The Impact of Serum Interleukin-17 on Chronic Hepatitis C and Its Sequelae

Elham Ahmed Hassan[1*], Abeer Sharaf EL-Din Abd El-Rehim[1], Asmaa Omar Ahmed[2], Nahla Mohamed Elsherbiny[3] and Noha Abd El-Rehim Abo Elhagag[4]

[1]Department of Tropical Medicine and Gastroenterology, Faculty of Medicine, Assiut University, Assiut, Egypt
[2] Departments of Clinical Pathology, Faculty of Medicine, Assiut University, Assiut, Egypt
[3]Department of Medical Microbiology and Immunology, Faculty of Medicine, Assiut University, Assiut, Egypt
[4]Department of Pathology, Faculty of Medicine, Assiut University, Assiut, Egypt

Abstract

Background: Recently, interleukin-17 (IL-17) cytokine family plays important roles in the host immunity in chronic inflammatory conditions, autoimmune and viral liver diseases. However, studies on the IL-17 role in the immunopathogenesis of hepatitis C virus (HCV) infection are limited.

Objectives: to assess serum IL-17 levels in chronically HCV infected patients and their relations to the severity of liver disease.

Patients and methods: The study included 200 chronically HCV infected patients; 100 chronic hepatitis C, 100 liver cirrhosis including 35 with hepatocellular carcinoma (HCC) and 30 controls. Serum IL-17 levels were quantified by ELISA.

Results: Serum IL-17 levels were significantly higher in chronically HCV infected patients than controls and cirrhotics had the highest levels (P<0.001). These levels were positively related with inflammation grade and fibrosis stage. Serum IL-17 was significantly higher in HCC than controls. IL-17 was significantly correlated with prothrombin time, ALT, serum albumin, viral load, and alpha fetoprotein-L3.

Conclusion: IL-17 levels were increased with increasing liver disease progression and chronicity. Thus, IL-17 may be an important biological marker for the immunopathogenesis of chronic hepatitis, liver fibrosis and HCC.

Introduction

Hepatitis C Virus (HCV) infection affects an estimated 180 million people globally, and is a leading cause of chronic hepatitis, cirrhosis, liver cancer, and primary indication for liver transplantation [1,2]. This RNA virus primarily infects hepatocytes and induces an immune-related necroinflammatory hepatic reaction, frequently leading to significant fibrosis or cirrhosis [3]. However, many aspects of immune dysregulations in hepatitis C are still unclear, especially the inflammatory pathways.

IL-17A (IL-17) is one of T cell–derived cytokines produced by Th17 CD4 T cells specifically memory CD4+ T cells [4]. IL-17 is also produced by a wide variety of cell types, including neutrophils, CD8+T cells, γδ T cells, NKT cells and Tregs [5].

IL-17 is a powerful chemoattractant for neutrophils and has been reported to be involved in many immune processes, most notably in inducing and mediating proinflammatory responses e.g. several autoimmune diseases, allergic diseases, asthma and pulmonary infection [6-8].

Several groups of investigators recognized the role of IL-17 in hepatitis B and its sequlae [9-11]. However, there has been limited data of the role of IL-17 in HCV infected patients, especially in relation to hepatic inflammation and fibrosis and hepatic cell carcinoma. In addition, Egyptian studies on the role of IL-17 in the immunopathogenesis of HCV are limited. Therefore, the aims of this study were to assess serum IL-17 levels in HCV-positive patients with chronic liver disease and their relation to the severity of liver disease.

Patients and Methods

Study design

This was a prospective study carried out at Assiut University Hospital (AUH), Egypt, from February 2013 to February 2014. The study was approved by the Ethics Committee of AUH and an informed, written consent was obtained from all the participants before enrollment.

Study population

The study group comprised 200 chronically HCV infected patients and they were divided into:

- 100 patients with chronic hepatitis C "CHC"

- 100 patients with liver cirrhosis including 35 with HCC.

Diagnosis of chronic hepatitis "C" was based on laboratory findings of fluctuations of serum transaminases levels for more than 6 months and positive HCV antibodies and serum HCV-RNA for HCV

infection and absence of sonographic findings of liver cirrhosis and histopathologic evidence of chronic hepatitis by liver biopsy.

