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IL-12/Th1 and IL-23/Th17 Biliary Microenvironment in Primary Biliary Cirrhosis: Implications for Therapy

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Abstract

The interleukin (IL)-12/IL-23 mediated Th1/Th17 signaling pathway has been associated with the etiopathogenesis of primary biliary cirrhosis (PBC). To address the cytokine microenvironment specifically in the liver, we examined the localized expression of cytokine subunits and their corresponding receptors using previously optimized immunohistochemistry with an extensive panel of antibodies directed at IL-12p70, IL-12p35, IFN-γ, IL-12RB2, IL-23p40, IL-23p19, IL-17 and IL-23R using liver from PBC (n=51) and non-PBC (n=80) control liver disease patients. Multiple portal tracts in each patient were blindly evaluated and individually scored. We report herein that although IL-12/Th1 and IL-23/Th17 staining were detected in all of the liver sections,

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they were primarily localized around the damaged interlobular bile ducts in PBC. Most importantly, Th17 skewing was prominent in advanced PBC patients with intensive secretion of IL-23p19 by inflamed hepatocytes around IL-23R, IL-12RB2, and IFN-γ expressing degenerated cholangiocytes. Our novel finding on the direct association of Th17 skewing and disease severity illustrates the significance of the IL-23/Th17 pathway in the perpetuation of IL-12/Th1-mediated immunopathology in PBC. Furthermore, localized IL-23p19 production by hepatocytes may enhance pro-fibrotic Th17 signaling and pro-inflammatory IFN-γ production that contribute to PBC pathology. In conclusion, our data emphasize the pathogenic relevance of IL-12/Th1 and IL-23/Th17 in the evolution of PBC. Of significance, however, the shift from a Th1 to a Th17 imbalance at advanced stages of the disease suggests the necessity to consider modulation of the IL-23/Th17 pathway as a potential target for therapeutic intervention.

Keywords

immunohistochemistry; Th1; IL-12; Th17; IL-23; primary biliary cirrhosis

Recent studies on both patients with and murine models of primary biliary cirrhosis (PBC) have demonstrated that the interleukin (IL)-12/Th1 signaling pathway is one of the central players in etiopathogenesis. For example, IL-12A and IL-12RB2 variants were strongly associated with PBC in two independent genome wide association studies (1, 2). Furthermore, utilizing a PBC-like disease animal model, namely dnTGFbRII mice, IL-12p40 KO-dnTGFbRII mice manifest a dramatic reduction in histological autoimmune cholangitis and significant decreases in levels of intrahepatic pro-inflammatory cytokines (3). Of importance was the finding that the IL-23/Th17 signaling pathway (another member of the IL-12 family) is also associated with the pathogenesis of PBC. Thus, increased frequencies of Th17 lymphocytic infiltrates were documented to be present in liver sections from PBC patients and liver tissues from the IL-2 receptor α knockout mice, another murine model of PBC (4).

Members of the IL-12 family of hetero-dimeric cytokines are pivotal in the initiation and regulation of cell-mediated immunity (5). IL-12 and IL-23, which are mainly produced by antigen presenting cells, are responsible in promoting Th1 and Th17 immune responses, respectively (6–8). Distinction between the IL-12/IL-23 cytokines is complex because the two cytokines share a common p40 chain and they also share a common IL-12RB1 chain in their respective cognate receptors. IL-12 is comprised of two proteins encoded by two separate genes, IL-12A (p35) and IL-12B (p40). IL-12 signals through a hetero-dimeric receptor composed of the IL-12RB2/IL-12RB1 chains (9) and stimulates the growth and function of T cells and natural killer cells, which in turn produce interferon (IFN)-γ and tumor necrosis factor (TNF)-α (10). The cytokine IL-23 on the other hand consists of two subunits, p19 and the shared p40 chain (11) and this cytokine signals through a hetero-dimeric receptor consisting of the IL-23R chain and the shared IL-12RB1 (12). IL-23 mediates the differentiation of Th17 cells from naïve CD4 T cells along with IL-6, IL-17, and transforming growth factor (TGF)-β (13).

The complexities in the IL-12/IL-23 molecules, the corresponding receptors, and their reported roles in the pathogenesis of PBC have prompted us to carefully examine the localized expression of these molecules and receptors utilizing an extensive panel of IL-12/IL-23 specific reagents in liver sections of PBC and for purposes of disease specificity non-PBC liver disease controls. The reagents utilized include antibodies with specificity for IL-12p70, IL-12p35, IL-23p40, IL-23p19, IFN-γ, IL-17, IL-12RB2, and IL-23R. Sections of portal tracts were blindly evaluated for reactivity against each of these antibodies and individually scored. The data obtained demonstrate that liver tissues from a majority of PBC patients were positive for both Th1 and Th17 cytokines at similar sites with comparable average intensity. However, the Th1/Th17 balance skewed toward Th17 in liver tissues from PBC patients at advanced stages. These finding indicates that Th17 signaling increases with disease progression. We hypothesize that while the IL-12/Th1 pathway may play a dominant role in the initiation of the disease process, it is the IL-23/Th17 pathway that plays a dominant role in the subsequent effector stages of the disease. Thus, from a therapeutic standpoint, the targeting of the IL-12/Th1 pathway may not be itself effective and modulation of the IL-23/Th17 pathway may be more judicious. Taken together, our data emphasize the particular role of the IL-23/Th17 pathway in the natural history of PBC and has important therapeutic implications for PBC.

Materials and Methods

Patients

Liver sections from 51 patients with well characterized PBC, including 22 subjects at early stages (Scheuer's stage I and II) and 29 subjects at advanced stages (Scheuer's stage III and IV), were studied. Liver sections from control patients with liver disorders, including 41 subjects with AIH, 26 subjects with HBV, and 13 subjects with HCV were studied in parallel. The clinical data on these patients is summarized in Table 1.

