Interleukin-33 Promotes Disease Progression in Patients with Primary Biliary Cirrhosis

Yongqiang Sun,^{1,2} Ji-Yuan Zhang,³ Sa Lv,³ Huan Wang,⁴ Man Gong,² Ning Du,² Honghong Liu,² Ning Zhang,² Jing Jing,² Chao Zhou,² Fan Zhang² and Zongren Wang¹

¹Department of Traditional Chinese Medicine, Xijing Hospital, Fourth Military Medical University, Xi'an, Shanxi Province, P.R. China

²Integrative Medical Center Liver Diseases, Beijing 302 Hospital, Beijing, P.R. China

³Research Center for Biological Therapy, Beijing 302 Hospital, Beijing, P.R. China

⁴Department of Dermatology, Tangdu Hospital, Fourth Military Medical University, Xi'an, Shanxi Province, P.R. China

Primary biliary cirrhosis (PBC) is a progressive autoimmune liver disease that can cause a series of complications, including cirrhosis, liver failure and hepatocellular carcinoma. Interleukin-33 (IL-33) is expressed in various non-hematopoietic cells and a certain population of immune cells, and exerts its biological effects by binding to the specific receptor, suppression of tumorigenicity 2 (ST2). A soluble form of ST2 (sST2) has been postulated to act as a decoy receptor for IL-33. In this study, we aimed to investigate the role of IL-33 in the pathogenesis of PBC. The study included 20 healthy controls and 68 patients with PBC. We thus found the increased serum IL-33 levels in PBC patients. Its elevated levels were positively correlated with serum alkaline phosphatase levels (a key parameter for the definition of PBC) and with Child-Pugh scores, which were used to determine the prognosis of liver cirrhosis. Moreover, the serum concentrations of sST2 were significantly higher in PBC patients compared with healthy subjects, irrespective of the disease severity. Importantly, the cells that express IL-33 and/or myeloperoxidase (a marker for neutrophils) were accumulated in the livers of PBC patients, and their number increased with the severity of liver lesions. Lastly, in vitro chemotaxis assays revealed that IL-33 enhanced the migration of neutrophils. These data suggest that IL-33 may affect the progress of PBC by recruiting neutrophils to the liver. This expanded knowledge of IL-33 in PBC patients is important for developing therapeutic strategies (e.g., neutralization of IL-33), selecting optimal clinical management, and predicting prognosis.

Keywords: disease progression; immune pathogenesis; interleukin-33; liver lesion; primary biliary cirrhosis Tohoku J. Exp. Med., 2014 December, **234** (4), 255-261. © 2014 Tohoku University Medical Press

Introduction

Primary biliary cirrhosis (PBC) is a progressive autoimmune liver disease that causes substantial loss of intrahepatic bile ducts and subsequently, cholestasis, advanced fibrosis, cirrhosis, liver failure, and hepatocellular carcinoma (Lindor et al. 2009; Selmi et al. 2011). A widely accepted notion is that PBC arises as a consequence of combinations of genetic predisposition and environmental mutagens. Collective evidence nevertheless has shown that immune cells, such as autoreactive pyruvate-dehydrogenase-complex-E2-specific CD4 or CD8 T cells, are clearly associated with its pathology or etiology (Shimoda et al. 1995; Kita et al. 2002; Takahashi et al. 2012). Currently, ursodeoxycholic acid is the only drug that has been specifically approved for the treatment of PBC. However, 40% of patients have incomplete responsiveness to ursodeoxycholic acid therapy (Leuschner et al. 2000), and this fact emphasizes the need to identify immunopathogenesis of PBC and to develop new therapeutic strategies.

Interleukin (IL)-33 is a cytokine belonging to the IL-1 family that was described in 2005 (Schmitz et al. 2005). It is expressed in various nonhematopoietic cells (e.g., endo-thelial cells, fibroblasts, bronchial and epithelial cells) and immune cells (e.g., macrophages and dendritic cells) (Kakkar and Lee 2008). IL-33 exerts its biological effects by binding to its specific primary receptor, suppression of tumorigenicity 2 (ST2), and the coreceptor, IL-1 receptor accessory protein. Soluble ST2 (sST2) has been postulated to act as a decoy receptor for IL-33, thereby attenuating its