Cirrhotic patients had diagnostic criteria of Liver Cirrhosis (LC) by clinical, biochemical and ultrasonographic findings. Severity of liver cirrhosis was assessed according to Child-Pugh classification [12]. Further, cirrhotic patients were divided according to hepatic focal lesion(s) by imaging and elevated alpha fetoprotein-L3 (AFP-L3).

The controls were 30 healthy volunteers and they had negative markers for HBV and HCV infections had no hepatic diseases and they were sex and age matched with patients. These patients were selected from outpatient clinics and inpatient wards of the department of Tropical Medicine and Gastroenterology, Assiut University Hospital (AUH), while controls were selected randomly from outpatient clinic and relatives of the patients. Patients with evidence of infection, co-infection of HCV and HBV, HIV, autoimmune hepatitis, alcoholic hepatitis, and hepatic metastasis or under antiviral therapy were excluded. At the study entry, a thorough medical history and clinical examination were taken and measurement of serum IL-17 level, other biochemical parameters and liver biopsy were undertaken.

Methods

Specimen collection and processing

Five milliliters of blood were withdrawn from each patient under complete aseptic conditions. Sera were separated and stored frozen at -70˚C until analysis.

Laboratory diagnosis

- Antibodies to HCV using a third-generation enzyme immunoassay, and HCV RNA was quantified by Taq Man Assay Reagents (Applied Biosystems) using the 7500 fast Real Time PCR system.

- Biochemical tests: liver function tests (Aspartate aminotransferase (AST), alanine transaminase (ALT), albumin, bilirubin and prothrombin time) and Alpha fetoprotein-L3 (AFP-L3 for cirrhotic patients with hepatic focal lesions).

- Serum IL-17 levels were measured by enzyme-linked immunosorbent assay (ELISA) (IL-17: R&D Systems; Quantikine ELISA; Human IL-17), according to the instructions of the manufacturer.

Liver histology

Liver biopsies were taken percutaneously with a 1.4 mm diameter Menghini needle and consisted of 3-5 mm long liver tissue cylinders. Biopsies were formalin fixed and then embedded in paraffin cut and stored until use. Biopsies were histologically examined using hematoxylin–eosin stain. The Scheuer score was used to classify hepatic necroinflammatory activity and fibrosis [13].

Statistical analysis

All statistical analyses were conducted using SPSS for windows version 17 (SPSS Inc., Chicago, IL, USA). The Kruskal Wallis and Mann-Whitney tests were used to compare the continuous variables. Categorical variables were expressed as percentage and compared using chi-square ($\chi 2$) or Fisher's exact test (two-tailed) test. Spearman's correlation coefficient (r) was used to find correlations. All data were summarized as mean and standard deviations. For all analyses, P value<0.05 was considered statistically significant.

Results

Characteristics of the study population

The baseline sociodemographic and biochemical characteristics of the study population were summarized in Table 1. The study included 200 chronically HCV infected patients; 128 males and 72 females with mean age of 47.7 ± 5.3 years. Control group was formed of 11 females and 19 males with a mean age of 45.5 ± 7.6 years.

Serum IL-17 levels

Compared with controls, serum IL-17 levels were significantly higher in chronically HCV infected patients and those with LC exhibited the highest IL-17 levels (P<0.001) as shown in Table 2. Patients with CHC had significantly higher levels than controls (P=0.03). Furthermore, serum IL-17 levels were significantly higher in patients with LC compared to CHC group (P<0.001). Additionally, cirrhotic patients with Child-Pugh class C had higher serum IL-17 levels (71.9 ± 41.9 pg/ml) than Child-Pugh class A and B (59.8 ± 29.5 and 65.5 ± 33.4 pg/ml respectively) but without statistical significant differences (P=0.07).

Among the cirrhotic group, patients with HCC (n=35) had significantly higher serum IL-17 levels than controls (67.3 ± 40.4 vs. 19.4 ± 7.3 pg/ml, P<0.001) and insignificantly higher values than patients without HCC (n=56) and patients with hemangioma(s) (n=