Immunopathology

Liver biopsy samples from patients were fixed in 4% paraformaldehyde for 2 to 3 days. The fixed tissues were embedded in paraffin, cut into 4-μm slices, deparaffinized, and stained with hematoxylin and eosin. The histopathology slides were blindly evaluated by a pathologist using a light microscope. After deparaffinization, sections were soaked in target retrieval–buffered saline (Tris, pH 6.1), in a plastic pressure cooker containing no metals, irradiated in a microwave oven for 10 minutes, and soaked in 3% H_2O_2 methanol solution for 5 minutes. Sections were blocked with 5% bovine serum albumin in Tris-buffered saline for 1 minute and incubated with primary antibodies for more than one hour under intermittent microwave irradiation (14). After washing with Tris-buffered saline containing 1% Tween 20 (TBS-T) for 1 minute, specimens were incubated with peroxidase-conjugated (Envision System, Dako Cytomation, Carpinteria, CA) or alkaline phosphatase–conjugated (Simple Stain System, Nichirei, Japan) secondary antibodies for more than one hour under intermittent microwave irradiation. After washing with TBS-T, sections were immersed in 3,3′-Diaminobenzidine (DAB) (Vector, Burlingame, CA), washed with running water, and counterstained with hematoxylin. In addition to single immunostaining, double immunostaining was performed using select specimens. In brief, all applied antibodies were

denatured in boiling water for more than 10 minutes after the DAB reaction. Specimens were subsequently immunostained using a different set of primary antibodies. After washing with TBS-T, sections were immersed in 3-amino-9-ethylcarbazole (AEC; peroxidase substrate kit, Nichirei), washed with running water, and counterstained with hematoxylin. An enhanced immunohistochemical staining procedure (iAEP method) was used for sensitive detection of cytokine receptor proteins as previously described (15). Optimal concentrations of antibodies used for Th1 and Th17 staining included IL-12p35 (rabbit polyclonal, Novus Biologicals, Littleton, CO), IL-12p70 (mouse/IgG1 monoclonal, R&D Systems, Minneapolis, MN), IL-23p19 (mouse/IgG1 monoclonal, BioLegend, San Diego, CA), IL-23p40 (rabbit polyclonal, Abcam, Cambridge, MA, or goat polyclonal, Thermo Scientific, Pittsburgh, PA), IL-12RB2 (rabbit polyclonal, Sigma-Aldrich, St. Louis, MO), IL-23R (rabbit polyclonal, Millipore, Temecula, CA or LifeSpan Biosciences, Seattle, WA), IFN-γ (goat polyclonal, R&D Systems), and IL-17 (goat polyclonal, R&D Systems). Th1 staining is defined on the basis of sections that stained positive for IL-12p35 and Il-12RB2, whereas Th17 staining is defined on the basis of sections that stained positive for IL-23p19 and IL-23R.

Statistical Analysis

The Th1/Th17 ratio was calculated and averaged for data derived on tissues from each subject. The data were subsequently transformed by taking the binary logarithm (Log_2) of each ratio. Differences of the Th1/Th17 ratio were analyzed by an unpaired 't' test with two-tailed *p*-values. The correlation between Th1/Th17 ratio and disease stage was determined by non-parametric Spearman's correlation coefficient. The level of correlation was interpreted by the non-parametric Spearman correlation coefficient (rs) and ranged between ±1.0. Statistical analysis was performed using Prism software Version 6.0 (Graphpad Software, La Jolla, CA, USA). *P*-values less than 0.05 were considered statistically significant.

Results

Histopathology

All biopsy samples were H&E stained and identifiable multiple portal tracts from each of the sections were blindly evaluated. Scheuer's staging was used throughout. All of the PBC cases demonstrated infiltration of lymphocytes and other mononuclear cells in one or more portal tracts with or without biliary damage (Figure 1). In most cases, diseased and normal portal tracts co-existed in the same specimen (Figure 1). Epitheloid granulomas were also observed in portal tracts and/or hepatic parenchyma in approximately 1/3 of the cases examined (Figure 1).

Immunohistochemistry

A total of 255 portal tracts from 51 PBC, 167 portal tracts from 41 AIH, 108 portal tracts from 26 HBV, and 74 portal tracts from 13 HCV patients were evaluated. IL-12/Th1 cytokines and/or cognate receptors were expressed primarily by the inflammatory cells infiltrating the portal tracts. While these Th1 cytokine positive cells were scattered around interlobular bile ducts in the portal tracts of PBC liver sections, they were to a large extent

located at the periphery of inflamed portal tracts or the area of interface in sections from hepatitis disease controls (Figure 2). Similarly, IL-23/Th17 cytokines and/or cognate receptors were expressed by the inflammatory cells localized to the portal tracts. However, in PBC there was an intense aggregation of such Th17 positive cells around interlobular bile ducts (Figure 3).

Taken together, the inflammatory IL-12/Th1 and IL-23/Th17 cytokines were generally located around the damaged interlobular bile ducts in PBC. Interestingly, the cholangiocytes of patients with PBC also demonstrate elevated expression of IL-12RB2 and IL-23R, suggesting that there is active interaction between Th1/Th17 cells and cholangiocytes in PBC patients.

