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Cytokine expression during chronic versus occult hepatitis B virus infection in HIV co-infected individuals

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Abstract

Chronic hepatitis B virus infection is characterized by persistent detectable levels of hepatitis B surface antigen (HBsAg) and HBV DNA in the serum. In contrast, HBsAg is not detectable during occult HBV infection, despite the presence of HBV DNA. An altered host immune response could play a role in the development of occult HBV infection; however, potential differences in immune responses among chronic and occult HBV-infected patients have not been evaluated *in vivo*. In the current study, we evaluated serum levels of regulatory, apoptotic, and fibrotic/anti-fibrotic cytokines/markers as indicators of immune responses in 25 chronic and 12 occult HBV-infected patients. More than half of the patients in both chronic and occult HBV infection groups had IL-2, IL-4, IL-13, and IFN- γ levels below detectable limits. In contrast, most patients had detectable levels of IL-8, IL-10, IP-10, sFas, sFasL, and TGF- β 1. Of these, only sFas was significantly different between the two groups, with lower levels observed during occult compared to chronic HBV infection ($p = 0.01$). As a surrogate marker of apoptotic inhibition, decreased sFas during occult HBV infection suggests that apoptosis occurs at different rates in occult compared to chronic HBV infection and therefore, may contribute to persistence of occult HBV infection.

Keywords

Cytokine; HBV/HIV co-infection; Occult hepatitis B virus; Soluble Fas (sFas)

1. Introduction

Globally, 350 million people are infected with chronic HBV with approximately 330,000 new cases per year in the United States alone. HBV is a non-cytopathic virus; therefore, liver damage—leading to fibrosis, cirrhosis and hepatocellular carcinoma (HCC)—is considered to be the direct result of the immune response to viral infection [1,2]. Due to their shared routes of transmission, HIV is common among persons with HBV. Moreover, liver disease has become a major cause of morbidity and mortality in HBV/HIV co-infected individuals due to prolonged survival with advances in antiretroviral therapy (ART) [3].

Numerous cytokines are involved in cell-mediated and humoral immune responses, as well as antiviral activity, viral clearance, apoptosis, and fibrogenesis. In acute HBV infection, a

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strong cell-mediated Th1 response, characterized by IL-2 and IFN- γ , is mounted against HBV and is involved in viral clearance [1,4] and immunopathogenesis of liver disease [1,5]. IFN- γ is also a major contributor of viral clearance by activating macrophages, as well as rendering neighboring uninfected cells resistant to infection [6]. In addition, in some cases, an increase in IL-10 production has been linked to persistence of chronic HBV and hepatitis C virus (HCV) infections [7]. On the other hand, a strong humoral response is characterized by increased IL-4. Chronic HBV infection develops in individuals that do not mount sufficient immune responses to clear acute HBV infection, which occurs in less than 1% of infected immunocompetent adults [8] and in about 90% of infected neonates [5]. Both cell-mediated and humoral immune responses against HBV are weak in chronically infected individuals compared to individuals with acute HBV infection [4,9]. Several factors could be involved in the development of chronic HBV infection [1], including: (1) CD8+ T-cell exhaustion or partial tolerance [10]; (2) decreased CD4+ T-cell responsiveness through dendritic cell (DC) impairment [11,12] or increased PD-1 expression [2,13]; (3) impairment of innate immune responses through decreased natural killer (NK) cell function [14]; and (4) increased number of regulatory T-cells leading to enhanced suppression of HBV-specific T-cell responses and decreased viral clearance [8,15,16], similar to that observed in HCV infection [17].