Received August 13, 2014; revised and accepted October 28, 2014. Published online November 14, 2014; doi: 10.1620/tjem.234.255. Correspondence: Zongren Wang, Department of Traditional Chinese Medicine, Xijing Hospital, Fourth Military Medical University, Xi'an, Shanxi Province 710032, P.R. China.

e-mail: yanfengyun2011@126.com

Subjects

biological activity (Jovanovic et al. 2012; Milovanovic et al. 2012). The role of the IL-33/ST2 axis has been extensively studied in Th2-driven chronic inflammatory diseases, such as asthma, rheumatoid arthritis, inflammatory bowel disease and allergic rhinitis (Milovanovic et al. 2012). In the field of hepatology, IL-33 has been identified as an important factor contributing to liver injury and was previously shown in *in vitro* models to be induced by chronic hepatitis B and C, and fibrosis (Wang et al. 2012a, b).

As yet, it is still unclear whether IL-33 is associated with the severity of disease progression and liver lesions in PBC patients. The primary aim of this study was to analyze the characteristics of IL-33 in the serum and livers of PBC patients. The secondary aim was to examine the possible pathogenic mechanisms by which IL-33 may act.

Methods

Sixty-eight treatment-naive patients with PBC and twenty healthy controls (HC) were enrolled in this study. These patients were diagnosed with PBC based on the presence of an anti-mitochondrial antibody titer > 1:40, and serum alkaline phosphatase (ALP) at least twice the upper limit of normal in the absence of biliary obstruction, which was in accordance with the American Association for the Study of Liver Diseases practice guidelines (Lindor et al. 2009). The Child-Pugh scores used to determine the prognoses of liver cirrhosis were calculated at the time of enrollment and higher scores indicated poor prognosis (Pugh et al. 1973). Paraffin-embedded liver tissues were obtained from ultrasound-guided needle liver biopsies from 16 patients who had approved the use of excess tissue from biopsies. Healthy liver tissues were collected from 6 healthy donors whose livers were subsequently used for liver transplantation. Blood samples were taken from seven healthy subjects for isolation of neutrophils. The liver lesions grading systems (Scheuer 1973) was used to describe the pathologic features of chronic nonsuppurative destructive cholangitis. Briefly, stage I-inflammatory destruction of interlobular bile ducts; stage II-proliferation of ductules; stage III-fibrosis; and stage IV-cirrhosis (Scheuer 1973). Patients with concurrent hepatitis B virus, hepatitis C virus or human immunodeficiency virus infection, or alcoholic liver disease were excluded. The Institutional Committee for Human Research of Beijing 302 Hospital approved this study and written informed consent was obtained from each subject. The patients' baseline clinical data are shown in Table 1.

Immunohistochemical staining

Anti-IL-33 antibody (ab118503) and anti-myeloperoxidase (MPO) antibody (ab45977) were purchased from Abcam (Cambridge, UK) and used for immunohistochemical staining. Paraffin-embedded, formalin-fixed liver tissue sections (5 μ m) were incubated with anti-IL-33 antibody or anti-MPO antibody overnight at 4°C after blocking endogenous peroxidase activity with 0.3% H₂O₂. 3-Amino-9-ethylcarbazole (red color) was used as the substrate, followed by counterstaining with hematoxylin, according to previously described protocols (Zhang et al. 2010). The percentage of IL-33 positive cells was analyzed semi-quantitatively based on the red-positive area under lower-power fields (200×) with the software, IPP6.0. As shown in Table 2, the quantitative scores of IL-33 in liver tissues were calculated by adding the score of positive areas with the score of intensity of positive areas. For counting MPO positive cells, high-power fields (400×) were used and the numbers of MPO positive cells were counted in three different areas by two independent investigators.

IL-33 mRNA quantification

Liver tissue RNA was extracted using the RNeasy Mini kit (Qiagen) according to the manufacturer's instructions. The RNA was reverse-transcribed to complementary DNA using oligo (dT) primers at 42°C for 30 min and at 95°C for 5 min. Quantitation of the IL-33 mRNA was determined by staining with the fluorogenic dye SYBR Green as previously described (Marvie et al. 2010). Each measurement was performed in duplicate. The primers used for IL-33 were as follows: forward 5'-aatcaggtgacggtgttg-3', reverse 5'-acactcaggat cagtcttg-3'. Beta-actin was used to normalize the samples in each PCR reaction. The primers used for beta-actin were as follows: for-

Table 1. Clinical characteristics of the participants enrolled in the study.

