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Anti-Mitochondrial Antibodies and Primary Biliary Cirrhosis in TGF-β Receptor II Dominant-Negative Mice[1]

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Primary biliary cirrhosis (PBC) is an autoimmune disease of the liver, characterized by lymphocytic infiltrates in portal tracts, selective destruction of biliary epithelial cells, and anti-mitochondrial Abs (AMAs). The elucidation of early events in the induction of tissue inflammation and autoimmunity in PBC has been hampered by the cryptic onset of the disease, the practical limitations in accessing the target tissue, and the lack of a suitable animal model. We demonstrate in this study that a mouse transgenic for directed expression of a dominant-negative form of TGF-β receptor type II (dnTGFβRII), under the direction of the CD4 promoter, mimics several key phenotypic features of human PBC, including spontaneous production of AMAs directed to the same mitochondrial autoantigens, namely PDC-E2, BCOADC-E2, and OGDC-E2. The murine AMAs also inhibit PDC-E2 activity. Moreover, there is lymphocytic liver infiltration with periportal inflammation analogous to the histological profile in human PBC. Additionally, the serum cytokine profile of affected mice mimics data in human PBC. The concomitant presence of these immunopathological features in the transgenic mice suggests that the TGF-βRII pathway is implicated in the pathogenesis of PBC. Finally, these data point away from initiation of autoimmunity by mechanisms such as molecular mimicry and more toward activation of an intrinsically self-reactive T cell repertoire in which necessary regulatory T cell influences are lacking. ***The Journal of Immunology,*** **2006, 177: 1655–1660.**

Primary biliary cirrhosis (PBC) is a progressive autoimmune liver disease characterized by portal inflammation and immune-mediated destruction of the intrahepatic bile ducts. The damage of bile ducts is associated with cholestasis and progressive development of hepatic lesions which include fibrosis, cirrhosis, and eventually lead to liver failure. Serologically, PBC is characterized by the presence of anti-mitochondrial Abs (AMAs) in 90–95% of patients preceding the occurrence of symptoms by many years (1). AMAs react with an epitope on the E2 subunit of the pyruvate dehydrogenase enzyme complex (PDC-E2) and, in a smaller proportion of cases, with the E2 subunit of functionally related 2-oxo-acid dehydrogenase complexes, branched chain 2-oxo-acid dehydrogenase (BCOADC-E2), and 2-oxo-glutarate dehydrogenase (OGDC-E2) (2). All of these enzymes contain two sites (the inner and outer domains) at which lipoic acid is attached to a lysine residue and acts as a swinging arm to transfer chemical groups between other members of the respective enzyme complexes. The major epitope for both autoantibodies and for reactive CD4 and CD8 T cells (3, 4) resides in the inner lipoyl domain of these enzyme complexes (5).

We report in this study that mice transgenic for directed expression of a dominant negative form of TGFβ receptor type II (TGFβRII), under the CD4 promoter lacking the CD8 silencer (6), spontaneously develop features characteristic of PBC. These features include the development of significant titers of autoantibodies with specificity against PDC-E2, BCOADC-E2, and OGDC-E2 that, as in PBC, inhibit enzyme function in vitro and react with the inner lipoyl domain epitope of the respective E2 subunits. Histologically there is extensive $CD4^+$ and $CD8^+$ lymphocytic infiltration in portal tracts, associated with biliary destruction. Moreover, mice manifest increased serum IFN-γ and elevated TNF-α, as seen in PBC (7, 8). The fact that TGFβRII-transgenic mice have specific impairment of TGFβ signaling, exclusively in T lymphocytes, provides the unique opportunity to identify a mechanism for the development of autoimmune biliary disease that could be applicable to human PBC. A previous report indicating the involvement of the TGFβRII pathway in the development of liver fibrosis (9) combined with the data presented herein suggest that lack or aberrant activity of TGF-β signaling contributes to a loss of self tolerance to autoantigenic proteins in the liver, which in turn leads to autoimmunity. Moreover, it points away from initiation of autoimmunity by mechanisms such as molecular mimicry and more toward activation of an intrinsically self-reactive T cell repertoire in which necessary regulatory T cell (T reg) influences are lacking.

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[4] Abbreviations used in this paper: PBC, primary biliary cirrhosis; AMA, anti-mitochondrial Ab; Treg, regulatory T cell; dn, dominant negative; MPO, myeloperoxidase; SP, set point.