While several cytokines possess antiviral activities, other cytokines have been implicated in viral persistence and immunopathogenesis of the disease. For example, it has been demonstrated that apoptosis of HBV-infected hepatocytes occurs primarily through the Fas-mediated pathway; soluble Fas (sFas) and soluble Fas ligand (sFasL) are inhibitors of this apoptotic pathway [18], allowing for the persistence of viral infection. While they are involved in mediating liver cell injury, their presence is increased during chronic HBV infection and correlates with persistence of chronic HCV infection [19], as well as with development of HCC [20]. In addition, IL-8 has been examined as a marker of liver damage due to its ability to inhibit the antiviral activity of IFN- α [21,22]. Finally, TGF- β 1 is a major regulator of liver fibrosis. It is involved in the control of multiple cellular processes, such as growth, proliferation and differentiation, as well as control of the immune system, down regulating the Th1 response [23], and has been implicated in the development of HCC [24].

While chronic HBV infection is diagnosed by the detection of HBsAg and HBV DNA in the serum, occult HBV infection is defined by the lack of detectable circulating HBsAg in the presence of low levels of HBV replication [25]. One potential mechanism that may explain the disparity between these two distinct forms of HBV infection, is an altered host immunologic response [26]. A stronger immune response during occult HBV infection relative to chronic HBV infection could partially explain the decreased HBV DNA levels and lack of detectable HBsAg in these patients. Since this mechanism has not been investigated *in vivo* to date, we measured the serum cytokine levels of several apoptotic, fibrotic/antifibrotic and immunologic response markers to test the hypothesis that their expression levels differ between chronic and occult HBV infections.

2. Materials and methods

2.1. Patient population

Nine hundred and nine HIV-positive individuals from the University of Cincinnati Infectious Disease Center (UC IDC) were previously evaluated in a retrospective study to determine HBV status [25]. HBV DNA was quantified using real-time PCR performed in triplicate and compared to a standard panel to determine viral titer (lower limit of detection [LLD] of 100 IU/mL). Serologic markers of HBV infection (HBsAg, anti-HBc and anti-HBs) were subsequently detected by ELISA (BioChain, Hayward, CA) for patients positive for HBV DNA. Thirty-one chronic (HBsAg+/HBV DNA+) and 12 occult (HBsAg-/HBV

DNA+) HBV infections were identified. HCV status was evaluated using standard clinical enzyme-linked immunosorbent assays (ELISA). As samples were collected from 1989 to 2004, HIV RNA levels were not assessed for all individuals. When available, HIV RNA levels were determined by either qualitative or quantitative reverse transcriptase polymerase chain reactions (rtPCR).

2.2. Multiplex immunoassay

In the current retrospective analysis, serum samples were evaluated, as peripheral blood mononuclear cells (PBMCs) were not routinely collected. Serum samples were available for 25 persons with chronic HBV infection and all 12 persons with occult HBV infection.

LINCoplex multiplex immunoassays (Linco Research, St. Charles, MO) were used to determine cytokine levels from patient serum in duplicate. LINCoplex human cytokine/chemokine panel—7 plex required 25 μ L of serum to measure levels of IL-2, IL-4, IL-8, IL-10, IL-13, IFN- γ , and IP-10 (LLD of 16 pg/mL). The LINCoplex human sepsis/apoptosis panel—2 plex required 25 μ L of 1:10 diluted serum to measure levels of sFas and sFasL (LLD of 48 pg/mL). Finally, the LINCoplex TGF- β 1—1 plex kit required 50 μ L of 1:50 diluted serum to measure TGF- β 1 (LLD of 320 pg/mL). Average values were calculated for each patient and compared to a standard curve to obtain the concentration for each cytokine.

2.3. Statistical analysis

Wilcoxon rank-sum tests were performed to compare demographics for chronic and occult HBV infected patients. Fisher's exact test was performed to compare serum cytokine detection rates between chronic and occult HBV infected patients. Additional statistical analysis was performed on cytokines for which at least 50% of the patients had detectable levels (IL-8, IL-10, IP-10, sFas, sFasL, and TGF- β 1). Observed cytokine concentrations were log-transformed to approximate a normal distribution and were evaluated using Wilcoxon rank-sum tests. *P* values <0.05 were considered statistically significant for all Fisher's exact and Wilcoxon rank-sum tests. Spearman rank tests were utilized to evaluate correlations between each cytokine, as well as between each cytokine and age, gender, race, and HBV DNA titer; however, HIV RNA level was not included due to the limited number of subjects with available data. The Bonferroni correction for multiple comparisons was utilized; *p* values of <0.0014 between cytokines and <0.00125 between cytokines and age, gender, race, and HBV DNA titer, were considered statistically significant.

