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Increased interleukin-32, interleukin-1, and interferon-γ levels in serum from hepatitis B patients and in HBV-stimulated peripheral blood mononuclear cells from healthy volunteers

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ABSTRACT

Background: Few studies showed the changes in cytokine profiles after infection by hepatitis B virus (HBV), the most common viral liver disease worldwide. This study examined the relationship between interleukin (IL)-32, IL-1, and interferon (IFN)-γ levels and HBV load.
Methods: IL-32, IL-1, and IFN-γ levels in hepatitis B patients serum and HBV-stimulated PBMCs were measured by ELISA. Gene transcripts in PBMCs from hepatitis B patients and HBV-stimulated PBMCs from healthy controls were measured by real-time PCR.
Results: IL-32, IL-1, and IFN-γ protein levels in serum from hepatitis B patients were significantly higher than those in healthy volunteers ($P < 0.05$). Hepatitis B patients showed significantly higher expression of IL-32, IL-1, and IFN-γ transcripts than healthy volunteers ($P < 0.05$). IL-32, IL-1, and IFN-γ levels in PBMCs stimulated by different amounts of HBV were significantly higher than those in HBV-unstimulated PBMCs ($P < 0.05$). Real-time PCR results were consistent with the ELISA results.
Conclusions: The levels of IL-32, IL-1, and IFN-γ protein and transcripts in serum and PBMCs from hepatitis B patients were higher than those in healthy volunteers. Similarly, both were higher in PBMCs from healthy volunteers stimulated by HBV *in vitro*. However, the changes in cytokine levels were not proportional to the viral load.

Contents

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Introduction

Hepatitis B is a prominent infectious disease caused by hepatitis B virus (HBV). Global assessment by Aparna Schweitzer showed that some 248 million people have been infected with hepatitis B [1]. Although HBV infections are decreasing, 686,000 people die from hepatitis B infections every year [2]. Continuous replication of HBV in host liver cells is crucial for HBV infection and disease progress [3]. The HBV viral load in serum is a reliable and direct indicator of virus replication [4]. According to the World Health Organization's guidelines for HBV guidelines issued in 2015, HBV DNA nucleic acid test (NAT) following a reactive HBsAg serological test result, is recommended to help further guide who to treat or not treat if there is no evidence of cirrhosis, and to monitor for treatment response [5]. Kim et al. performed a study of IL-18-inducible genes and identified human interleukin-32 (IL-32) as a member of the interleukin family [6]. IL-32 is produced by a variety of cells, including mitogen-stimulated human peripheral blood lymphocytes (PBMCs) [7], epithelial cells stimulated by human interferon (IFN)-γ, and NK cells stimulated by IL-12 and IL-18 [6,8]. IL-32 and IL-1 are pro-inflammatory cytokines that play a role in diseases caused by infection by avian influenza [9], HIV [10], hepatitis B [11], hepatitis C [12], andhuman papilloma viruses[13]. IFN-γ is secreted by T cells and NK cells, and has antiviral, immunoregulatory, and anti-tumor properties [14,15].

Here, we examined the relationship between serum levels of IL-32, IL-1, and IFN-γ and the HBV viral load in patients with hepatitis B. We used real-time PCR and ELISA to measure IL-32, IL-1, and IFN-γ transcript and protein levels, respectively, in serum and in the culture supernatants of PBMCs derived from HBV patients and healthy volunteers. The results provide insights into the early detection, diagnosis, and prevention of hepatitis B.

Materials and methods

Instruments and reagents

A Biological Safety Cabinet (Class II, Type A2, Thermo Fisher, Shanghai, China), a centrifuge (Allegra 64R, Beckman Coulter, Fullerton, CA, USA), and a real-time PCR Gene amplification instrument (ABI 7500, Carlsbad, CA, USA) were used for the study. A reverse transcription PCR Kit, SYBR® Premix Ex Taq™, and Trizol regent were purchased from TaKaRa (Shiga, Japan). Tissue culture plates, EP tubes, and tips were purchased from Corning (Corning, NY, USA). Fetal bovine serum was purchased from Hyclone (Logan, UT, USA).

Collection of human blood samples

Blood samples were obtained from 113 patients (79 male and 34 female; age range, 18–68 years) with hepatitis B and from 60 healthy volunteers (33 male and 27 female; age range, 21–60 years) attending a public hospital in Tai'an, Shandong province. The study was approved by the local ethics committee. The hepatitis B patients were hospitalized from January 2015 to February 2016. The inclusion criteria for hepatitis B patients were as follows: i) the patients did not receive any antiviral treatment or immunotherapy; ii) the patients did not receive liver-protective treatment in the 6 months prior to the study; and iii) the patients agreed to blood sample collection. Blood samples were collected from patients and controls, and added to vacuum blood collection tubes with separate gels and centrifuged to obtain supernatants. Hepatitis B patients were classified into three groups according to viral load: high, intermediate, and low. Information on patients and controls is listed in Table 1.

Detection of IL-32, IL-1, and IFN-γ in serum from HBV patients and healthy volunteers

IL-32, IL-1, and IFN-γ were detected using commercial ELISA kits in accordance with the manufacturer's instructions. Samples, standards, and blanks were run in triplicate. Finally, the OD_{450nm} was measured, and the concentrations of IL-32, IL-1, and IFN-γ were calculated from the standard curves.

Detection of the gene transcripts in PBMCs from hepatitis B patients and healthy volunteers by real-time PCR

Isolation of PBMCs

Fresh blood samples with EDTA anticoagulants (2 ml) were mixed with an equal volume of PBS buffer (pH 7.4) prior to layering onto the surface of human lymphocyte separation medium (Ficoll) (Solarbio, Beijing, China, 2 ml). After centrifugation at 1500 g for 25 min, the PBMC cell layer was collected by centrifugation and washed three times with PBS buffer.

Extraction of total RNA

PBMCs were lysed with Trizol reagent (0.5 ml per 1×10^6–1×10^7 cells). Chloroform (0.1 ml) was then added to each tube and homogenized by shaking vigorously for 15 s. Tubes were then incubated at room temperature for 3 min. After centrifugation for 15 min (4 °C, 12000 × *g*), the supernatant was transferred to a new tube and 1 ml of ethanol (75%) was added. RNA was precipitated by gently inverting the tubes. Total RNA was collected by centrifuging the solution for 5 min at 4 °C (7500 g). The supernatant was discarded, and the RNA pellet was air dried for 10 min at room temperature before being dissolved in 20 μl of DEPC water and storage at −80 °C.

Reverse transcription

Total RNA was reverse-transcribed using the following reaction system: 5 × buffer (2.0 μl), RT mix (0.5 μl), random primers (1.0 μl),

Table 1
Information on patients and controls.

	Healthy controls	Patients infected by HBV		
		Low HBV load (copies/ml)	Intermediate HBV load (copies/ml)	High HBV load (copies/ml)
Number (male/female)	60 (33/27)	29 (19/10)	46 (36/10)	38 (24/14)
Age (mean ± SD)	32 ± 8.33	42.11 ± 9.14	45.86 ± 12.55	38.87 ± 12.69
ALT (U/L)	21.3 ± 6.82	40.43 ± 27.31	65.95 ± 63.79	132.4 ± 159.3
AST (U/L)	20.11 ± 7.31	38.82 ± 26.43	56.88 ± 31.21	89.43 ± 106.47
ALB (g/L)	47 ± 4.13	44.9 ± 7.04	42.4 ± 8.29	45.08 ± 6.89
HBV-DNA	0	$2.39 \times 10^3 \pm 1.99 \times 10^3$	$1.45 \times 10^6 \pm 2.21 \times 10^6$	$1.29 \times 10^8 \pm 1.49 \times 10^8$

Note: SD: standard deviation. ALT: alanine aminotransferase. AST: aspartate aminotransferase. HBV: hepatitis B virus. DNA: deoxyribonucleic acid.

Table 2
Sequences of the primers used to amplify IL-32, IL-1, and IFN-γ.

Target gene	Primer sequences
IL-32	Forward primer P1: 5′-GCGAATTCATGTGCTTCCCGAAG-3′ Reverse primer P2: 5′-GCAAGCTTTCATTTTGAGGATTGGG-3′
IL-1	Forward primer P1: 5′-CGGGATCCGCCGCCACCATGGCAGAAGTACC-3′ Reverse primer P2: 5′-CCCTCGAGTTAGGAAGACACAAA-3′
IFN-γ	Forward primer P1: 5′-CGGAATTCATGTGTTACTGCCAGGAC-3′ Reverse primer P2: 5′-CGGGATCCTTACTGGGATGCTCTTCG-3′

Note: IL: interleukin. IFN: interferon.

and total RNA (6.5 μl [1 μg]). The PCR amplification parameters were as follows: 42 °C for 30 min, 99 °C for 5 min, and 5 °C for 5 min, followed by holding at 4 °C.

Real-time PCR

The reverse-transcribed products were diluted with DEPC water and used as templates for real-time PCR. The reaction mixture comprised SYBR Premix Ex Taq (10 μl), forward and reverse primers (0.4 μl each), Rox reference Dye II (0.4 μl), ddH_2O (6.8 μl), and template (2.0 μl). PCR amplification was carried out as follows: initial denaturation at 95 °C for 30 s, 40 cycles of amplification (including denaturation) at 95 °C for 5 s, and annealing and extension at 60 °C for 34 s. The primer sequences are listed in Table 2.

Detection of IL-32, IL-1, and IFN-γ transcripts and protein in HBV-stimulated PBMCs from healthy volunteers

PBMCs were isolated from anticoagulated blood (6.0 ml) obtained from healthy volunteers, and the cell density was adjusted to 1.5×10^6/ml. The PBMCs were inoculated into 12-well cell culture plates, and RPMI 1640 medium (1.0 ml) containing 10% calf serum and 20 g/ml Con A was added to each well. After incubating at room temperature for 40 min, RPMI 1640 medium (1.0 ml) containing 10% calf serum and differing amounts of HBV virus (6.20×10^3, 4.97×10^5, or 3.26×10^7 copies/ml; representing low, intermediate, or high HBV viral loads, respectively) was added. Three replicates were included for each viral load. The negative control contained no HBV virus. After 60 h incubation at 37 °C, 0.5 ml of supernatant was taken from each well and used to measure IL-32, IL-1, and IFN-γ by ELISA. In the meantime, PBMCs were collected and total RNA was isolated as described above. IL-32, IL-1, and IFN-γ transcripts were examined by real-time PCR as described above.

Statistical analysis

Data are presented as the mean and standard deviation (mean ± SD). All statistical analyses were performed using SPSS v16 (SPSS Inc., Chicago, IL). Differences between groups were analyzed using single factor analysis of variance. Two groups were compared using least squares differences. Differences were considered statistically significant at $P < 0.05$.

Results

IL-32, IL-1, and IFN-γ levels in serum from hepatitis B patients

The amount of IL-32, IL-1, and IFN-γ in the serum of 113 hepatitis B patients with low, intermediate, or high HBV viral load, and in serum from 60 healthy volunteers, is shown in Table 3. The amounts of all three cytokines were significantly higher in hepatitis B patients than in healthy volunteers ($P < 0.05$). The changes in the levels of the three cytokines were not proportional to viral load.

Expression of IL-32, IL-1, and IFN-γ in PBMCs from hepatitis B patients and healthy volunteers

Analysis of the IL-32, IL-1, and IFN-γ transcripts in PBMCs revealed a significant difference between hepatitis B patients and healthy volunteers ($P < 0.05$). Expression of all three cytokines was higher in hepatitis B patients. However, there were no significant differences between the low, intermediate, or high HBV viral load groups ($P > 0.05$) (Fig. 1).

Amount of IL-32, IL-1, and IFN-γ protein in HBV-stimulated PBMCs from healthy volunteers

The amount of IL-32, IL-1, and IFN-γ protein in HBV-stimulated PBMCs from healthy volunteers (Table 4) was significantly higher than that in the control group (no exposure to HBV). However, there was no consistent trend between IL-32, IL-1, and IFN-γ levels and viral load.

Expression of IL-32, IL-1, and IFN-γ transcripts in HBV-stimulated PBMCs from healthy volunteers

The amounts of IL-32, IL-1, and IFN-γ transcripts in HBV-stimulated PBMCs from healthy volunteers stimulated with differing HBV loads were significantly higher than those of controls not stimulated with HBV ($P < 0.05$); however, there was no significant difference between transcript levels expressed by the stimulated groups ($P > 0.05$) (Fig. 2).

Discussion

Since Kim et al. first identified IL-32 in 2005, many studies have examined the relationship between IL-32 and disease. For example, IL-32 is closely related to the occurrence and development of many diseases, including autoimmune diseases [16], tumors [17], and diseases caused by bacteria [18] or viruses [11,12,13,19]. IL-1, the same as IL-6, is a pro-inflammatory cytokine. It is not only essential to innate immune defense, but is also an important mediator of adaptive immune response to viral infections [20]. Interferon-γ is

Table 3
Amount of IL-32, IL-1, and IFN-γ protein in serum samples from patients and healthy volunteers.

HBV-DNA content (copies/ml)	Gender (male/female)	IL-32 (ng/l)	IL-1 (ng/l)	IFN-γ (ng/l)
0	33/27	117.93 ± 32.19	111.84 ± 34.84	81.25 ± 20.07
$2.39 \times 10^3 \pm 1.99 \times 10^3$	19/10	410.78 ± 71.23*	366.80 ± 93.41*	174.13 ± 103.91*
$1.45 \times 10^6 \pm 2.21 \times 10^6$	36/10	394.94 ± 85.45*	324.50 ± 89.81*,▲	181.22 ± 106.38*
$1.29 \times 10^8 \pm 1.49 \times 10^8$	24/14	359.90 ± 77.74*,▲,#	324.09 ± 107.44*,▲	145.07 ± 112.80*

Note: IL: interleukin. IFN: interferon. HBV: hepatitis B virus. DNA: deoxyribonucleic acid.
* Compared with the respective healthy control group (P < 0.05).
▲ Compared with the low HBV viral load group (P < 0.05).
Compared with the intermediate viral load group (P < 0.05).

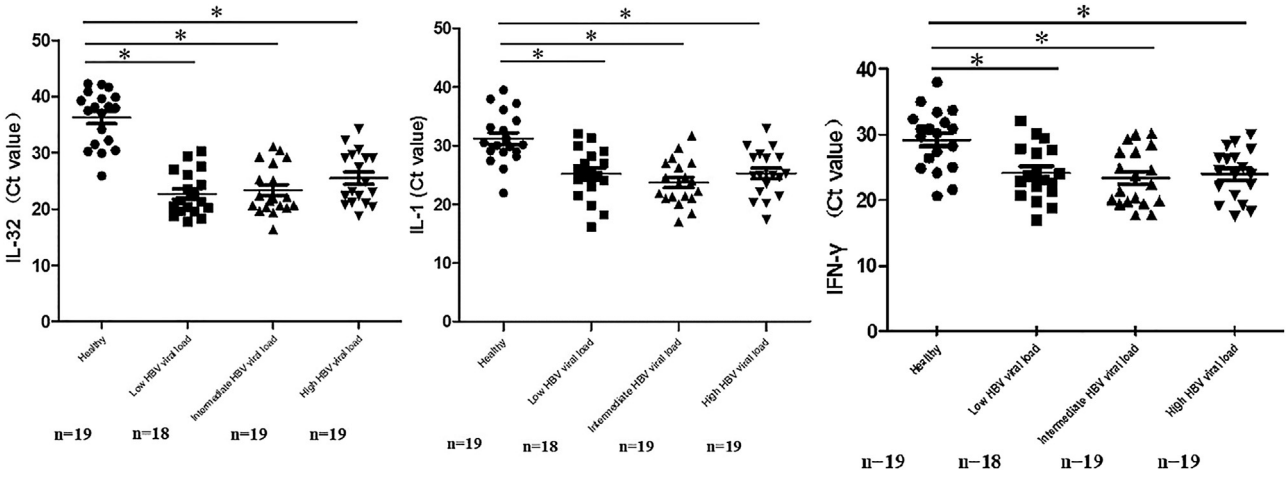

Fig. 1. Ct values for IL-32, IL-1, and IFN-γ mRNA in PBMCs isolated from hepatitis B patients and healthy volunteers. *Compared with the healthy volunteer group (P < 0.05).
Note: IL: interleukin. IFN: interferon. HBV: hepatitis B virus. CT.

Table 4
Amount of IL-32, IL-1, and IFN-γ protein in PBMCs from healthy volunteers stimulated by HBV.

Group	HBV-DNA (copies/ml)	IL-32 (ng/l)	IL-1 (ng/l)	IFN-γ (ng/l)
Control	0	60.51 ± 17.86	71.27 ± 13.89	47.28 ± 15.34
Stimulated by low HBV load	6.20×10^3	91.25 ± 11.49*	104.13 ± 42.56*	75.92 ± 24.06*
Stimulated by intermediate HBV load	4.97×10^5	119.17 ± 28.87*,#	128.69 ± 37.61*,#	86.75 ± 24.05*
Stimulated by high HBV load	3.26×10^7	98.31 ± 29.71*	102.83 ± 27.66*	85.95 ± 24.27*

Note: IL: interleukin. IFN: interferon. HBV: hepatitis B virus. DNA: deoxyribonucleic acid.
* Comparison between each stimulated group and the control group (P < 0.05).
# Comparison between groups stimulated with an intermediate HBV load and the group stimulated with a low HBV load (P < 0.05).

an antiviral cytokine, and often used in the treatment of HBV. However, little is known about the relationship between these three cytokines and HBV infection in hepatitis B patients. The results of this study demonstrate that the serum of hepatitis B patients harbors higher levels of IL-32, IL-1, and IFN-γ transcripts and protein than that of healthy controls. Also, HBV-stimulated PBMCs from healthy volunteers harbor higher transcript and protein levels than unstimulated PBMCs from healthy controls, suggesting that these cytokines have utility for the early detection and monitoring of HBV infection.

We examined blood samples from hepatitis B patients and performed *in vitro* infection of PBMC cells from healthy volunteers to examine changes in cytokine levels. We found that the amount of IL-32, IL-1, and IFN-γ protein in serum from hepatitis B patients was significantly higher than that in healthy volunteers. Among hepatitis B patients with different HBV loads, IL-32 levels decreased as the HBV load increased. The amount of IL-32 in the serum of hepatitis B patients with a high HBV load was significantly higher than that in serum from patients with an intermediate (P < 0.05) or low (P < 0.01) HBV load; however, there was no significant difference between hepatitis B patients with intermediate or low HBV loads. Similarly, the amount of IL-1 in hepatitis B patients fell as the HBV load increased, and there was a significant difference between patients with high or intermediate HBV loads and patients with a low HBV load (P < 0.05); there was no significant difference between patients with intermediate or low HBV loads. By contrast, the amount of IFN-γ did not differ significantly between hepatitis B patients harboring different HBV loads.

Consistent results were obtained from *in vitro* analyses of HBV-stimulated PBMCs isolated from healthy volunteers. HBV stimulation led to a significant increase in the amount of IL-32, IL-1, and IFN-γ expressed by PBMC cells when compared with controls without HBV stimulation (P < 0.05). The amount of IL-32 and IL-1 secreted by PBMCs stimulated with an intermediate HBV load was significantly higher than that of PBMC cells stimulated with a low or high HBV load (P < 0.05). There was no significant difference between PBMC cells stimulated with low or high HBV loads (P > 0.05). The amount of IFN-γ secreted by PBMC cells stimulated by different HBV loads was similar.

At the gene transcript level, we detected significantly higher numbers of IL-32, IL-1, and IFN-γ transcripts in PBMCs from hepatitis B patients (P < 0.05). Also, numbers were higher in HBV-stimulated PBMCs from healthy volunteers than in PBMCs not stimulated with HBV (P < 0.05). However, transcript numbers were

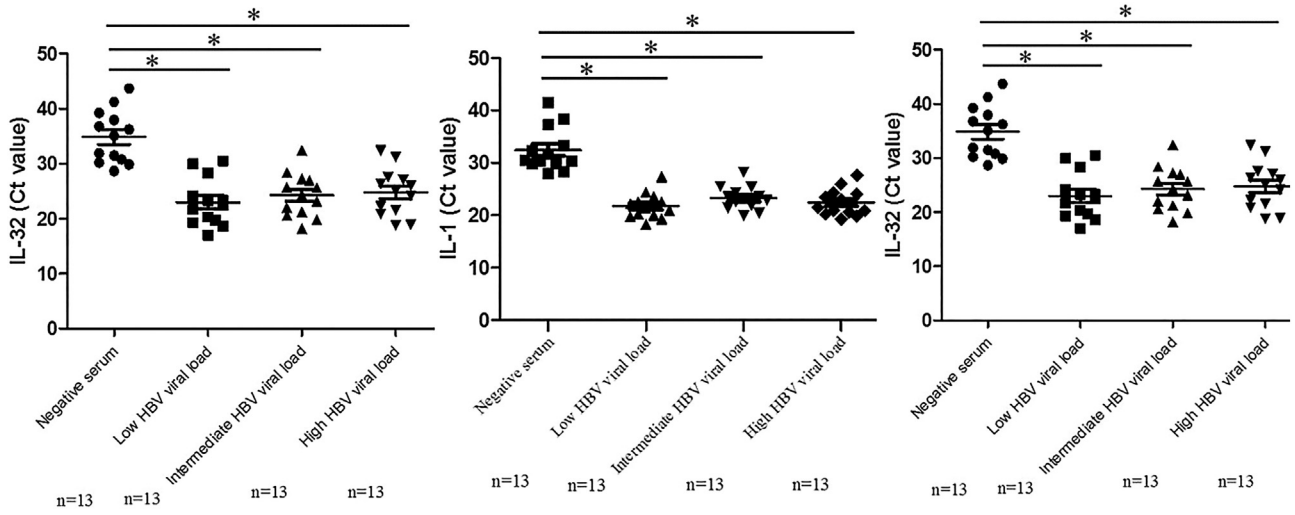

Fig. 2. Ct values for IL-32, IL-1, and IFN-γmRNA in HBV-stimulated PBMCs from healthy volunteers. *Compared with the control group ($P < 0.05$).
Note: IL: interleukin. IFN: interferon. HBV: hepatitis B virus.

not significantly different among PBMCs from hepatitis B patients stimulated with different HBV loads or PBMC cells from healthy volunteers stimulated by different HBV loads ($P > 0.05$), suggesting that HBV infection leads to complex regulation of cytokine abundance.

We speculate that infection by HBV stimulates proliferation of immune cells, which increase cytokine production to inhibit viral replication. IL-32 and IL-1 might function as pro-inflammatory cytokines, whereas IFN-γ probably interferes with the virus replication. Notably, we found that the amount of IL-32 and IL-1 protein fell in hepatitis B patients with higher HBV loads, a finding not reported in the literature. Thus, it is possible that a feed-back inhibition mechanism regulates production of IL-32 and IL-1. When the HBV load reaches a certain level, HBV itself might inhibit production of IL-32 and IL-1. Another possibility is that IL-32 and IL-1 are consumed during the process of virus inhibition. The relationship between the HBV load, IL-32, IL-1, and IFN-γ levels, and the mechanisms regulating HBV inhibition will be the focus of future studies.

Author contributions

Tian Zhao-ju designed the experiments; Tian Zhao-ju, Shen-Yu, Li Xin-rui, Wei Ya-nan, and Ren Qi-kui performed the experiments; Tian Zhao-ju and Fan Hua analyzed the data; Tian Zhao-ju, Shen-Yu, and Li Xin-rui wrote the paper.

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Competing interests

None declared.

Ethical approval

Not required.