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Materials and Methods

Dominant-negative (dn) TGFβRII mice

dnTGFβRII mice were originally developed by R. A. Flavell (6). The dnTGFβRII mice were bred onto the C57BL/6 background (The Jackson Laboratory) at the University of California animal facility (Davis, CA). Nineteen dnTGFβRII mice and 17 control littermates were used for the described experiments. dnTGFβRII and wild-type littermates were fed with the sterile rodent *Helicobacter* Medicated Dosing System (three-drug combination) diets (Bio-Serv) and maintained in individually ventilated cages under specific pathogen-free condition. dnTGFβRIIRag1$^{-/-}$ mice were generated by backcrossing female B6 Rag1$^{-/-}$ with male dnTGFβRII mice. The resulting F_2 generation was genotyped and selected for dnTGFβRIIRag1$^{-/-}$ mice. All studies were performed with approval from the University of California Animal Care and Use Committee.

Production of recombinant mouse mitochondrial proteins

cDNA encoding the full-length protein of mouse OGDC-E2 and BCOADC-E2, as well as amino acids 1–391 of PDC-E2, which includes the inner lipoyl domain on this protein, were generated with standard reverse transcription and PCR, using total RNA isolated from mouse liver. The PDC-E2 cDNA was engineered into the *Escherichia coli* expression vector pCAL-n-Flag (Stratagene) with a 6× His purification tag on the C terminus of the partial protein. The OGDC-E2 and BCOADC-E2 cDNA were engineered into the *E. coli* expression vector pET32a. The recombinant proteins were expressed in *E. coli* Rosetta-gami 2(DE3) (Novagen). The expressed His-tagged fusion proteins were purified with Ni-NTA affinity chromatography (Qiagen) under denaturing conditions.

ELISA and immunoblotting for AMA

Ninety-six-well ELISA plates (Nunc) were coated overnight with 100 μl of rAMA Ags (PDC-E2, BCOADC-E2, OGDC-E2) at a concentration of 10 μg/ml. A reference standard was composed of pooled AMA-positive mouse sera, used to construct a standard curve by serial dilutions of the reference serum and an ELISA performed as described (10). Plates were read at 450 nm. Threshold values are set by mean + 2 SD of values for B6 controls. For immunoblotting, ~20 μg of the purified mouse rPDC-E2 protein was loaded on a 4–20% Novex mini protein gel (Invitrogen Life Technologies) and fractionated at 170 V for 1 h and probed as described with known positive and negative sera (10). The signal was detected with a FluorTech 8900 gel doc system (Alpha Innotech) equipped with a chemiluminescent filter.

Inhibition of pyruvate dehydrogenase (PDC) enzyme activity

A predetermined serum dilution (1/100) was incubated with PDC (Sigma-Aldrich) for 10 min at room temperature. The solution was then added to a PDC reaction mixture containing 5 mM sodium pyruvate, 2.5 mM NAD+, 0.2 mM thiamine pyrophosphate, 0.1 mM coenzyme A, 0.3 mM DTT, 1 mM magnesium chloride, 50 mM potassium phosphate buffer (pH 8.0). UV absorbance change per minute at 340 nm was monitored for 5 min. Enzyme activity measurements without addition of serum were run in parallel to define 100% activity. Enzyme inhibition activity was assayed in parallel on sera from dnTGFbRII mice, B6 mice, patients with PBC and negative human controls.

Liver tissue preparation

dnTGFbRII and control mice were sacrificed at age 24–28 wk. Whole liver tissue was explanted and immediately fixed with a 1:1 solution of formalin (18.75%) and methanol (100%) for 1 to 2 h at room temperature. Paraffin-embedded tissue sections were then cut into 5-μm slices for routine hematoxylin (DakoCytomation) and eosin (American Master Tech Scientific) staining.

Immunohistochemistry

Abs against cell markers, including CD4 (1/200 dilution), CD8 (1/200), CD19 (1/50; all obtained from eBioscience), pDCA1 (1/200; Miltenyi Biotec), DX5 (1/50; Biolegend), and myeloperoxidase (MPO; 1/10; Cell Sciences) were used for immunohistochemical staining of the portal tract infiltrates. Anti-cytokeratin 7 (1/10; Research Diagnostics) was used as a biliary cell marker. Briefly, after deparaffinization sections were incubated in a Decloaking Chamber (Biocare Medical; set point (SP)1 123°C for 2 min, SP2 85°C for 10 s, SP limit 10°C) soaked in 3% H_2O_2 methanol solution for 5 min, then 15 min in 1× Universal blocking solution (BioGenex) and 20 min in 10% goat serum to prevent nonspecific staining. Primary Abs were applied for 1 h at room temperature 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) or polyvalent anti-mouse Ig (DakoCytomation, ready to use) or goat anti-hamster IgG (GeneTex; 1/100 dilution) was applied as secondary Ab for 1 h at room temperature 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).

For phenotypic definition of the MPO-positive cells, double enzyme immunohistochemical staining was performed. Briefly, anti-F4/80 (Caltag Laboratories; 1/25) was applied as a primary Ab as described above, and polyclonal HRP-conjugated rabbit anti-rat Ig (DakoCytomation) was used as the secondary Ab. After development with DAB, sections were boiled in water for 10 min to denature excess Ab. Anti-MPO (Cell Sciences; 1/10) was then applied for 1 h at room temperature in a moist chamber. After washing with PBST, alkaline phosphatase-conjugated polyvalent anti-mouse Ig (DakoCytomation) was used for development and Mayer's hematoxylin for counterstaining.

Flow cytometry

Lymphoid cells from PBS-perfused liver were separated by centrifugation with Histopaque-1077 (Sigma-Aldrich). FcR were blocked by incubation with 2.4G2 mAb (eBioscience) and cells were stained with fluorochrome-conjugated Abs specific for CD3, CD4, CD8, NK1.1 (eBioscience), and α-GalCer-loaded CD1d tetramers at 4°C in PBS/0.2% BSA for 30 min. The stained cells were analyzed on a five-color FACScan flow cytometer (BD Immunocytometry Systems). Acquired data were analyzed with CellQuest (BD Biosciences) and FlowJo software (Tree Star).

Measurement of serum cytokines

Serum samples were collected at different ages and kept at −70°C until assayed. Thawed serum samples were diluted 1/2 (for IFN-γ, TNF-α, and IL-6) or 1/5 (for IL-12p40) with PBS. Concentrations of IFN-γ, TNF-α, and IL-6 were measured with a cytometric bead assay using the mouse inflammation cytokine kit, a cytometric bead assay (BD Biosciences). Concentrations of IL-12p40 were measured using the mouse IL-12p40 ELISA kit, respectively (R&D Systems).

Results

dnTGFβRII-transgenic mice (dnTGFβRII) were first developed for studies aimed at dissection of the role of this receptor in T lymphocyte function (6). During the characterization of these dnTGFβRII mice, histological studies revealed mononuclear cell infiltration of various tissues and specifically clusters of macrophages and lymphocytes in the parenchyma and portal tracts of the liver. Analysis of these histological features indicated that the pathological findings in the dnTGFβRII mice appeared very similar to those seen in human PBC and prompted us to examine the sera of these mice for PBC-like serologic reactivity. Positive results for Abs to recombinant human PDC-E2 specifically directed against the inner lipoyl domain of the molecule provided the impetus to carry out the studies described herein.

dnTGFβRII mice develop species-specific AMAs

Although the murine orthologs of the human PBC autoantigen show a high degree (> 99%) of similarity with the human protein, we needed to ensure that the detected reactivity was a true autoimmune response, directed also against the rodent enzymes as well as to recombinant human PDC-E2, BCOADC-E2, and OGDC-E2. Hence, all three murine genes were cloned and expressed as recombinant proteins. The cloned proteins encompassed the entire length of the native protein for BCOADC-E2 and OGDC-E2. For PDC-E2 a partial protein was produced that included the major epitope sequence of the inner lipoyl domain. AMA reactivity in mice was demonstrated by ELISA (Fig. 1*A*), immunoblot and immunofluorescence (data not shown) at all time points examined starting at 4 wk of age. Transgenic mice displayed an increase in reactivity toward all tested Ags over time. By weeks 22–24, 100, 95, and 68% of the mice sera samples were positive for Abs against PDC-E2, OGDC, and BCOADC, respectively. The mean titers for anti-PDC-E2, anti-OGDC-E2, and anti- BCOADC-E2 were all significantly higher for weeks 22–24 than weeks 4–10