3. Results

3.1. Characterization of patient population

Patient demographics for the 25 patients with chronic HBV infection and the 12 with occult HBV infection are listed in Table 1. The median age for chronic HBV patients was 34.7 years and 32.9 years for occult HBV patients. Fifty-six percent of chronic HBV patients were African-American and 44% were Caucasian, while 42% of occult HBV patients were African-American and 50% were Caucasian. One hundred percent of chronic HBV patients and 75% of occult HBV patients were male. Median alanine aminotransferase (ALT) levels were 49.0 U/L in chronic HBV patients and 26.0 U/L in occult HBV patients. One occult HBV patient was also co-infected with HCV. All patients were previously diagnosed serologically with HIV infection. At the time of serum sample collection, HIV RNA was detectable by either a qualitative or a quantitative assay in 17 of 19 (84%) chronic HBV patients tested and in 7 of 7 (100%) occult HBV patients tested. Among those individuals with quantitative HIV viral loads, the median HIV RNA level was 6.3×10^4 copies/mL for chronic HBV patients and 1.7×10^5 copies/mL for occult HBV patients. Four chronic HBV

patients (16%), as well as 3 occult HBV patients (25%), were receiving antiretroviral therapy at the time of sample collection. Overall, 3 chronic HBV patients (12%) were taking at least one medication that inhibits both HIV and HBV. The median CD4+ cell count in occult HBV infections was 167 cells/mL compared to 306 cells/mL in chronic HBV infections, although this difference was not significantly different. The median HBV DNA level was significantly lower among persons with occult HBV infection, 8.2×10^3 IU/mL, compared to 8.0×10^6 IU/mL among persons with chronic HBV infection ($p = 0.019$).

3.2. Serum cytokine detection rates

Cytokine expression levels were below the LLD in greater than 50% of samples for both the chronic and occult HBV patient groups for IL-2, IL-4, IL-13, and IFN- γ (Fig. 1). IL-2 was detectable in 4% of chronic HBV patients, while it was below the detectable limit for all 12 occult HBV patients. IL-4 was detectable in 32% of chronic HBV patients and 42% of occult HBV patients, while both IL-13 and IFN- γ were detectable in 40% of chronic HBV patients and 8% of occult HBV patients. Due to the limited data available for analysis, these cytokines were excluded from further statistical evaluation. However, it is interesting to note that undetectable levels in chronic and occult HBV-infected patients were decreased compared to previously reported serum levels in healthy (HIV negative, HBV negative, HCV negative) individuals [20,27–33]. Previously published serum cytokine levels in healthy individuals, listed in Table 2, were all within the detectable ranges of the multiplex immunoassays. IL-8 and IL-10, on the other hand, were detectable in 72% and 84% of chronic HBV patients, as well as in 67% and 58% of occult HBV patients, respectively. Both sFas and sFasL had 100% detection rates in chronic HBV patients and 100% and 92% detection, respectively, in occult HBV patients. Finally, 76% of chronic HBV patients had detectable TGF- β 1, as well as 83% of occult HBV patients. There were no statistically significant differences between the detection rates in chronic versus occult HBV patients for any cytokine measured.

3.3. Serum cytokine expression levels

Decreased levels of IL-8, IL-10, IP-10, sFas and sFasL were observed in occult HBV patients compared to chronic HBV patients (Table 3). In contrast, increased levels of TGF- β 1 were observed in occult HBV infection compared to chronic HBV infection (Table 3). For both occult and chronic HBV infections, sFas levels were increased compared to previously published levels in uninfected individuals (600–1500 pg/mL) [20,30–32]. However, a statistically significant decrease in sFas levels was observed among occult HBV patients compared to chronic HBV patients (geometric mean = 7089.0 pg/mL versus 10242.3 pg/mL; $p = 0.01$) (Table 3 and Fig. 2). Additional analysis did not identify any statistically significant correlations between cytokines or between individual cytokines and age, gender, race, and HBV DNA level (data not shown).