	Healthy control (20 cases)	PBC (68 cases)	Child A (21 cases)	Child B (29 cases)	Child C (18 cases)
NLR	1.33 ± 0.15	2.35 ± 0.30	1.86 ± 0.24	1.93 ± 0.17	2.88 ± 0.33
ALB (g/l)	39.27 ± 0.89	31.97 ± 0.73	35.60 ± 0.73	27.68 ± 0.84	25.80 ± 2.08
Age	49.80 ± 1.78	54.01 ± 1.28	50.29 ± 1.48	58.27 ± 2.00	61.00 ± 5.82
AST/ALT	1.47 ± 0.24	1.93 ± 0.13	1.65 ± 0.12	2.37 ± 0.28	1.85 ± 0.23
ALP (U/L)	128.67 ± 20.89	283.21 ± 28.69	300.47 ± 39.03	280.92 ± 49.99	163.40 ± 36.39
GGT (U/L)	28.53 ± 3.09	200.56 ± 27.64	249.68 ± 44.32	155.08 ± 27.87	54.60 ± 10.27
TBIL (µmol/l)	14.33 ± 3.78	52.73 ± 9.70	21.45 ± 3.47	61.41 ± 9.77	246.99 ± 79.57
CHE (U/L)	9,342.56 ± 389.90	$4,277.43 \pm 245.43$	$5,529.58 \pm 249.94$	2,957.16 ± 259.83	$1,\!362.40\pm150.74$
IgA (g/l)	2.04 ± 0.69	2.98 ± 0.22	2.36 ± 0.18	3.68 ± 0.42	5.02 ± 1.19
IgG (g/l)	13.08 ± 0.98	19.98 ± 0.84	19.03 ± 1.13	20.91 ± 1.43	23.55 ± 1.42
IgM (g/l)	2.21 ± 0.36	3.30 ± 0.30	3.44 ± 0.46	3.20 ± 0.37	2.61 ± 0.75
γPro (%)	15.30 ± 0.65	25.87 ± 1.08	22.57 ± 1.20	29.87 ± 1.68	30.03 ± 4.94

NLR, neutrophil to lymphocyte ratio; ALB, albumin; ALT, alanine aminotransferases; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, r-glutamyltransferase; TBIL, total bilirubin; CHE, cholinesterase; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; γPro, γ-Protein.

Tuble 2. Quantuative scoring of the 55 minutubilistoenemistry.							
Score	0	1	2	3			
Percentage of positive areas	0	< 5%	5-20%	> 20%			
Score	1	2		3			
Intensity of positive areas	Weak	Moderate		Strong			

Table 2. Quantitative scoring of IL-33 immunohistochemistry.

Total quantitative scores of IL-33 in liver tissues were calculated by adding the score of the percentage of positive areas to the score of the intensity of positive areas.



Fig. 1. Serum concentrations of IL-33.

(A) Pooled data from the serum of IL-33 in 20 healthy controls and 68 treatment-naive PBC patients relative to Child-Pugh scores. (B) Pooled data from the serum ST2 in 20 healthy controls and 68 treatment-naive PBC patients relative to Child-Pugh scores. (C-F) Correlations between IL-33 levels and ALP, ALT, AST, or GGT in 68 PBC patients. One dot represents one individual. **P < 0.001. ALP, alkaline phosphatase; ALT, alanine aminotransferases; AST, aspartate aminotransferase.

ward 5'-cactcttccagccttcctt-3', reverse 5'-tgtgttggcgtacaggtct-3'. RNA samples from 5 healthy individuals were used in each RT-PCR as quality controls.

Chemotaxis

Blood from healthy subjects was lysed with fluorescenceactivated cell sorting lysing solution (BD Pharmingen) and neutrophils were further purified using easySepTM human neutrophil enrichment kit following the manufacturer's recommendations (Stem Cell Technologies). Cells were loaded in the upper chamber with a 5 μ m pore size semi-permeable polycarbonate membrane. Medium with serum from HCs and PBC patients, or in the presence of IL-33 (1 ng/ ml) were loaded in the lower chambers. After incubation for 5 h at 37°C, the total number of neutrophils in the lower chamber was counted using Vi-CELL. Each condition was performed in duplicate. The results were expressed as index of migration compared to the negative control.