IL-12/Th1 and IL-23/Th17 signaling was balanced in PBC

Since IL-12/Th1 and IL-23/Th17 staining were both present in PBC and disease control patients, we further examined the intensity of Th1 and Th17 signaling in all the sections. In order to quantify the intensity of Th1/Th17 signaling in each subject, we individually evaluated and scored staining pattern of each portal tract according to the level of positivity of Th1/Th17 immunostaining around the portal tract area. The scoring system was based on five indices: the positivity of Th1/Th17 immunostaining in (1) biliary epithelial cells, (2) lymphocytes, (3) histiocytes (epithelioid cells) or other mononuclear cells, (4) vascular endothelial cells, and (5) hepatocytes and other cells. Th1 and Th17 staining pattern of each portal tract was individually scored as “1” if the staining is positive, whereas the negative staining pattern was scored as “0”.

While multiple portal tracts were evaluated, as expected, the number of portal tract evaluated in each tissue specimen was different due to specimen variation. The Th1/Th17 ratio was calculated and averaged for sections from each patient (Supplementary Tables 1–4). The average of Th1/Th17 ratio in PBC is close to but lower than the zero base line (Figure 4), indicating that the average intensity of Th1/Th17 signaling in PBC patients was balanced but slightly prone to Th17. There was no significant difference in Th1/Th17 ratio between AIH and PBC. However, the Th1/Th17 ratio in HBV was higher than that in PBC ($p < .01$), while the ratio in HCV was lower ($p < .001$). These results suggest that Th1/Th17 staining in HBV was more Th1 predominant while Th1/Th17 staining in HCV was more Th17 predominant.

IL-23/Th17 skewing in advanced PBC patients

Although the average intensity of Th1/Th17 signaling in PBC patients was balanced, we next examined the data on the intensity of Th1/Th17 signaling according to the disease stage. These samples were classified as early (Scheuer’s stage I and II) and advanced (Scheuer’s stage III and IV) PBC patients. In order to visualize the skewed Th1/Th17 balance between early and advanced disease stages, PBC patients were categorized into three groups based on their average Th1/Th17 ratios using a Log_2 scale: (1) Patients with ratios larger than 0.2 were classified as “Th1 predominant”; (2) Patients with ratios between ± 0.2 were classified as “Th1 and Th17 balanced”; (3) Patients with ratios smaller than -0.2 were classified as “Th17 predominant”.

Regardless of the disease stage, patients that were Th17 predominant (37%) were significantly higher than those that were Th1 predominant (18%, $p < .05$), yet the majority of total PBC patients (45%) were Th1 and Th17 balanced (Figure 5A). In early PBC, most of the patients (54%) were still Th1 and Th17 balanced. Interestingly, the percentage of patients who were Th1 predominant was the same (23%) compared with the Th17 predominant group (Figure 5B). In advanced PBC, however, the majority of PBC patients (48%) were Th17 predominant. In contrast, the percentage of patients that were Th1 predominant and Th1 and Th17 balanced decreased to 14% and 38%, respectively. The decreased percentage in both of the groups appeared secondary to the increased percentage in the Th17 predominant group, from 23% in early PBC to 48% in advanced PBC (Figure 5C).

In addition to the imbalance toward Th17 in advanced PBC, there was also a significant negative correlation (Spearman rs = 0.2959) between disease stage and Th1/Th17 ratio ($p < .05$), suggesting that the Th1/Th17 balance skewed toward Th17 when the disease became severe. This result is consistent with our observation that the Th1/Th17 balance shifted to Th17 in advanced PBC. Given that the shift in the Th1/Th17 balance toward Th17 was only noticeable in advanced PBC patients, the elevated percentage of Th17 predominant patients in total PBC could be attributed to the Th1/Th17 imbalance in advanced PBC.

IFN-γ was secreted by degenerated bile ducts

Interestingly we also noted two very unique immunostaining patterns in liver sections from PBC patients. First, the expression of IL-12RB2 and the presence of IFN-γ positive mononuclear cells were both observed around the damaged interlobular bile ducts in PBC patients (Figure 6A). Interestingly, IFN-γ was also present in the biliary lumen of the degenerated bile ducts (Figure 6B), suggesting these biliary epithelial cells were capable of producing inflammatory IFN-γ.

IL-23p19 was extensively produced by inflamed hepatocytes in PBC specifically

The expression of IL-23R and the infiltration of IL-17 positive cells were also detected around biliary epithelial cells especially the degenerated cholangiocytes of interlobular bile ducts in PBC patients (Figure 6C). Notably, IL-23p19 was expressed by the hepatocytes around the inflamed portal tracts in PBC patients, especially in those hepatocytes that were surrounded by infiltrating immune cells. On the other hand, staining for IL-23p19 was less intense in liver sections from patients with AIH, and rarely noted in sections from HBV patients (Figure 7).

Discussion

IL-12 and IL-23 are both pleiotropic cytokines with pro-inflammatory effects that are both implicated as playing a major role in a number of autoimmune diseases (16, 17). However, whether IL-12/IL-23 acts directly on biliary epithelial cells and subsequently mediates the damage of small bile ducts in PBC remains elusive. An understanding of the pathogenic effects of the IL-12/23 signaling axis in the liver could facilitate the development of therapeutic intervention. Our study has several advantages. First, we included a large sample

size of liver tissues from subjects with PBC and non-PBC liver disease for detailed immunohistochemical analysis. Second, we used an extensive panel of specific antibodies for comprehensive analysis of the Th1/Th17 pathway and a modified immunohistochemical procedure to further enhance IL-12/23 receptor staining (15). Third, multiple portal tracts in each of the patients were blindly evaluated and individually scored. Thus, the derived Th1/Th17 ratio is an objective reflection of the liver tissue specific cytokine microenvironment of each donor patient.