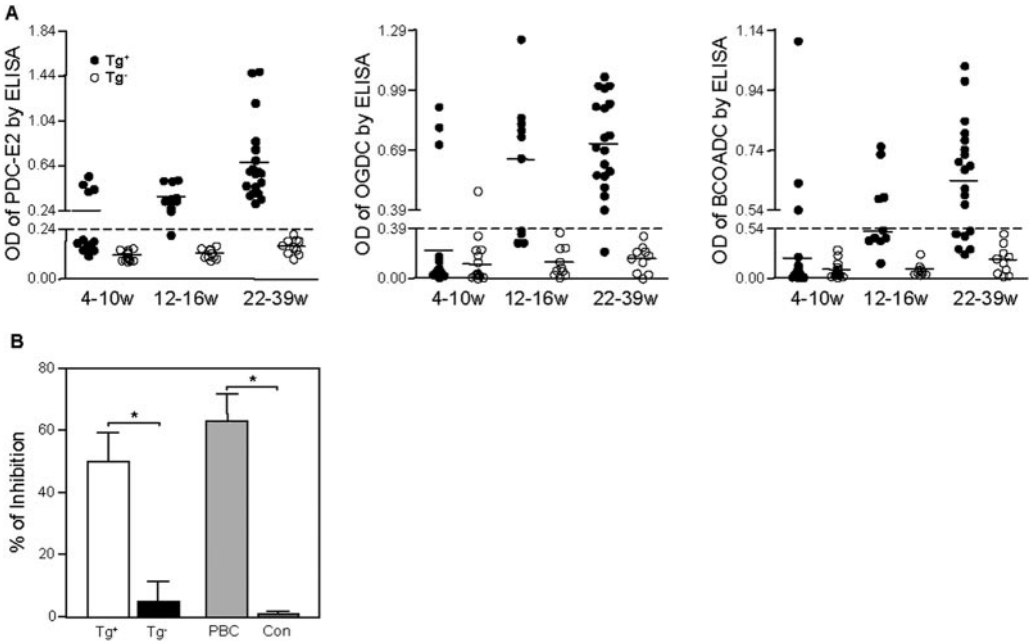

FIGURE 1. dnTGF-βRII mice produce Abs to mitochondrial Ags. *A*, ELISA for sera from dnTGFbRII mice and control B6 littermates. Wells were coated with recombinant proteins PDC-E2, OGDC-E2, or BCOADC-E2. Serum samples were diluted 1/500. Sera from 4- to 10-wk-old mice (dnTGFβRII $n = 11$, B6 $n = 10$), 12- to 16-wk-old mice (dnTGFβRII $n = 10$, B6 $n = 9$), and 22- to 39-wk-old mice (dnTGFβRII $n = 19$, B6 $n = 10$) were tested. Threshold values are set by mean + 2 SD of values for B6 controls. *B*, PDC enzyme inhibition assay. PDC was preincubated with 1/100 diluted sera from 20-wk-old dnTGFbRII mice ($n = 7$) and control B6 mice ($n = 4$), along with sera from patients with PBC ($n = 6$) and normal human control, followed by enzyme activity assay. ∗, $p < 0.001$. Tg^+, dnTGFβRII transgenic mice; Tg-, littermate controls.

($p < 0.0005$, Mann-Whitney *U* test). Comparable autoantibody reactivity in the control littermate mice was not detected. This time sequence resembles that of the natural history of PBC in humans where the disease is not observed in childhood and typically presents in the fourth or fifth decade of life.

A particular characteristic of human PBC is the ability of sera that contain anti-mitochondrial Ab, to specifically inhibit enzymatic activity of PDC-E2 in vitro, typically showing inhibition in the range of 50–65%. Sera from the dnTGFβRII mice inhibited murine PDC-E2 enzyme activity (median, 50.5% $n = 7$), whereas there was no comparable reactivity of sera from the B6 littermate controls ($p < 0.001$, Fig. 1*B*).

Lymphocytic liver infiltrate and selective biliary ductular cell destruction in dnTGFβRII mice

The liver histology of human PBC is characterized by the presence of lymphocytic infiltration associated with selective small bile duct destruction, eventually leading to cirrhosis (11). The severity and temporal progression vary widely among patients and do not correlate with AMA titers (12). The liver of 6- to 7-mo-old dnTGFβRII mice was examined and compared with livers of normal B6 littermates. Moderate to severe lymphoid cell infiltration was detected within the portal tracts in association with bile duct damage (Fig. 2, *A–C*), occurring only in the dnTGFβRII mice, and mirroring what is characteristically seen in PBC. Direct bile duct destruction was determined by the detection of scattered cytokeratin 7-positive cells in the portal infiltrates (Fig. 2*D*). Moreover, in liver tissues from some of the mice, biliary cell destruction was sufficiently advanced that identification of an intact bile duct structure was impossible; yet another feature similar to that seen in advanced human PBC (Fig. 2*B*).

Immunohistochemical analysis demonstrated that the infiltrate included varying numbers of $CD4^+$ and $CD8^+$ lymphocytes (Fig. 2, *E* and *F*), B lymphocytes ($CD19^+$) (Fig. 2*G*) and NK cells ($DX5^+$) (Fig. 2*I*). Additionally monocytes/macrophages ($MPO^+F4/80^+$), characteristically present in liver tissues of PBC patients, could also be identified in the affected portal tracts of the dnTGFβRII mice, but not in control littermates (Fig. 2*K*). A particular feature seen in human PBC is the presence of infiltrating eosinophils and granulomas in portal tracts, but these were not evident in the livers of the transgenic mice.

Lymphocytic infiltrates in the liver are represented by $CD4^+$ and $CD8^+$ lymphocytes

The lymphocytic infiltrate detected in the liver of patients with PBC comprises both $CD4^+$ and $CD8^+$ lymphocytes (13) and, additionally, there is a relative increase in the resident NKT cell population compared with healthy controls (14). We analyzed the composition of the intrahepatic lymphoid cell population within the liver of transgenic mice and control littermates to establish quantitative and qualitative differences. In the liver, both the total number of $CD3^+$ lymphocytes (Table I) and the percentage of $CD3^+$ cells among the total lymphoid cell infiltrate (Fig. 3*A*) were significantly increased in the transgenic mice. Although the total number of intrahepatic $CD4^+$ lymphocytes was enlarged (Table I), the percentage of $CD4^+$ cells in the $CD3^+$ population did not increase (Fig. 3*C*). In contrast, the $CD8^+$ population was significantly amplified ($p < 0.05$) in total number (Table I) as well as percentage in the $CD3^+$ population compared with controls (Fig. 3*C*). These features resulted in a change in the ratio of CD4 to CD8 T cells biased toward the $CD8^+$ population (Table I). This finding is particularly interesting, considering previous reports of an increase in precursors of CTLs in the blood of patients in the early stages of PBC vs advanced stages and the 10-fold increase of autoantigen-specific CTLs within the liver as compared with the circulating pool, reported in the same study (4). In addition, the transgenic mice showed a marked increase in the number of

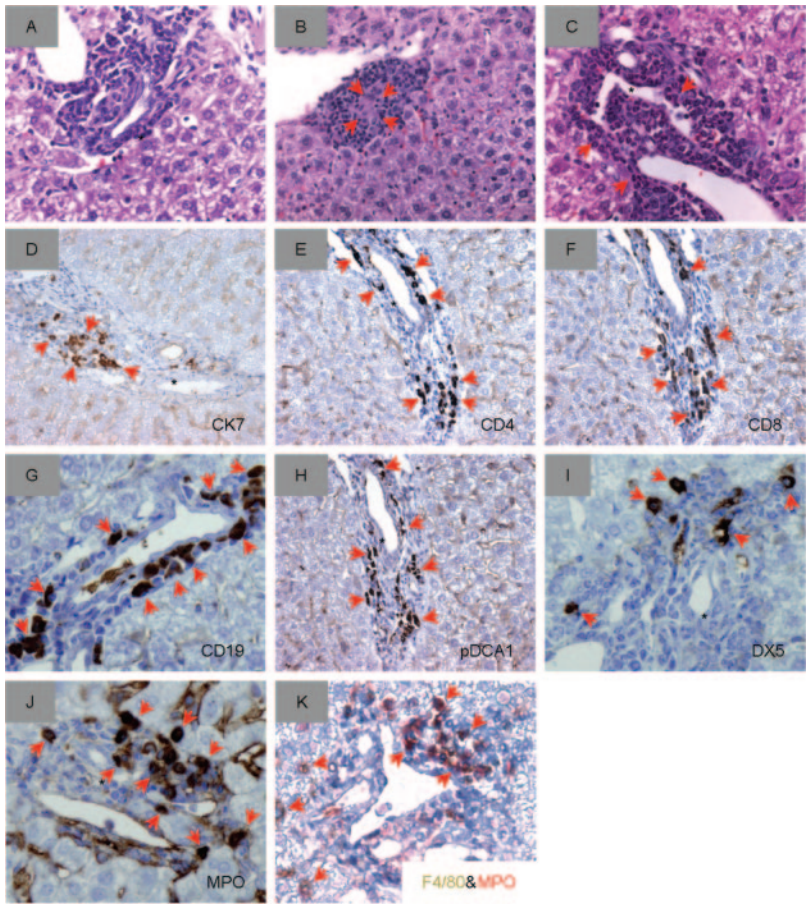

FIGURE 2. Histological features of the 24–28 wk liver. *A–C*, Different degrees of lymphocytic infiltration (red arrows) surrounding the small bile ducts were detected within the portal tracts (H&E staining); D-L, individual cells (red arrows) of cell types composing the portal tract infiltrates were stained with Abs against cell markers: *D*, biliary cells identified by cytokeratin 7; *E*, $CD4^+$ lymphocytes; *F*, $CD8^+$ lymphocytes; *G*, $CD19^+$ B cells; *H*, $pDCA1^+$ plasmacytoid dendritic cells; *I*, $DX5^+$ NK cells; *J*, MPO^+ macrophages; *K*, macrophages stained with MPO, confirmed by costaining with F4/80 (black arrows, MPO^+ cells; red arrows, $MPO^+F4/80^+$ cells).

intrahepatic NKT cell population, resembling the increase described in human PBC (14); although the percentage of NKT cells in intrahepatic lymphocytes did not increase in the mouse model (Fig. 3*E*). Interestingly, this increase was associated with a decrease of the NKT cell population within the spleen of the transgenic mice (Fig. 3*F*, Table I).

Similar analyses were conducted for lymphocyte populations in the spleen, which also showed a significant change in $CD4^+$-$CD8^+$ ratio in the transgenic mice (Table I).

Cytokine profiles of dnTGFβRII mice

A transcriptional up-regulation of IFN-γ, TNF-α, and IL-6 within biliary epithelial cells, associated with up-regulation of their cognate receptors, has been demonstrated in PBC (7, 8). We thus analyzed the serum levels of these and various other cytokines in dnTGFβRII mice and B6 littermates of different ages. The levels of IFN-γ, TNF-α, IL-6, and IL-12p40 at ages of 14–16, 22–26, and 28–32 wk were all significantly increased in dnTGFβRII mice compared with B6 littermates ($p < 0.001$) (Fig. 4). Of note, the levels of IFN-γ and TNF-α in the older transgenic mice were lower than those in younger mice, while those of IL-6 and IL-12p40 did not decline with age.

Absence of disease in dnTGFβRIIRag1$^{-/-}$ mice

dnTGFβRIIRag1$^{-/-}$ mice were observed for 6 mo. No AMAs could be detected in the serum and, more importantly, the liver histology appeared normal and similar to wild-type B6 controls without evidence of hepatic or biliary-specific pathology (data not shown).

Discussion

The murine model of PBC described herein, based on a deficiency in a pathway involved in peripheral tolerance, generates a new pathogenetic scenario for autoimmunity that differs from the loss

Table I. *Phenotype of mononuclear cells in the liver and spleen*

	Liver		Spleen	
	Tg^+	Tg^-	Tg^+	Tg^-
Total cell number ($n \times 10^6$)	17.79 ± 8.48**	1.78 ± 0.20	77.10 ± 21.30	91.80 ± 18.70
$CD3^+$	9.47 ± 3.23**	0.62 ± 0.05	27.88 ± 8.52	26.29 ± 4.84
$CD4^+$	3.10 ± 1.45**	0.28 ± 0.02	8.49 ± 2.45*	12.99 ± 2.68
$CD8^+$	4.40 ± 1.49**	0.20 ± 0.06	16.98 ± 7.97	9.56 ± 2.04
CD1d $tetramer^+$	0.68 ± 0.40*	0.18 ± 0.05	0.37 ± 0.11**	1.17 ± 0.16
$CD4^+/CD8^+$	0.71 ± 0.24**	1.55 ± 0.42	0.57 ± 0.24**	1.37 ± 0.16

*, $p < 0.05$; **, $p < 0.01$, compared with Tg.