Enzyme-linked immunosorbent serologic assay (ELISA)

The concentrations of serum IL-33 and sST2 in PBC patients and HCs were determined by ELISA using human IL-33 and sST2

ELISA kits, according to the manufacturer's instructions (Roche Diagnostics). Briefly, individual serum samples at 1:4 dilutions were subjected to ELISA analysis, and the concentrations of serum IL-33 in individual samples were calculated according to the standard curve established using the recombinant IL-33 and sST2. The detection limits of IL-33 and sST2 ELISA kit were 0-16 ng/L and 1.6 ng/L, respectively.

Statistical analysis

Data analysis was performed with SPSS 16.0 software for Windows (SPSS Inc. Chicago, IL, USA). Multiple comparisons were done between the various groups using the Kruskal-Wallis H nonparametric test and the Mann-Whitney U test. Comparisons within groups were performed with the Wilcoxon matched-pair *t*-test. Correlations between variables were evaluated with the Spearman rank correlation test. For all tests, a two-sided P value < 0.05 was considered significant.

Results

PBC patients show increased serum concentrations of IL-33 As shown in Fig. 1A, the serum concentrations of IL-33 were significantly higher in patients at Child A, B, and C (57.24 ± 6.55 , 74.93 ± 6.56 and 75.61 ± 7.95 ; for Child A, Child B and Child C patients, respectively) when compared with HC (32.24 ± 8.37). In addition, the levels of IL-33 in Child B and C patients were increased compared to Child A patients, whereas there was no significant difference between Child B and C patients.

Serum sST2 has been assumed to act as a decoy receptor for IL-33, thereby attenuating its biologic activity. Therefore, we measured the serum concentrations of sST2. Fig. 1B shows that the sST2 concentrations were significantly higher in patients compared with HC subjects (144.94 \pm 224.60). However, the sST2 concentration was similar in PBC patients irrespective of the severity of liver disease (1,343.84 \pm 583.28, 1,438.31 \pm 485.42, and 1,439.37 \pm 503.73 for Child A, Child B and Child C, respectively).

Fig. 1C shows that the serum concentrations of IL-33 were significantly correlated with the levels of ALP (R = 0.421, P < 0.001). No noteworthy correlations were observed between the concentrations of IL-33 and ALT, AST, and GGT levels (Fig. 1D-F).

Increased quantitative scores of IL-33 are markedly associated with the severity of liver lesions

We examined the distribution of IL-33 positive cells in the liver of healthy individuals and PBC patients. As shown in Fig. 2A (left), a small number of IL-33 positive cells were observed in the liver of healthy individuals. Interestingly, increased numbers of IL-33 positive cells were found not only accumulated in the portal areas but also in the lobules of the liver in PBC patients (Fig. 2A middle and right). Most importantly, the quantitative scores of IL-33 in liver tissues were distributed differentially in patients with different degree of liver lesions. Specifically, significantly higher quantitative scores of IL-33 were observed in the liver tissues from stage III or IV patients compared to stage I or II patients (Fig. 2B).

To further investigate the relationship between IL-33 and degree of liver lesions, we quantified the mRNA levels of IL-33 in liver biopsies from patients using real-time qPCR. As showed in Fig. 2C, IL-33 mRNA levels were significantly higher in patients with advanced liver lesions than control liver or livers from patients with less advanced lesions.

The presence of neutrophils increases with advancement of liver lesions and are associated with IL-33 levels in patients

We examined the distribution of MPO positive cells in the livers of healthy individuals and patients. As shown in Fig. 3A (left), there were a small number of MPO positive cells. Interestingly, increased numbers of MPO positive cells were found accumulated in the portal and lobular areas in the livers of patients (Fig. 3A middle and right). We also found that MPO3 positive cells were differentially distributed in the livers of PBC patients. Specifically, significantly more MPO positive cells were observed to have infiltrated



Fig. 2. Quantitative scores of IL-33 in the livers of patients with PBC. (A) Immunohistochemical staining for IL-33 in the livers of 6 healthy controls (magnification, 200×) and 16 PBC patients (magnification, 200×). Positive cells and areas are stained red. (B) Quantitative scores for IL-33 in the livers of 6 healthy controls and 16 PBC patients with various liver lesions. The methodology for obtaining the quantitative scores of IL-33 is provided in Table 2. One dot represents one individual. Horizontal bars represent the median quantitative scores for IL-33. **P < 0.001. (C) IL-33 mRNA levels in 5 healthy controls and 12 PBC patients, these 12 patients were divided into stage I (n = 2), stage II (n = 4), stage III (n = 3) and stage IV (n = 3), respectively. **P < 0.001.



Fig. 3. Neutrophils in the livers of patients with PBC.
(A) Immunohistochemical staining for MPO positive cells in livers of HC (magnification, 400×) and PBC patients (magnification, 400×). Positive cells are stained red. (B) Numbers of MPO positive cells in the livers of 6 HC and 16 PBC patients of various histology stages. One dot represents one individual. Horizontal bars represent the median numbers of MPO positive cells. **P < 0.001. (C) Correlations between numbers of MPO positive cells and quantitative scores of IL-33. One dot represents one individual.





(A) Effects of serum samples from PBC patients with or without IL-33 on the chemotaxis of neutrophils. (B) The data are representative of seven independently performed experiments with serum samples from seven healthy subjects and serum samples from four PBC patients. **P < 0.001.

the portal and lobular areas of stage III or IV patients compared to stage I or II patients (Fig. 3B). In addition, as shown in Fig. 3C, the numbers of MPO positive cells in livers were closely correlated with the quantitative scores of IL-33 positive cells.

Increased IL-33 enhances chemotaxis of neutrophils

To define the effect of IL-33 on neutrophils, chemotaxis assays were performed, as shown in Fig. 4A, B. Serum from patients induced more neutrophils to migrate compared to serum from HC. When IL-33 was added to the serum from PBC patients, the neutrophil chemotaxis index was further increased compared to neutrophils cultured in serum from PBC patients (Fig. 4B).

Discussion

PBC is characterized by a number of well-defined abnormalities in immune dysfunction. Firstly, there is a

multi-lineage loss of tolerance to pyruvate-dehydrogenasecomplex E2, including the presence of autoreactive CD4 and CD8 cells and a highly directed B cell response (Shimoda et al. 1995; Kita et al. 2002). There is also evidence that the pathogenesis of the disease may involve different cytokines at different stages of disease activity (Takahashi et al. 2012). Despite these accumulating data, the effects of IL-33 in patients with PBC remain unknown. It is therefore of clinical importance to investigate the characteristics of IL-33 in PBC patients. We found that serum IL-33 levels were increased in PBC patients. The quantitative scores of IL-33 and neutrophils increased in the livers of PBC patients, and increased with severity of liver lesions. We also noted that increased quantitative scores of IL-33 in liver tissues were associated with neutrophil infiltration. In vitro, the administration of IL-33 increased the chemotaxis index of neutrophils. These data extended the knowledge about the properties of IL-33 from a mouse fibrosis model to human fibrosis and cirrhosis.

IL-33 is a cytokine involved in type 2 immunity and inflammatory responses, and has major effects on innate and adaptive cells, including innate lymphoid cell-2, T helper 2 cells, and alternatively activated M2 polarized macrophages. Recently, an increasing body of literature has shown that IL-33 is related to the pathogenic mechanisms of fibrosis (Marvie et al. 2010; Volarevic et al. 2012; McHedlidze et al. 2013). For example, IL-33 expression correlates with ST2 expression, and with collagen expression in fibrotic livers in mice and humans (Marvie et al. 2010). IL-33-dependent innate lymphoid cells have also been reported to mediate hepatic fibrosis. However, very little is known about the characteristics of IL-33 in the pathogenesis of PBC. In view of this, we, for the first time, characterized IL-33 in a cohort of patients with PBC. The present study indicates that the overexpression of IL-33 may represent a potential mechanism leading to disease progression of PBC. There are four aspects of evidence to support this notion. First, the increased serum IL-33 levels in PBC patients were positively correlated with serum ALP levels, which is a key parameter for the definition of PBC. In addition, the serum concentrations of IL-33 increased with Child-Pugh scores, which were used to determine the prognosis of liver cirrhosis. Third, sST2, which could attenuate the biologic activity of IL-33, did not increase with Child-Pugh scores. Lastly and most importantly, we found that the quantitative scores for IL-33 and IL-33 mRNA levels were positively correlated with advanced histology stages. For example, stage III liver lesions are characterized by a distortion of hepatic architecture with numerous fibrous septa and stage IV is characterized by liver cirrhosis with the existence of regenerative nodules. Taken together, these results indicate that the overexpression of IL-33 is closely associated with disease progression in patients with PBC. Notably, although the quantitative scores for IL-33 were increased in livers of PBC patients, we cannot definitely claim that this improvement is related to a type of lymphocyte or hematopoietic cell. It is of interest to identify the cell types responsible for the IL-33 production.

The present observations related to neutrophils are consistent with previous literature on neutrophil biology in a mouse model of cholestatic liver damage (Gujral et al. 2003; Sitia et al. 2004). These reports indicated that accumulated neutrophils in liver were significantly associated with PBC progression. In addition, IL-33 has been shown to induce neutrophil migration to the inflammatory tissues (Alves-Filho et al. 2010; Verri et al. 2010; Hueber et al. 2011), a finding consistent with our current data which suggested that serum from PBC patients induced more neutrophils to migrate compared to HC. These results indicated that the overexpression of IL-33 in the liver, at least in part, contributes to the accumulation of neutrophils. Future studies are necessary to investigate the mechanisms that underlie neutrophil-induced liver damage and fibrosis in PBC patients.

Taken together, our data demonstrated, for the first time, that IL-33 may participate in the modulation of PBC progression. These findings extend the knowledge about the properties of IL-33 from the previous mouse fibrosis model to patients with PBC and suggest that the neutralization of IL-33 could be a potential therapeutic strategy for PBC treatment.

Acknowledgments

This study is supported by the grant of Beijing Nova Program of China (Z121107002512071) and the National Natural Science Foundation of China (No. 81302593 and No. 81271848).

Conflict of Interest

The authors declare no conflict of interest.

References

- Alves-Filho, J.C., Sônego, F., Souto, F.O., Freitas, A., Verri, W.A. Jr., Auxiliadora-Martins, M., Basile-Filho, A., McKenzie, A.N., Xu, D., Cunha, F.Q. & Liew, F.Y. (2010) Interleukin-33 attenuates sepsis by enhancing neutrophil influx to the site of infection. *Nat. Med.*, 16, 708-712.
- Gujral, J.S., Farhood, A., Bajt, M.L. & Jaeschke, H. (2003) Neutrophils aggravate acute liver injury during obstructive cholestasis in bile duct-ligated mice. *Hepatology*, 38, 355-363.
- Hueber, A.J., Alves-Filho, J.C., Asquith, D.L., Michels, C., Millar, N.L., Reilly, J.H., Graham, G.J., Liew, F.Y., Miller, A.M. & McInnes, I.B. (2011) IL-33 induces skin inflammation with mast cell and neutrophil activation. *Eur. J. Immunol.*, 41, 2229-2237.
- Jovanovic, I.P., Pejnovic, N.N., Radosavljevic, G.D., Arsenijevic, N.N. & Lukic, M.L. (2012) IL-33/ST2 axis in innate and acquired immunity to tumors. *Oncoimmunology*, 1, 229-231.
- Kakkar, R. & Lee, R.T. (2008) The IL-33/ST2 pathway: therapeutic target and novel biomarker. *Nat. Rev. Drug Discov.*, 7, 827-840.
- Kita, H, Lian, Z.X., Van de Water, J., He, X.S., Matsumura, S., Kaplan, M., Luketic, V., Coppel, R.L., Ansari, A.A. & Gershwin, M.E. (2002) Identification of HLA-A2-restricted CD8(+) cytotoxic T cell responses in primary biliary cirrhosis:

T cell activation is augmented by immune complexes crosspresented by dendritic cells. J. Exp. Med., **195**, 113-123.