| Variable	Control (n = 30)	CHC (n = 100)	LC (n = 100)
Age (years) mean ± SD	45.5 ± 7.6	46.5 ± 10.8	52.3 ± 7.7
Sex (n) Male Female	19 (63.3%) 11 (36.7%)	60 (60%) 40 (40%)	68 (68%) 32 (32%)
Child-Pugh score (mean ± SD)	-	-	12.4 ± 1.7
Child-Pugh class (A/B/C) (n)	-		(21/32/47)
MELD score mean ± SD	-	-	18.3 ± 8.2
ALT (IU/L) mean ± SD	22.2 ± 8.9	55.3 ± 20.2	60.5 ± 58.7
AST (IU/L) mean ± SD	24.8 ± 8.6	32.5 ± 5.7	97 ± 45.9
Serum albumin (g/dl) mean ± SD	4.3 ± 0.2	4.8 ± 0.4	2.1 ± 0.4
Serum bilirubin (µmol/l) mean ± SD	10.2 ± 3.4	8.7 ± 2.1	53.1 ± 39.8
Prothrombin time (seconds)	13 ± 1.1	11.9 ± 1.1	20.4 ± 4.1
Viral load (log10 copies/ml) mean ± SD	-	5.8 ± 1.3	4.4 ± 1.4
Hepatocellular carcinoma (n)	-	-	35 (35%)
Hepatic hemangioma (n)	-	-	9 (9%)

CHC: Chronic hepatitis C; LC: Liver cirrhosis; ALT: Alanine transaminase; AST: Aspartate transaminase; HCV: Hepatitis C virus

Table 1: Clinical and laboratory characteristics of the study population.

	Case (n)	IL-17 (pg/ml)
Controls	30	19.4 ± 7.3
CHC	100	41.6 ± 33.6[ab]
LC*	65	65.9 ± 38.9[a]

*Cases of LC without hepatocellular carcinoma
[a]p<0.05 compared with controls, [b]p<0.05 compared with LC
CHC: Chronic hepatitis C; LC: Liver cirrhosis

Table 2: Comparison of mean serum IL-17 levels among the different studied groups.
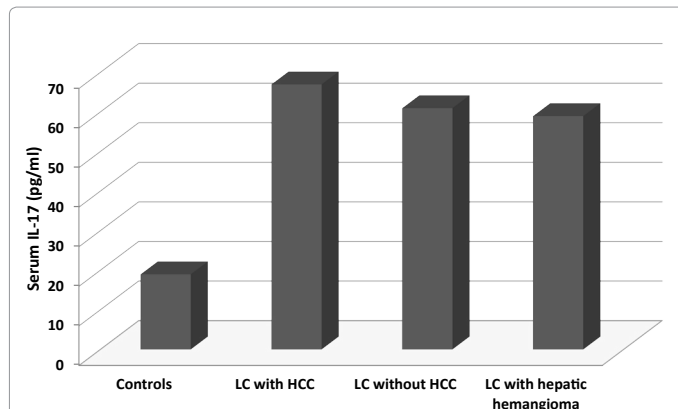
Figure 1: Comparison of mean serum IL-17 levels among cirrhotic patients showed serum IL-17 levels were higher in patients with HCC than those without HCC and with hepatic hemangioma (P>0.05 for both) and controls (P<0.001)
LC: Liver cirrhosis; HCC: Hepatocellular carcinoma.

9) (67.3 ± 40.4 vs. 61.7 ± 35.7 and 59. 7 ± 35.4 pg/ml, P = 0.4 and 0.1 respectively, Figure 1).

Table 3 showed that CHC patients with the highest serum IL-17 levels had the highest necroinflammatory activity (P = 0.02) and highest fibrosis stage (P=0.04). Additionally, there were significant correlation between serum IL-17 and grades of inflammation (r=0.98, P<0.001) and stages of fibrosis (r=0.57, P=0.02). Serum IL-17 levels were significantly correlated with ALT, viral load in CHC patients and prothrombin time and negatively correlated with serum albumin in cirrhotic patients (Table 4). Regarding HCC, serum IL-17 level positively correlated with AFP-L3 (r=0.55 and P=0.02), however, no correlation was found between serum IL-17 levels and tumor size or number (r=0.13, P=0.3 and r=- 0.21, P=0.2 respectively).

Discussion

Hepatitis C Virus (HCV) infection primarily infects hepatocytes and induces an immune-related necroinflammatory hepatic reaction, frequently leading to significant fibrosis or cirrhosis [3]. The present study was conducted to investigate the involvement of the Th17 immune response which is not fully understood by determining serum levels of the IL-17 cytokine, in patients with chronic hepatitis C infection as well as cirrhosis with or without HCC aiming to reach an immunotherapeutic target at the future for the prevention of liver tissue damage. As a major function of most cytokines is to act in intercellular communication, it has been demonstrated that cytokine levels in serum and plasma provide at best an indirect measure of this key cytokine function [14].