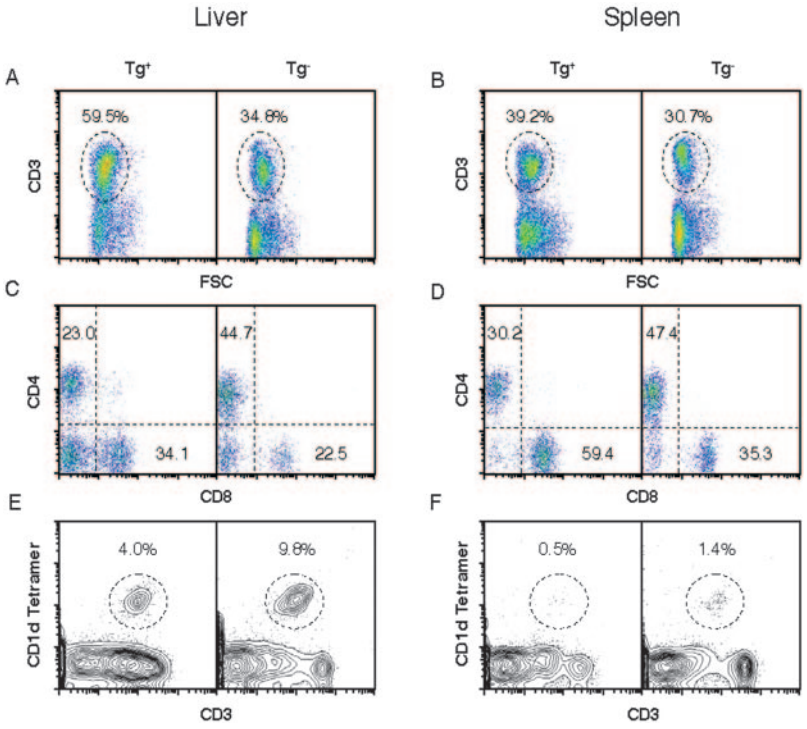

FIGURE 3. Lymphoid cells isolated from liver and spleen were stained with fluorochrome-conjugated mAbs and analyzed by flow cytometry. *A* and *B*, Frequency of $CD3^+$ lymphocytes in total lymphocyte population. *C* and *D*, The percentage of $CD4^+$ and $CD8^+$ cells in total $CD3^+$ population. *E* and *F*, The percentage of CD1d $tetramer^+$ NKT cells in total lymphocyte population.

of tolerance resulting from molecular mimicry or chemical modifications of a native epitope. The selective deficiency of the TGF-βR-signaling pathway exclusively in T lymphocytes accounts for the impairment of a system essential to peripheral tolerance. Circulating Treg cells depend on TGF-β for their regulatory activity, thus allowing the emergence of tissue-specific autoreactive effector T cells if impaired. To assess whether the liver pathology in dnTGFβRII mice was due to an immune response or a TGF-β-mediated lesion in the biliary target tissue, we generated dnTGFβRIIRag1$^{-/-}$ mice. As discussed above, there was no evidence of liver pathology in these mice. This new colony represents a valuable tool for use in adoptive transfer and consequent focus on effector mechanisms. Such experiments are ongoing and are preferable to the use of anti-CD4- or anti-CD8-treated animals due to the pleiotropic sites of action of these depleting Abs. Cell transfer will allow not only dissection of the effector mechanism, but also the critical question of specific donor cell age requirements. Additional future work will also be directed at the role of bacterial flora. A number of T cell-depleted animal models of inflammatory bowel disease demonstrate reduction in their bowel pathology in a germfree environment (15). Therefore, the specific contribution of bacterial flora to disease development in this model should be addressed because PBC is frequently used as an example of molecular mimicry (16).