In our study, IL-12/Th1 cytokines and the cognate receptors IL-12RB2 were all expressed in the portal tract area in PBC and disease controls. However, the presence of these cytokines and receptors in PBC was primarily around interlobular bile ducts in the portal tracts, while the presence of these cytokines and receptors in disease controls was mostly localized at the periphery of inflamed portal tracts or the area of interface hepatitis. IFN-γ positive mononuclear cells were also found around the degenerated bile ducts, suggesting that IL-12 and IFN-γ are both potentially associated with biliary inflammation and injury in PBC. This finding is consistent with a previous report that highlighted the role of IL-12 mediated IFN-γ and Th1 response in the development of organ-specific autoimmunity (18).

Several susceptibility gene loci for PBC, including IL-12A, IL-12RB2, and STAT4 that are critical in the IL-12/Th1 signaling pathway, were recently identified via genome-wide association studies from three different populations (19). The pathogenic roles of IL-12 and IFN-γ have also been addressed in our murine models of PBC. For example, mice immunized with xenobiotic 2-octynoic acid coupled to bovine serum albumin (2OA-BSA), and transgenic mice with abrogated transforming growth factor-β signaling in T cells (dnTGF-βRII) developed lymphocytic cell infiltration in liver portal tracts with associated bile duct damage that resembles the liver pathology found in human PBC (20, 21). Of note, autoimmune cholangitis induced by 2OA-BSA immunization was ameliorated in IFN-γ−/− and IL-12p35−/− mice (22). While deletion of IFN-γ remarkably reduced inflammatory cell infiltrates in liver and prevents bile ducts from subsequent destruction, the deletion of IL-12p35 only reduced liver infiltrates and biliary damage to a lesser degree. Similarly, deletion of IL-12p35 in dnTGF-βRII could partially suppress disease severity and delayed the onset of liver inflammation (23).

Independent of the IL-12/IFN-γ axis, IL-23 mediated IL-17 and Th17 responses are essential for the induction of autoimmune inflammation (24, 25). Similar to IL-12/Th1 staining patterns, IL-23/Th17 cytokines, the corresponding receptors IL-23R, and effector IL-17 positive cells were also present in each of the liver section examined, including PBC and disease controls, but specifically surrounding interlobular bile ducts in the portal tracts of PBC. The unique localization of these Th17 effector molecules strongly suggests that they are involved in the pathogenesis of PBC. We note that we have also taken advantage of our two well-developed animal models of PBC, 2OA-BSA immunized mice and the dnTGF-βRII mice, to examine the pathogenic role of IL-23/Th17 pathway. Although we demonstrated that 2OA-BSA immunization induced portal inflammation and bile duct damage were ameliorated in IL-23p19$^{-/-}$ and IL-17$^{-/-}$ mice compared with controls (22), the protective effects of IL-23p19/IL-17 deletion were absent in our dnTGF-βRII murine model (26). However, deletion of cytokines that are associated closely with Th17 cells,

including IL-23p40, IL23-p19, IL-17, and IL-6, led to significantly lower serum titer of anti-nuclear antibodies, i.e. anti-gp210, compared to dnTGF-βRII mice (27). These data suggest that IL-23/Th17 cytokines orchestrate anti-gp210 production but may not mediate biliary pathology in this particular murine model of PBC. The IL-23/IL-17 axis has been implicated in the pathogenesis of PBC in other studies; stimulation of IL-17 induces pro-inflammatory cytokines in cultured human biliary epithelial cells (28). Of significance, the expression of IL-6 and IL-1β was specifically enhanced in the bile ducts of PBC and the infiltration of IL-17 positive cells was found scattered around impaired bile ducts according to their immune-histochemical staining, which is consistent with our observation. Rong et al. also demonstrated that both Th17-related cytokines, especially IL-17 and IL-23, and Th17 cell populations were increased in the peripheral blood of patients with PBC compared to disease and healthy controls (29). These data and our current results suggest that the pathogenic effects of the IL-23/IL-17 axis are closely associated with the chronic inflammation of bile ducts in PBC.

Neither blockage of the IL-12/IFN-γ pathway in dnTGF-βRII IL-12p35$^{-/-}$ mice nor abrogation of the IL-23/IL-17 pathway in dnTGF-βRII IL-23p19$^{-/-}$ mice completely abolished autoimmune cholangitis (23, 26). We suggest that IL-12/IFN-γ and IL-23/IL-17 both coordinate the perpetuation of local inflammation in PBC. Of interest, we identified a negative correlation, for the first time, between Th1/Th17 ratio and disease severity of PBC. This novel finding is consistent with a recent study, which reported that the number of IL-23 and IL-17 positive mononuclear cells in portal areas of liver tissues from advanced PBC subjects was significantly higher than that in early PBC (30). Furthermore, the number of IL-17 positive cells correlated with the degree of liver fibrosis that characterizes the advanced stage of PBC (31). Given the fact that the Th1/Th17 balance shifts toward Th17 in advanced PBC patients, we postulate that Th1 plays a key role at the onset of disease, while Th17 is necessary for the perpetuation of ongoing pathology. Furthermore we also detected the expression of IL-23p19 by hepatocytes around the inflamed portal tracts in PBC. Thus, hepatocytes may be involved in bile duct injury through expression of pro-inflammatory cytokines such as IL-23. IL-23 not only mediates Th17 differentiation through the well-defined IL-23/IL-17 axis, but also promotes IFN-γ production through a mostly unknown IL-23/IFN-γ axis (32). It should be noted that cholangiocytes in PBC express IL-12RB2 and IL-23R concurrently. Therefore, the expression of IFN-γ we observed in the biliary lumen of degenerated bile duct cells could not only be attributed to IL-12/Th1 signaling, but also to the expression of IL-23/Th17. This pathogenic role of hepatocytes, to our best knowledge, has never been addressed before. We should note that the current data are largely descriptive and do not provide mechanistic proof regarding the role of IL-12/Th1 and IL-23/Th17 in human PBC. With these comments in mind, the imbalance towards Th17 in advanced PBC could serve as a potential target of immunotherapy for late stage PBC patients. However, to achieve successful therapeutic effects, blockade of IL-12/Th1 and IL-23/Th17 signaling should both be considered.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