We found that serum IL-17 levels were significantly higher in chronically HCV infected patients than controls and the highest serum IL-17 levels were observed in LC patients. This higher serum IL-17 in

patients with chronic liver disease goes with what has been reported previously that serum IL-17 level was increased in liver injuries following chronic hepatitis and cirrhosis supporting a role for IL-17 as a chronic disease inducer in the pathogenesis and/or progression of liver fibrosis [15]. However, IL-17A levels were not significantly different between Child A and B and C. This lack of difference may be attributed to the small sample size within the cirrhosis subgroups.

In this study, serum IL-17 levels of CHC patients were positively related with the grades of liver inflammation and fibrosis; the higher the grade, the higher the serum IL-17 levels indicating that IL-17 takes part in chronic inflammation and in the development of hepatic fibrosis. These results were compatible with previous studies that reported a close correlation between virus-induced liver inflammation, infiltration in CHC infections and activation of Th17 cells and the amount of liver damage caused by the antiviral immune response [15,16]. IL-17 exerts its proinflammatory effects through recruitment of neutrophils and monocytes, T cell infiltration facilitation and production of variety of inflammatory factors as IL 1,6, TNF- α...etc. It also induces the expression of several inflammation- associated genes in primary hepatocytes [6,17-19]. The relation of IL-17 to liver fibrosis remains elusive. Liver fibrosis is a severe, life-threatening clinical condition resulting from non-resolving hepatitis of different origins. IL-17 may have a pro-fibrogenic effect through two independent mechanisms [9,20]: first, IL-17 stimulates Kupffer cells to express inflammatory cytokines IL-6, IL-1β, and TNF-α, as well as the major fibrogenic cytokine TGF-β1which in turn, induce activation of Hepatic Stellate Cells (HSC) into myofibroblasts. Second, IL-17 directly stimulates HSCs to express collagen Type I and promotes their activation into fibrogenic myofibroblasts via Stat3 signaling pathway. Moreover, it has been reported elsewhere that intrahepatic IL-17 expression was positively correlated with the serum indices of hepatic fibrosis that is an important pathological process in the development of liver cirrhosis [6].

Inflammation grades	n	Serum IL-17 level	P	Fibrosis stage	n	Serum IL-17 level	P
G0-1	18	21.4 ± 9.2		**S0-1**	23	18.7 ± 8.2	
G2	33	34.2 ± 18.4	0.04	**S2**	32	32.3 ± 17.5	0.02
G3	37	41.9 ± 24.6		**S3**	35	43.1 ± 24.1	
G4	12	52 ± 27.8		**S4**	10	55.6 ± 33.7	

Table 3: Serum IL-17 level and different grades of liver inflammation and fibrosis in CHC patients.

		CHC	LC
ALT	r	0.85	0.11
	p	<0.001	0.7
AST	r	0.13	0.12
	p	0.4	0.6
Serum albumin	r	0.02	- 0.54
	p	0.7	0.001
Serum bilirubin	r	0.19	0.22
	p	0.5	0.2
Prothrombin time	r	0.27	0.45
	p	0.1	0.03
HCV viremia	r	0.47	0.30
	p	0.02	0.07
Child-Pugh score	r	-	0.32
	p	-	0.06

CHC: Chronic hepatitis C; LC: Liver cirrhosis; ALT: Alanine transaminase; AST: Aspartate transaminase; HCV: Hepatitis C virus

Table 4: Correlation between serum IL-17 levels and clinical and laboratory characteristics among the studied cases.

We reported significant correlations between the serum concentration of IL-17 and that of some indicators of liver function e.g. ALT, prothrombin time and serum albumin suggesting that IL-17 is, to a certain extent, associated with the extent of liver damage. Additionally, this significant correlation between serum IL-17 and ALT was in accordance with several studies which showed that IL-17 level correlated directly with severity of liver inflammation [10,15]. This is probably because IL-17 activates a variety of immune cells to release inflammatory mediators as mentioned in details before, leading to repeated inflammation of the liver and deterioration of liver function [20]. However, Du et al.'s earlier report showed no correlation between serum IL-17 and ALT where the latter can be easily affected by drugs which decrease its level [6].