4. Discussion

Occult HBV infection is frequently identified via retrospective testing, and long-term clinical follow-up of patients with occult HBV infection has yet to be adequately explored. Similarly, immunologic evaluation of occult HBV infection is absent from the available literature. To our knowledge, our study represents the first evaluation of serum cytokine expression profiles during occult HBV infection. The cytokines evaluated—IL-2, IL-4, IL-8, IL-10, IL-13, IFN- γ , IP-10, sFas, sFasL, and TGF- β 1—are involved in promoting antiviral activity and viral clearance, inhibition of antiviral activity, the development of viral persistence, apoptotic inhibition, and/or fibrogenesis.

Detection rates in this study were below 50% for 4 of 10 cytokines measured (IL-2, IL-4, IL-13, and IFN- γ) in both chronic and occult HBV infection groups (Fig. 1) and has been observed previously in the setting of chronic HBV infection for IL-2, IL-4, and IFN- γ [34]. While positive responses cannot be evaluated for these cytokines, cytokine levels below detectable limits may indicate that either these cytokines do not play a role in occult HBV infection, or that their absence is critically important for viral persistence, since published normal levels would be detectable. This may suggest that in occult HBV infection, both Th1 (indicated by IL-2 and IFN- γ) and Th2 responses (indicated by IL-4) are weak, similar to what has been reported in chronic HBV infection [4,9]. While HIV could also contribute to overall cytokine expression in our population [35], all individuals were HIV co-infected. Moreover, it is unlikely that antiretroviral therapy significantly impacts these results as only a small number of individuals were receiving ART in either HBV patient group.

Consistent with a previous report, IL-10 levels appeared slightly lower in individuals with chronic HBV infection compared to normal controls [36], although the differences were not statistically significant. Increased IP-10 levels compared to normal controls was also observed in all HBV/HIV co-infected individuals in this study. Increases in IP-10 have been identified previously in chronic HBV infection [37], as well as in productive HIV infection, where IP-10 synthesis was induced [38,39].

Although our study was limited by the low detection rates for certain cytokines and the relatively small number of samples evaluated, sFas levels were significantly decreased during occult HBV infection ($p = 0.01$) (Table 3 and Fig. 2). As sFas inhibits Fas-mediated apoptosis of HBV-infected hepatocytes, decreased sFas levels suggest less apoptotic inhibition, although apoptosis may still occur through alternative pathways [18]. During chronic HBV infection, increased sFas levels have been observed previously [40]. Similarly, in this study, sFas levels were significantly increased in patients with chronic HBV infection, compared to sFas levels previously measured in uninfected controls [20,30–32]. These data suggest that while apoptotic inhibition does occur during both chronic and occult HBV infections, the magnitude of apoptotic inhibition may vary between these two distinct types of HBV infection. Decreased apoptotic inhibition in occult HBV infection could improve viral clearance and partially explain the low levels of viral replication often observed during occult HBV infection. Moreover, increased apoptosis of infected hepatocytes could potentially increase or accelerate liver damage, although the clinical consequences of occult HBV infection remain understudied.

Future longitudinal studies are necessary to further evaluate the role of apoptosis in occult HBV infection, as well as to characterize the immune responses that may distinguish occult HBV infections from chronic HBV infections *in vivo*.