PBC	primary biliary cirrhosis
AIH	autoimmune hepatitis
HBV	hepatitis B virus
HCV	hepatitis C virus
H&E	hematoxylin and eosin

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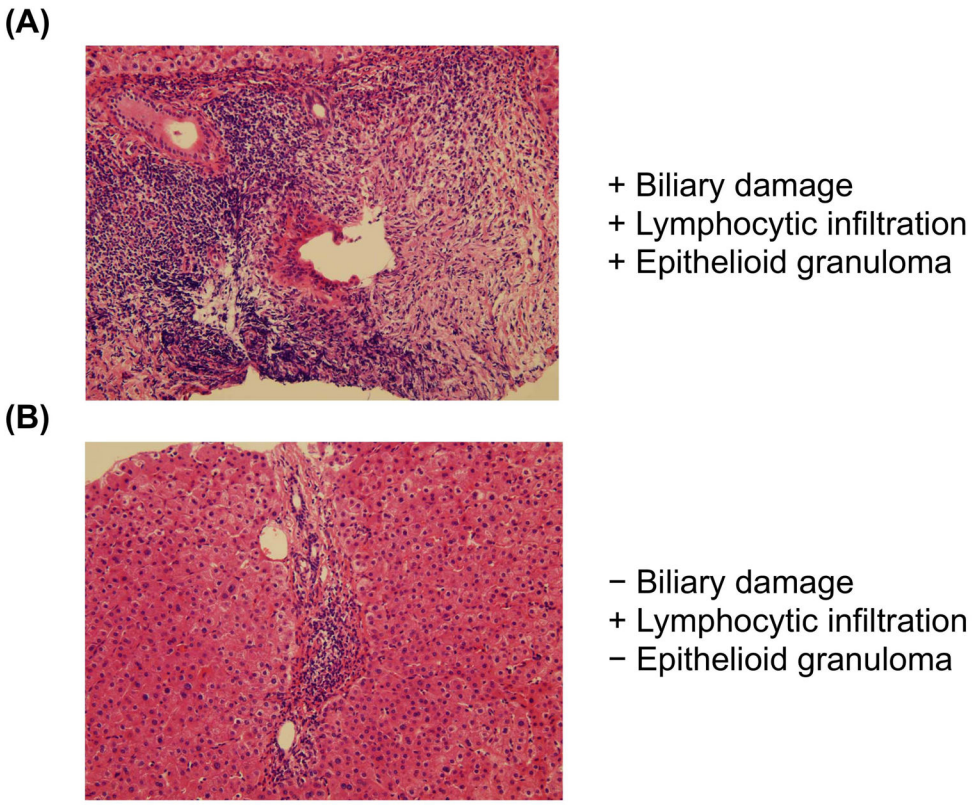

Figure 1.

Liver H&E of two representative portal sections from PBC subjects. Lymphocytic infiltration was observed in all of the PBC subjects. Epithelioid granulomas were also observed near damaged bile duct and/or hepatic parenchyma.