Our study revealed a positive linear correlation between serum IL-17 and viral load that was similar with previous studies [21]. This finding which is in agreement with that of Hou et al. suggested that that IL-17 may contribute to the immunopathogenesis of chronic viral hepatitis by persistent viral infection due to IL-17 up-regulated anti-apoptotic molecules and thus enhancing the survival of virus infected cells and blocking target cell destruction by cytotoxic T cells [22,23]. Also, Oyoshi et al. [24], reported that IL-17 contributes to viral replication in a model of disseminated vaccinia infection rather than providing host defense against viral infection. However, Balanescu et al. [5], reported the demonstrated lack of correlation between serum IL-17 levels and viral markers owing to multiple IL-17 sources that might account for their serum levels and fluctuation of viral loads.

In the present study, we found that serum IL-17 levels were significantly higher in patients with HCC compared to controls and were insignificantly higher than in those without HCC and hepatic haemangioma(s). This result was agreed with Liao at al. [25] who reported that serum IL-17 levels to be higher in cirrhotic patients with HCC than those with hepatic haemangioma which suggested that IL-17 takes part in the pathogenesis of HCC reflecting the systemic immune status of individuals with tumor. The contribution of IL-17 and Th17-related immunity during carcinogenesis has been demonstrated recently [25,26]. The potential mechanisms involve angiogenesis and promation of tumor growth by cytokine induction in the tumor microenvironment and activating the oncogenic signal Stat3 [27-29]. As we reported that serum IL-17 levels were positively correlated with serum AFP, it may act as a noninvasive marker for HCC screening and recurrence monitoring [29]. Our study was in accordance with Wu et al. [11] where the increased serum IL-17 level was not significantly influenced by the tumor intrinsic characteristics (tumor size and number). Liao et al. [30] found that IL-17 was over expressed in HCC tumors cells and associated with HCC recurrences and poor survival [31].

In conclusion, IL-17 levels were increased with increasing liver disease progression and chronicity. Thus, IL-17 may be an important biological marker for the immunopathogenesis of chronic hepatitis, liver fibrosis and HCC. Thus, Blocking of IL-17 expression may be a potential target for controlling the inflammatory response in chronic hepatitis and liver fibrosis, hence, the development of cirrhosis and HCC. Further studies are recommended to establish the role of infiltrating IL-17+ cells in the pathogenesis of liver diseases in conjunction with the measurements of serum IL-17 cytokine.

References

1. Rosen HR (2011) Clinical practice. Chronic hepatitis C infection. N Engl J Med 364: 2429-2438.

2. Davis GL, Albright JE, Cook SF, Rosenberg DM (2003) Projecting future complications of chronic hepatitis C in the United States. Liver Transpl 9: 331-338.

3. Tsai SL, Liaw YF, Chen MH, Huang CY, Kuo GC (1997) Detection of type 2-like T-helper cells in hepatitis C virus infection: implications for hepatitis C virus chronicity. Hepatology 25: 449-458.

4. Huang W, Na L, Fidel PL, Schwarzenberger P (2004) Requirement of interleukin-17A for systemic anti-Candida albicans host defense in mice. J Infect Dis 190: 624-631.

5. Bălănescu P, Lădaru A, Voiosu T, Nicolau A, Ene M, et al. (2012) Th17 and IL-17 immunity in chronic hepatitis C infection. Rom J Intern Med 50: 13-18.

6. Woltman AM, de Haij S, Boonstra JG, Gobin SJ, Daha MR, et al. (2000) Interleukin-17 and CD40-ligand synergistically enhance cytokine and chemokine production by renal epithelial cells. J Am Soc Nephrol 11: 2044-2055.

7. Toda M, Leung DY, Molet S, Boguniewicz M, Taha R, et al. (2003) Polarized in vivo expression of IL-11 and IL-17 between acute and chronic skin lesions. J Allergy Clin Immunol 111: 875-881.

8. Louten J, Boniface K, de Waal Malefyt R (2009) Development and function of TH17 cells in health and disease. J Allergy Clin Immunol 123: 1004-1011.

9. Du WJ, Zhen JH, Zeng ZQ, Zheng ZM, Xu Y, et al. (2013) Expression of interleukin-17 associated with disease progression and liver fibrosis with hepatitis B virus infection: IL-17 in HBV infection. Diagn Pathol 8: 40.

10. Wang L, Chen S, Xu K (2011) IL-17 expression is correlated with hepatitis B-related liver diseases and fibrosis. Int J Mol Med 27: 385-392.