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References

1. Chang JJ, Lewin SR. Immunopathogenesis of hepatitis B virus infection. *Immunol Cell Biol* 2006;85:16–23. [PubMed: 17130898]
2. Isogawa M, Furuichi Y, Chisari FV. Oscillating CD8+ T cell effector functions after antigen recognition in the liver. *Immunity* 2005;23:53–63. [PubMed: 16039579]

3. Thio CL, Seaberg EC, Skolasky JR, Phair J, Visscher B, Munoz A, et al. HIV-1, hepatitis B virus, and risk of liver-related mortality in the Multicenter Cohort Study (MACS). *The Lancet* 2002;360:1921–6.
4. Szkaradkiewicz A, Jopek A, Wysocki J, Grzymislawski M, Malecka I, Wozniak A. HBcAg-specific cytokine production by CD4 T lymphocytes of children with acute and chronic hepatitis B. *Virus Res* 2003;97:127–33. [PubMed: 14602204]
5. Jung MC, Pape GR. Immunology of hepatitis B infection. *Lancet Infect Dis* 2002;2:43–50. [PubMed: 11892495]
6. Guidotti LG, Chisari FV. Cytokine-mediated control of viral infections. *Virology* 2000;273:221–7. [PubMed: 10915592]
7. Blackburn SD, Wherry EJ. IL-10, T cell exhaustion and viral persistence. *Trends Microbiol* 2007;15:143–6. [PubMed: 17336072]
8. Norman JE, Beebe GW, Hoofnagle JH, Seeff LB. Mortality follow-up of the 1942 epidemic of hepatitis B in the US Army. *Hepatology* 1993;18:790–7. [PubMed: 8406352]
9. Chichester JA, Feitelson MA, Calkins CE. Transient inhibition of Th1-type cytokine production by CD4+ T cells in hepatitis B core antigen immunized mice is mediated by regulatory T cells. *Immunology* 2006;118:438–48. [PubMed: 16762029]
10. Reignat S, Webster GJM, Brown D, Ogg GS, King A, Seneviratne SL, et al. Escaping high viral load exhaustion: CD8 cells with altered tetramer binding in chronic hepatitis B virus infection. *J Exp Med* 2002;195:1089–101. [PubMed: 11994415]
11. Beckebaum S, Cicinnati VR, Zhang X, Ferencik S, Frilling A, Grosse-Wilde H, et al. Hepatitis B virus-induced defect of monocyte-derived dendritic cells leads to impaired T helper type 1 response in vitro: mechanisms for viral immune escape. *Immunology* 2003;109:487–95. [PubMed: 12871214]
12. van der Molen RG, Sprengers D, Binda RS, de Jong EC, Niesters HGM, Kusters JG, et al. Functional impairment of myeloid and plasmacytoid dendritic cells of patients with chronic hepatitis B. *Hepatology* 2004;40:738–46. [PubMed: 15349914]
13. Boettler T, Panther E, Bengsch B, Nazarova N, Spangenberg HC, Blum HE, et al. Expression of the interleukin-7 receptor alpha chain (CD127) on virus-specific CD8+ T cells identifies functionally and phenotypically defined memory T cells during acute resolving hepatitis B virus infection. *J Virol* 2006;80:3532–40. [PubMed: 16537621]
14. Sprengers D, van der Molen RG, Kusters JG, Hansen B, Niesters HGM, Schalm SW, et al. Different composition of intrahepatic lymphocytes in the immune-tolerance and immune-clearance phase of chronic hepatitis B. *J Med Virol* 2006;78:561–8. [PubMed: 16555293]
15. Xu D, Fu J, Jin L, Zhang H, Zhou C, Zou Z, et al. Circulating and liver resident CD4+CD25+ regulatory T cells actively influence the antiviral immune response and disease progression in patients with hepatitis B. *J Immunol* 2006;177:739–47. [PubMed: 16785573]
16. Stoop JN, van der Molen RG, Baan CC, van der Laan LJW, Kuipers EJ, Kusters JG, et al. Regulatory T cells contribute to the impaired immune response in patients with chronic hepatitis B virus infection. *Hepatology* 2005;41:771–8. [PubMed: 15791617]
17. Boettler T, Spangenberg HC, Neumann-Haefelin C, Panther E, Urbani S, Ferrari C, et al. T cells with a CD4+CD25+ regulatory phenotype suppress in vitro proliferation of virus-specific CD8+ T cells during chronic hepatitis C virus infection. *J Virol* 2005;79:7860–7. [PubMed: 15919940]
18. