et al. Oxidative stress-induced apoptosis of bile duct cells in primary biliary cirrhosis. *J Autoimmun*. 2007; 29:78–86. [PubMed: 17544621]
26. Abbas AK, Lohr J, Knoechel B. Balancing autoaggressive and protective T cell responses. *J Autoimmun*. 2007; 28:59–61. [PubMed: 17363216]
27. Tsonis IA, Avrameas S, Moutsopoulos HM. Autoimmunity and pathophysiology. *J Autoimmun*. 2007; 29:203–205. [PubMed: 17884336]
28. Green EA, Choi Y, Flavell RA. Pancreatic lymph node-derived CD4(+)CD25(+) Treg cells: highly potent regulators of diabetes that require TRANCE-RANK signals. *Immunity*. 2002; 16:183–191. [PubMed: 11869680]
29. Green EA, Flavell RA. The temporal importance of TNFalpha expression in the development of diabetes. *Immunity*. 2000; 12:459–469. [PubMed: 10843379]
30. Asseman C, Mauze S, Leach MW, Coffman RL, Powrie F. An essential role for interleukin 10 in the function of regulatory T cells that inhibit intestinal inflammation. *J Exp Med*. 1999; 190:995–1004. [PubMed: 10510089]
31. Fuss IJ, Boirivant M, Lacy B, Strober W. The interrelated roles of TGF-beta and IL-10 in the regulation of experimental colitis. *J Immunol*. 2002; 168:900–908. [PubMed: 11777988]
32. Kobayashi S, Yoshida K, Ward JM, Letterio JJ, Longenecker G, Yaswen L, Mittleman B, et al. Beta 2-microglobulin-deficient background ameliorates lethal phenotype of the TGF-beta 1 null mouse. *J Immunol*. 1999; 163:4013–4019. [PubMed: 10491004]
33. Tiegs G. Cellular and cytokine-mediated mechanisms of inflammation and its modulation in immune-mediated liver injury. *Z Gastroenterol*. 2007; 45:63–70. [PubMed: 17236122]
34. Lang KS, Burow A, Kurrer M, Lang PA, Recher M. The role of the innate immune response in autoimmune disease. *J Autoimmun*. 2007; 29:206–212. [PubMed: 17904335]
35. Kita H, Naidenko OV, Kronenberg M, Ansari AA, Rogers P, He XS, Koning F, et al. Quantitation and phenotypic analysis of natural killer T cells in primary biliary cirrhosis using a human CD1d tetramer. *Gastroenterology*. 2002; 123:1031–1043. [PubMed: 12360465]
36. Tsuneyama K, Yasoshima M, Harada K, Hiramatsu K, Gershwin ME, Nakanuma Y. Increased CD1d expression on small bile duct epithelium and epithelioid granuloma in livers in primary biliary cirrhosis. *Hepatology*. 1998; 28:620–623. [PubMed: 9731549]
37. Chuang Y, Lian Z, Yang G, Shu S, Moritoki Y, Ridgway WM, Ansari AA, et al. Invariant NKT cells exacerbate liver injury in a TGFbeta receptor II dominant-negative mouse model of primary biliary cirrhosis. *Hepatology*. 2008 in press.

Figure 1. T cell infiltration in dnTGFβRII mice

A. Lymphoid cells isolated from the liver of dnTGFβRII mice and control B6 mice at serial ages were stained with fluorochrome-conjugated mAbs (see Methods) and analyzed by flow cytometry. Total lymphoid cells (left panel) and the ratio of CD8⁺ T cell to CD4⁺ T cells (right panel) are shown. The data presented in the figure are the mean ± SD, with 4 to 8 mice at each time point. *, dnTGFβRII vs B6 mice, $P < 0.05$. B. Immunohistochemical analysis of liver of dnTGFβRII mice at 20 weeks of age using mAbs to CD4 and CD8.

Figure 2. Splenocytes of dnTGFβRII mice can transfer liver disease to Rag1^{-/-} recipient mice
 B6/Rag1^{-/-} (Ly5.2) mice received 1×10^7 splenic lymphoid cells isolated from either dnTGFβRII (Ly5.1) or B6 Ly5.1 control mice. A. Data display the mean weight of 6 mice per group and are representative of 2 independent experiments. B. Total cell number of lymphoid cells isolated from liver and spleen of B6/Rag1^{-/-} recipients 8 weeks after transfer. *, dnTGFβRII vs B6 mice, $P < 0.05$. C. Quantification of anti-PDC-E2 in the sera of recipient mice 2 and 4 weeks after transfer of dnTGFβRII spleen cells.

Figure 3. dnTGF β RII splenic lymphoid cells induce inflammation in multiple organs of Rag1 $^{-/-}$ recipient

B6/Rag1 $^{-/-}$ (Ly5.2) mice received 1×10^7 splenic lymphoid cells isolated from either dnTGF β RII (Ly5.1) or B6 Ly5.1 control mice. 6 weeks after transfer, recipients were sacrificed and liver, colon, and lung were fixed in 10 % formalin. Paraffin-embedded tissues were stained with H & E.

Figure 4. Absence of cell infiltration in naive $dnTGF\beta RIIRag1^{-/-}$ mice
 $dnTGF\beta RIIRag1^{-/-}$ mice were generated by breeding $dnTGF\beta RII$ mice with $B6/Rag1^{-/-}$ mice. These transgenic mice and control $B6/Rag1^{-/-}$ mice were sacrificed at 20 weeks of age. Tissues were fixed in 10 % formalin. Paraffin-embedded tissues were stained with H & E.

Figure 5. Characteristics of Rag1^{-/-} mice transferred with dnTGFβRII CD4⁺ and CD8⁺ T cells
 CD4⁺ and CD8⁺ T cells were isolated from spleen of dnTGFβRII (Ly5.1) mice by magnetic microbeads. Aliquots of 1×10⁶ CD4⁺ and CD8⁺ T cells were intravenously injected into B6/Rag1^{-/-} (Ly5.2) mice. A. Body weight of recipients transferred with CD4⁺ or CD8⁺ T cells. The error bars represent the means ± SD. B. Survival of the recipients (%) transferred with CD4⁺ or CD8⁺ T cells. C. T cell numbers in liver of recipients transferred with CD4⁺ and CD8⁺ T cells. Recipients were sacrificed at 8 weeks after transfer. Liver lymphoid cells were stained with fluorochrome-conjugated mAbs and analyzed by flow cytometry. Absolute numbers of CD4⁺ or CD8⁺ T cells from donor are shown as the means ± SD. Each group included 6 to 8 mice. *, CD4 group vs CD8 group, p<0.05.

Figure 6. Serum cytokine profiles of mice transferred with dnTGF β RII CD4⁺ and CD8⁺ T cells Sera were collected at various times after T cell transfer. Concentrations of IFN- γ and TNF- α were determined by CBA (see Materials and Methods). Each group included 4 to 6 mice. Data represent means $\pm$ SD.

Figure 7. Liver histological changes of recipient mice transferred with dnTGFβRII CD4⁺ and CD8⁺ T cells

B6/Rag1^{-/-} (Ly5.2) mice received CD4⁺ or CD8⁺ T cells as described in Fig. 5; 8 weeks after transfer, recipients were sacrificed and liver tissues were stained with H & E.

Representative photomicrographs of tissues are shown. A, C, E. Pattern of lymphoid cell infiltration in liver after transfer with CD4⁺ T cells. B, D, F. Pattern of lymphoid cell infiltration in liver after transfer with CD8⁺ T cells. Arrows show areas of inflammation in the liver. P, portal tract. C, central vein.

Figure 8. Histological changes of recipient mice transferred with dnTGF β RII CD4⁺ or CD8⁺ T cells

B6/Rag1^{-/-} (Ly5.2) mice received CD4⁺ or CD8⁺ T cells as described in Fig. 5; 8 weeks after transfer, recipients were sacrificed and sections of lung and colon were stained with H & E. Representative photomicrographs illustrate inflammation affecting colon and lung of recipients transferred with different T cell subsets.