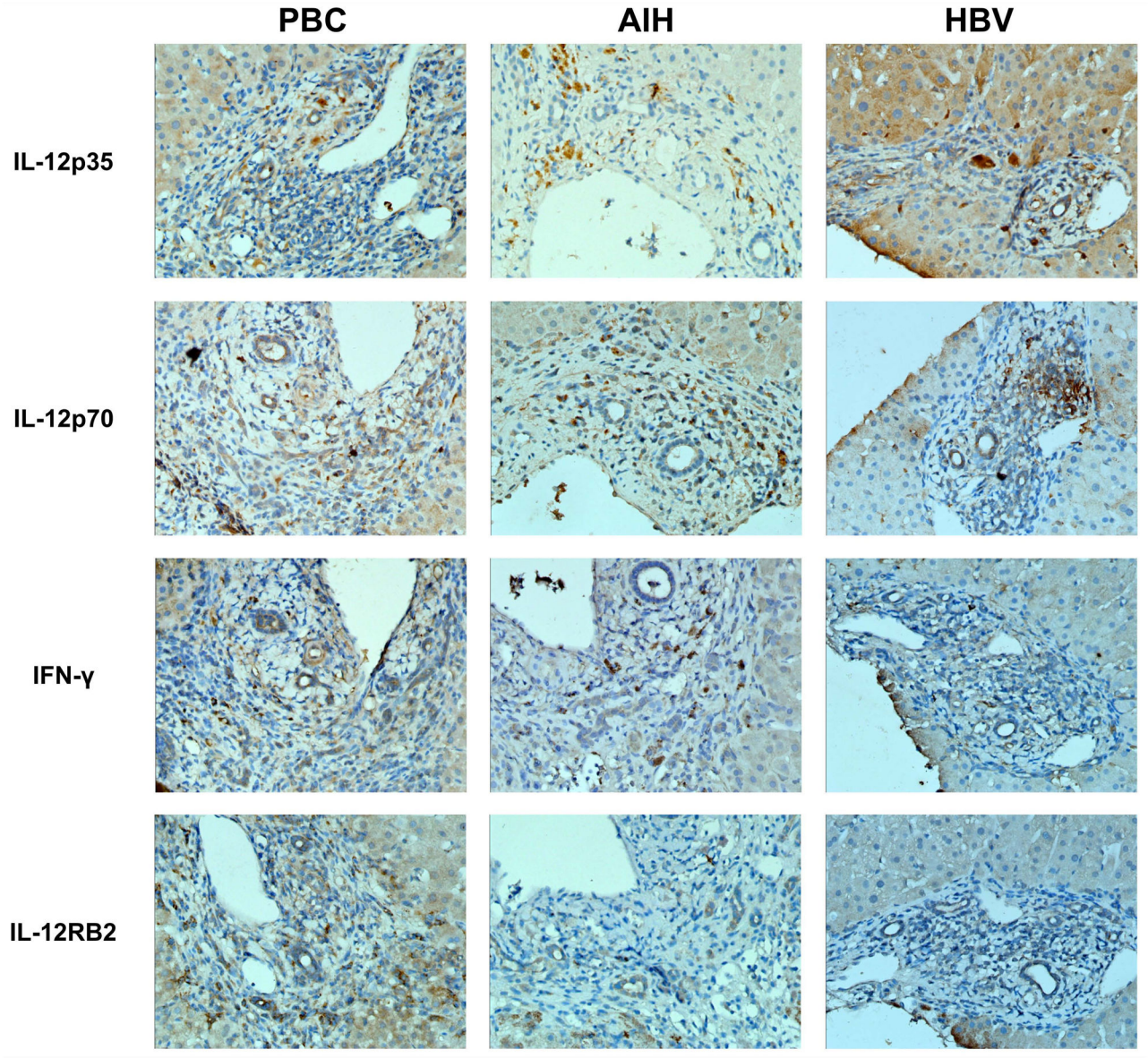

Figure 2.

Liver immunohistochemical staining of Th1 cytokines, including IL-12p35, IL-12p70, IFN-γ, and IL-12 receptor subunit IL-12RB2 in PBC and disease controls. Representative staining images from patients with PBC (n = 51), AIH (n = 41), and HBV (n = 26) are shown.

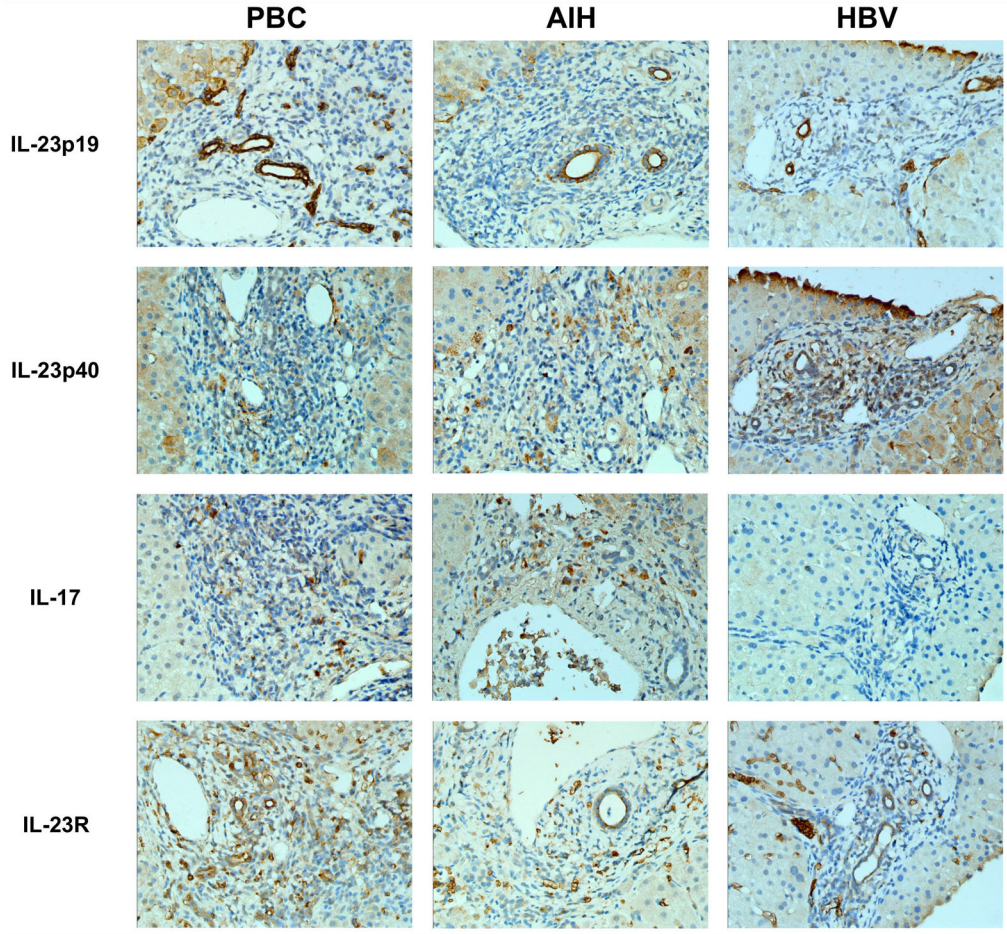

Figure 3.

Liver immunohistochemical staining for Th17 cytokines, including IL-23p19, IL-23p40, IL-17, and IL-23 receptor subunit IL-23R in PBC and disease controls. Representative staining from patients with PBC (n = 51), AIH (n = 41), and HBV (n = 26) are shown.

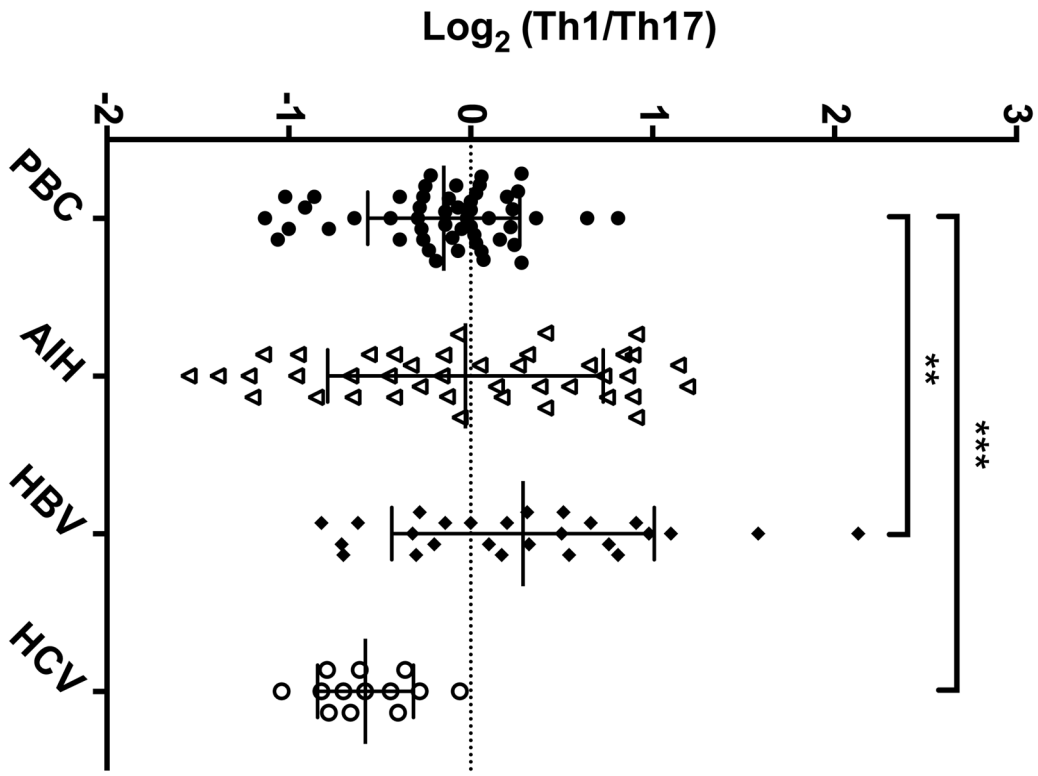

Figure 4.

The Th1/Th17 ratio in PBC (n =51), AIH (n = 41), HBV (n = 26), and HCV (n = 13). The Th1/Th17 ratio is very close to the base line zero in PBC and AIH, while the Th1/Th17 ratio is higher than zero in HBV and lower than zero in HCV. The Th1/Th17 ratio in HBV is higher than that in PBC (**$p < .01$), while the ratio in HCV is lower than that in PBC (***$p < .001$).

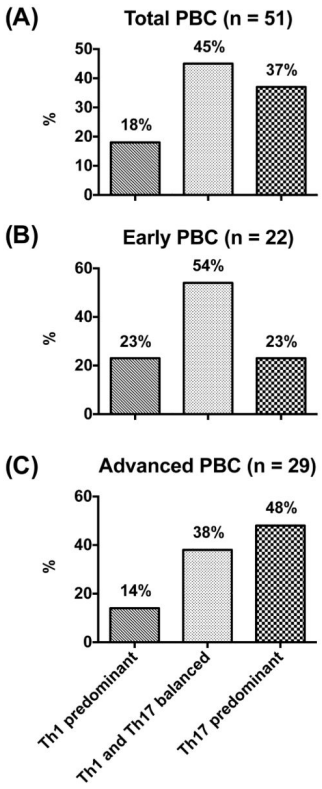

Figure 5.

The distribution of the Th1/Th17 ratio in (A) total PBC (n = 51), (b) early PBC (n = 22), and (C) advanced PBC (n = 29). The intensity of Th1/Th17 staining in the majority of total and early PBC patients was Th1 and Th17 balanced. However, the intensity skewed from Th1 to Th17 predominantly in advanced PBC. The Th1/Th17 ratio was negatively correlated (Spearman rs = 0.2959) with disease stage in PBC ($p < .05$).

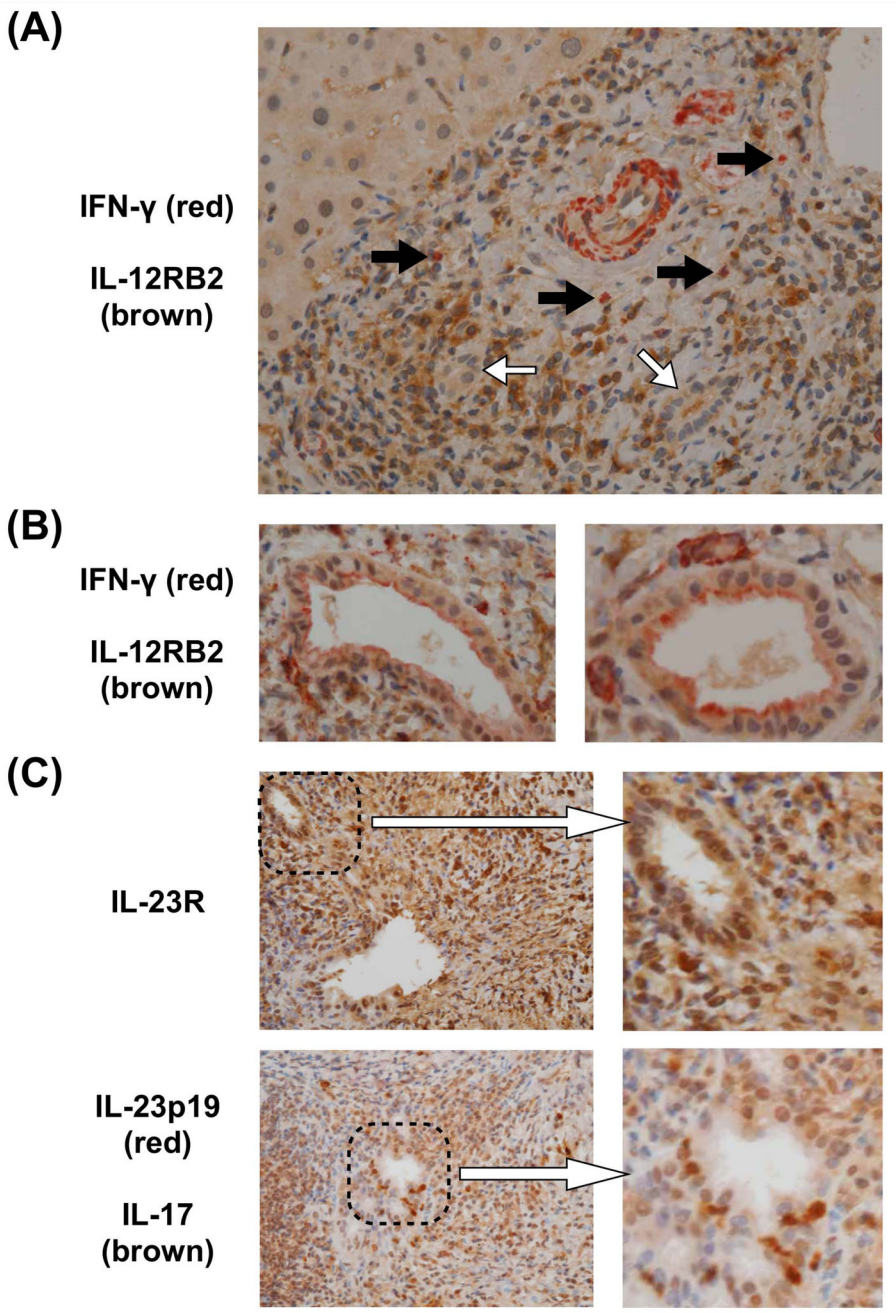

Figure 6.

(A) The expression of IL-12RB2 and the presence of IFN-γ positive mononuclear cells (black arrows) around damaged bile ducts (white arrows) in PBC. (B) The expression of IFN-γ in the biliary lumen of degenerated biliary epithelial cells in PBC. (C) The expression of IL-23R and the infiltration of IL-17 positive cells around damaged biliary epithelial cells in PBC. Liver sections were stained with anti-IL-23R individually, as well as anti-IL-23p19 (red) combined with anti-IL-17 (brown) or IFN-γ (red) combined with IL-12RB2 (brown) concurrently. Representative staining images from patients with PBC (n = 51) are shown.

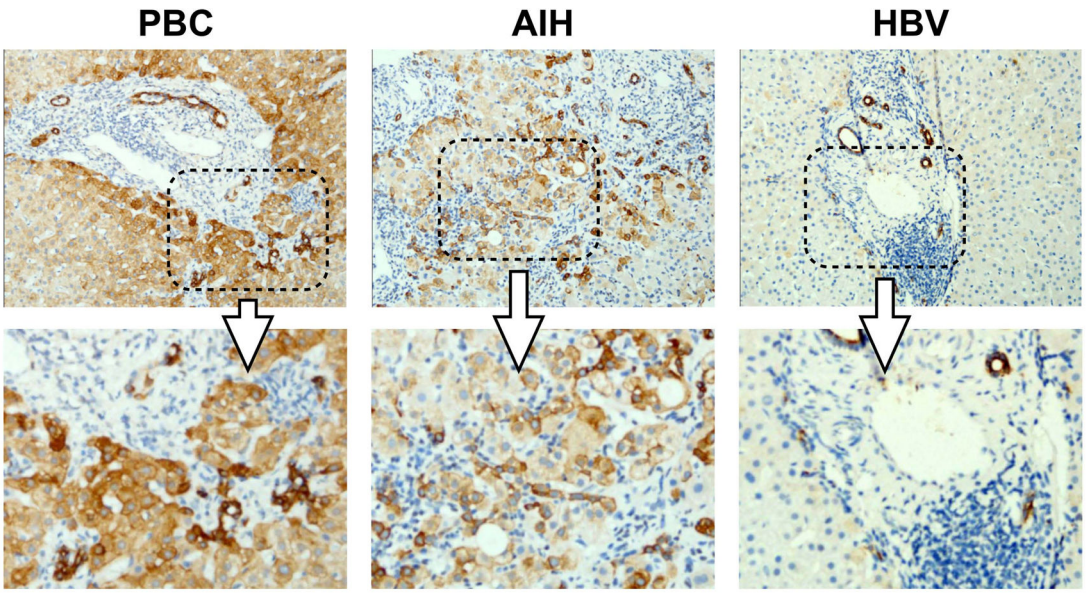

Figure 7.

Liver immunohistochemical staining for IL-23p19 in PBC and disease controls. Representative staining images from patients with PBC (n = 51), AIH (n = 41), and HBV (n = 26) are shown. The cholangiocytes, oval cells, and small hepatocytes are strongly positive especially in PBC.

Table 1

Clinical data of PBC, AIH, HBV, and HCV patients.

		PBC	AIH	HBV	HCV
Sex	Male	12	7	18	2
	Female	39	34	8	11
Age at Study	≤50	17	22	21	2
	51–60	20	14	3	4
	61–70	13	5	2	4
	>70	1	0	0	3
Clinical Stage	0	--	5	5	--
	1	15	6	6	7
	2	7	10	1	5
	3	18	15	6	1
	4	11	5	8	--
Total		51	41	26	13
Number of Portal Tracts Evaluated		255	167	108	74