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References

[1] Schweitzer A, Horn J, Mikolajczyk RT, Krause G, Ott JJ. Estimations of worldwide prevalence of chronic hepatitis B virus infection: a systematic review of data published between 1965 and 2013. Lancet (London, England) 2015;386:1546–55.
[2] GBD 2013 Mortality and Causes of Death Collaborators. Global, regional, and national age–sex specific all-cause and cause-specific mortality for 240 causes of death, 1990–2013: a systematic analysis for the global burden of disease study 2013. Lancet (Elsevier Ltd.) 2015;385:117–71.
[3] Schädler S, Hildt E. HBV life cycle: entry and morphogenesis. Viruses 2009;1:185–209.
[4] Vincenti D, Solmone M, Garbuglia AR, Iacomi F, Capobianchi MR. A sensitive direct sequencing assay based on nested PCR for the detection of HBV polymerase and surface glycoprotein mutations. J Virol Methods 2009;159:53–7.
[5] WHO guidelines on hepatitis B and C testing. ISBN 978-92-4-154998-1.
[6] Kim SH, Han SY, Azam T, Yoon DY, Dinarello CA. Interleukin-32: a cytokine and inducer of TNF alpha. Immunity 2005;22:131–42.
[7] Panelli MC, Wang E, Phan G, Puhlmann M, Miller L, Ohnmacht GA, et al. Gene-expression profiling of the response of peripheral blood mononuclear cells and and melanoma metastases to systemic IL-2 administration. Genome Biol 2002;3(7):1–17, research0035.
[8] Netea MG, Azam T, Ferwerda G, Girardin SE, Walsh M, Park JS, et al. IL-32 synergizes with nucleotide oligomerization domain (NOD) 1 and NOD2 ligands for IL-1beta and IL-6 production through a caspase 1-dependent mechanism. Proc Natl Acad Sci USA 2005;102(45):16309–14.
[9] Wei L, Yan L, Muhammad MM, Gong R, Pan Y, Rasool ST, et al. Activation of Iinterleukin-32 pro-inflammatory pathway in response to influenza A virus infection. PLoS One 2008;3(4):1–9.
[10] Rasool ST, Tang H, Wu J, Li W, Mukhtar MM, Zhang J, et al. Increased level of IL-32 during human immunodeficiency virus infection suppresses HIV replication. Immunol Lett 2008;117(2):161–7.
[11] Pan X, Cao H, Lu J, Shu X, Xiong X, Hong X, et al. Interleukin-32 expression induced by hepatitis B virus protein X is mediated through activation of NF-κB. Mol Immunol 2011;48(12/13):1573–7.
[12] Moschen AR, Fritz T, Clouston AD. Interleukin-32: a new proinflammatory cytokine involved in hepatitis C virus related liver inflammation and fibrosis. Hepatology 2011;53(6):1819–29.
[13] Lee S, Kim JH, Kim H, Kang JW, Kim SH, Yang Y, et al. Activation of the interleukin-32 pro-inflam matory pathway in response to human papillomavirus infection and over-expression of interleukin-32 controls the expression of the human papillomavirus oncogene. Immunology 2011;132(3):410–20.
[14] Numasaki M, Tagawa M, Iwata F, Suzuki T, Nakamura A, Okada M, et al. IL-28 elicits antitumor responses against murine fibrosarcoma. J Immunol 2007;78:5086–98.
[15] Kotenko SV, Gallagher Q, Baurin W, Lewis-Antes A, Shen M, Shah NK, et al. IFN-lambdas mediate antiviral protection through a distinctclass II cytokine receptor complex. Nat Immunol 2003;4:69–77.

[16] Joosten LA, Netea MG, Kim SH, Yoon DY, Oppers-Walgreen B, Radstake TR, et al. IL-32, a proinflammatory cytokine in rheumatoid arthritis. Proc Natl Acad Sci USA 2006;103:3298–303.
[17] Seo EH, Kang J, Kim KH, Cho MC, Lee S, Kim HJ, et al. Detection of expressed IL-32 in human stomach cancer using ELISA and immunostaining. J Microbiol Biotechnol 2008;18(9):1606–12.
[18] Mayordomo L, Marenco JL, Gomez Mateos J, Rejon E. Pulmonary military tuberculosis in a patient with anti-TNF-alpha treatment. Scand J Rheumatol 2002;31(1):45–55.
[19] Xu Q, Pan X, Shu X, Cao H, Li X, Zhang K, et al. Increased interleukin-32 expression in chronic hepatitis B virus-infected liver. Infect 2012;65(4):336–42.
[20] Bortolami M, Kotsafti A, Cardin R, Farinati F. Fas/FasL system, IL-1β expression and apoptosis in chronic HBV and HCV liver disease. J Viral Hepat 2008;15(7):515–22.