At a systemic level, TGF-β appears as an essential modulator of Foxp3 expression by Treg cells (17), conditioning their suppressive ability in the periphery. Recent studies from our group have identified selective defects in the circulating Treg population of patients with PBC as compared with healthy controls (18). In addition, it is reported that the population of $CD4^+CD25^+$ lymphocytes that coexpress Foxp3 and TGF-β decreases with age, specifically in female NOD mice, modifying both the degree of lymph node localization and target tissue infiltration (19). These results, unique to females, are of specific interest to PBC, considering also that the primary activation site of $CD8^+$ T cells, whether in the regional lymph nodes or liver, appears to be fundamental for the predominance of immunity over tolerance (20). Interestingly, TGF-β selectively targets circulating Treg cells and not thymic precursors. Nonetheless, the expression of CD4 by dendritic cells within the murine thymus (21) could modify the responsiveness of these cells to TGF-β, altering their maturation and potentially accounting for a defect in negative selection of the intrathymic T cells. These features appear of particular importance given the recent description of autoimmune cholangitis in a congenic strain of

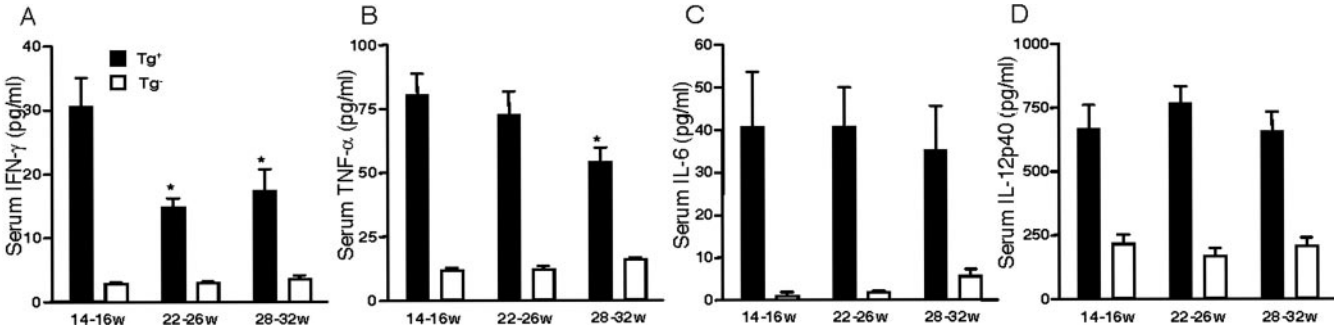

FIGURE 4. Serum cytokine profiles of dnTGFβRII mice. Concentrations of IFN-γ, TNF-α, IL-6, and IL-12p40 in sera of dnTGFβRII transgenic mice (Tg^+) and B6 control littermates (Tg^-) of different ages were determined by ELISA (*, $p < 0.05$ compared with 14–16 wk).

NOD mice, with features very similar to those of human PBC (22). The most interesting connection between the two models concerns the role of TGF-β. The genetic locus of autoimmune biliary disease in the NOD.c3c4 model appears within the *abd* locus. A candidate molecule within that region is *Cdkn2b*, which is induced by TGF-β and is a possible mediator of TGF-β cell cycle arrest (23). If the B6 variant of *Cdkn2b* mediates a decreased TGF-β response in NOD.c3c4 mice, it might provide a mechanistic connection between the NOD.c3c4 and dnTGFβRII models of PBC.

Considering the striking similarities of the murine cholangitis model described herein to human PBC, and its potential contribution to the analysis of novel pathways involved in pathogenesis, we need to annotate that this model does have some clear differences from human PBC. First, we could not detect the female bias characteristic of PBC within our population of affected animals; second, the consistent increase of circulating IgM is not shared by affected mice, which, on the contrary, develop an increase in IgA (6). However, these differences could well be secondary to species-specific differences in response to equivalent perturbations in the signaling cascade within the two species, similarly to the previously mentioned decrease of TGF-β/FoxP3-positive Tregs in NOD mice. Moreover, we cannot rule out that selected factors involved in human PBC might be absent or not consistently mimicked in the murine model, such as hormonal influences (24).

Another essential difference detected in the mouse model is the absence of eosinophilic infiltration and granuloma formation. These features are reported in a number of cases of human PBC, but due to the cryptic onset of the human disease, it has not been possible to effectively define the temporal relation of eosinophilia and granuloma formation with serological positivity (25). At present, we are unable to define whether the absence of these histological characteristics is due to not sufficiently advanced disease or to consistent lack of these features in the mouse model.

In conclusion, we describe herein a murine model in which immunoregulatory defects within the lymphocytic and phagocytic components of the immune system, potentially in association with a particular vulnerability of the biliary ductular target tissue, initiate an autoimmune response resembling that seen in PBC. This model, and the newly described NOD.c3c4 model, both facilitate analysis of causal events and help define the essential steps necessary to biliary disease.

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Disclosures

The authors have no financial conflict of interest.

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