11. Wu J, Du J, Liu L, Li Q, Rong W, et al. (2012) Elevated pretherapy serum IL17 in primary hepatocellular carcinoma patients correlate to increased risk of early recurrence after curative hepatectomy. PLoS One 7(12):e50035.

12. Pugh RN, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R (1973) Transection of the oesophagus for bleeding oesophageal varices. Br J Surg 60: 646-649.

13. Scheuer PJ (1991) Classification of chronic viral hepatitis: a need for reassessment. J Hepatol 13: 372-374.

14. Jason J, Archibald LK, Nwanyanwu OC, Byrd MG, Kazembe PN, et al. (2001) Comparison of serum and cell-specific cytokines in humans. Clin Diagn Lab Immunol 8: 1097-1103.

15. Chang Q, Wang YK, Zhao Q, Wang CZ, Hu YZ, et al. (2012) Th17 cells are increased with severity of liver inflammation in patients with chronic hepatitis C. J Gastroenterol Hepatol 27: 273-278.

16. Lemmers A, Moreno C, Gustot T, Maréchal R, Degré D, et al. (2009) The interleukin-17 pathway is involved in human alcoholic liver disease. Hepatology 49: 646-657.

17. Hammerich L, Heymann F, Tacke F (2011) Role of IL-17 and Th17 cells in liver diseases. Clin Dev Immunol 2011: 345803.

18. Albanesi C, Cavani A, Girolomoni G (1999) IL-17 is produced by nickel-specific T lymphocytes and regulates ICAM-1 expression and chemokine production in human keratinocytes: synergistic or antagonist effects with IFN-gamma and TNF-alpha. J Immunol 162: 494-502.

19. Miossec P, Korn T, Kuchroo VK (2009) Interleukin-17 and type 17 helper T cells. N Engl J Med 361: 888-898.

20. Harada K, Shimoda S, Sato Y, Isse K, Ikeda H, et al. (2009) Periductal interleukin-17 production in association with biliary innate immunity contributes to the pathogenesis of cholangiopathy in primary biliary cirrhosis. Clin Exp Immunol 157: 261-270.

21. Meng F, Wang K, Aoyama T, Grivennikov SI, Paik Y, et al. (2012) IL-17 signaling in inflammatory cells, Kupffer cells and Hepatic Stellate cells exacerbates liver fibrosis. Gastroenterology 143: 765–776.e1-3.

22. El Husseiny NM, Fahmy HM, Mohamed WA, Amin HH (2012) Relationship between vitamin D and IL-23, IL-17 and macrophage chemoattractant protein-1 as markers of fibrosis in hepatitis C virus Egyptians. World J Hepatol 4: 242-247.

23. Hou W, Kang HS, Kim BS (2009) Th17 cells enhance viral persistence and inhibit T cell cytotoxicity in a model of chronic virus infection. J Exp Med 206: 313-328.

24. Oyoshi MK, Elkhal A, Kumar L, Scott JE, Koduru S, et al. (2009) Vaccinia virus inoculation in sites of allergic skin inflammation elicits a vigorous cutaneous IL-17 response. Proc Natl Acad Sci U S A 106: 14954-14959.

25. Liao R, Sun J, Wu H, Yi Y, Wang JX, et al. (2013) High expression of IL-17 and IL-17RE associate with poor prognosis of hepatocellular carcinoma. J Exp Clin Cancer Res 32: 3.

26. Wang L, Yi T, Zhang W, Pardoll DM, Yu H (2010) IL-17 enhances tumor development in carcinogen-induced skin cancer. Cancer Res 70: 10112-10120.

27. He D, Li H, Yusuf N, Elmets CA, Athar M, et al. (2012) IL-17 mediated inflammation promotes tumor growth and progression in the skin. PLoS One 7: e32126.

28. Ye J, Livergood RS, Peng G (2013) The role and regulation of human Th17 cells in tumor immunity. Am J Pathol 182: 10-20.

29. Maniati E, Soper R, Hagemann T (2010) Up for Mischief? IL-17/Th17 in the tumour microenvironment. Oncogene 29: 5653-5662.

30. Wang L, Yi T, Kortylewski M, Pardoll DM, Zeng D, et al. (2009) IL-17 can promote tumor growth through an IL-6-Stat3 signaling pathway. J Exp Med 206: 1457-1464.

31. Spangenberg HC, Thimme R, Blum HE (2006) Serum markers of hepatocellular carcinoma. Semin Liver Dis 26: 385-390.