- Leuschner, M., Dietrich, C.F., You, T., Seidl, C., Raedle, J., Herrmann, G., Ackermann, H. & Leuschner, U. (2000) Characterisation of patients with primary biliary cirrhosis responding to long term ursodeoxycholic acid treatment. *Gut*, 46, 121-126.
- Lindor, K.D., Gershwin, M.E., Poupon, R., Kaplan, M., Bergasa, N.V. & Heathcote, E.J.; American Assosiation for Study of Liver Diseases (2009) Primary biliary cirrhosis. *Hepatology*, 50, 291-308.
- Marvie, P., Lisbonne, M., L'helgoualc'h, A., Rauch, M., Turlin, B., Preisser, L., Bourd-Boittin, K., Théret, N., Gascan, H., Piquet-Pellorce, C. & Samson, M. (2010) Interleukin-33 overexpression is associated with liver fibrosis in mice and humans. J. Cell. Mol. Med., 14, 1726-1739.
- McHedlidze, T., Waldner, M., Zopf, S., Walker, J., Rankin, A.L., Schuchmann, M., Voehringer, D., McKenzie, A.N., Neurath, M.F., Pflanz, S. & Wirtz, S. (2013) Interleukin-33-dependent innate lymphoid cells mediate hepatic fibrosis. *Immunity*, 39, 357-371.
- Milovanovic, M., Volarevic, V., Radosavljevic, G., Jovanovic, I., Pejnovic, N., Arsenijevic, N. & Lukic, M.L. (2012) IL-33/ ST2 axis in inflammation and immunopathology. *Immunol. Res.*, **52**, 89-99.
- Pugh, R.N., Murray-Lyon, I.M., Dawson, J.L., Pietroni, M.C. & Williams, R. (1973) Transection of the oesophagus for bleeding oesophageal varices. *Br. J. Surg.*, 60, 646-649.
- Scheuer, P.J. (1973) Liver Biopsy Interpretation, 2nd ed., Williams and Wilkins, Baltimore, MD, pp. 33-38.
- Schmitz, J., Owyang, A., Oldham, E., Song, Y., Murphy, E., McClanahan, T.K., Zurawski, G., Moshrefi, M., Qin, J., Li, X., Gorman, D.M., Bazan, J.F. & Kastelein, R.A. (2005) IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity*, 23, 479-490.
- Selmi, C., Bowlus, C.L., Gershwin, M.E. & Coppel, R.L. (2011) Primary biliary cirrhosis. *Lancet*, 377, 1600-1609.

- Shimoda, S., Nakamura, M., Ishibashi, H., Hayashida, K. & Niho, Y. (1995) 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., 181, 1835-1845.
- Sitia, G., Isogawa, M., Iannacone, M., Campbell, I.L., Chisari, F.V. & Guidotti, L.G. (2004) MMPs are required for recruitment of antigen-nonspecific mononuclear cells into the liver by CTLs. J. Clin. Invest., 113,1158-1167.
- Takahashi, T., Miura, T., Nakamura, J., Yamada, S., Miura, T., Yanagi, M., Matsuda, Y., Usuda, H., Emura, I., Tsuneyama, K., He, X.S. & Gershwin, M.E. (2012) Plasma cells and the chronic nonsuppurative destructive cholangitis of primary biliary cirrhosis. *Hepatology*, 55, 846-855.
- Verri, W.J. Jr., Souto, F.O., Vieira, S.M., Almeida, S.C., Fukada, S.Y., Xu, D., Alves-Filho, J.C., Cunha, T.M., Guerrero, A.T., Mattos-Guimaraes, R.B., Oliveira, F.R., Teixeira, M.M., Silva, J.S., McInnes, I.B., Ferreira, S.H., et al. (2010) IL-33 induces neutrophil migration in rheumatoid arthritis and is a target of anti-TNF therapy. *Ann. Rheum. Dis.*, 69, 1697-1703.
- Volarevic, V., Mitrovic, M., Milovanovic, M., Zelen, I., Nikolic, I., Mitrovic, S., Pejnovic, N., Arsenijevic, N. & Lukic, M.L. (2012) Protective role of IL-33/ST2 axis in Con A-induced hepatitis. J. Hepatol., 56, 26-33.
- Wang, J., Cai, Y., Ji, H., Feng, J., Ayana, D.A., Niu, J. & Jiang, Y. (2012a) Serum IL-33 levels are associated with liver damage in patients with chronic hepatitis B. *J. Interferon Cytokine Res.*, **32**, 248-253.
- Wang, J., Zhao, P., Guo, H., Sun, X., Jiang, Z., Xu, L., Feng, J., Niu, J. & Jiang, Y. (2012b) Serum IL-33 levels are associated with liver damage in patients with chronic hepatitis C. *Mediators Inflamm.*, 2012, 819636.
- Zhang, J.Y., Zhang, Z., Lin, F., Zou, Z.S., Xu, R.N., Jin, L., Fu, J.L., Shi, F., Shi, M., Wang, H.F. & Wang, F.S. (2010) Interleukin-17-producing CD4(+) T cells increase with severity of liver damage in patients with chronic hepatitis B. *Hepatology*, 51, 81-91.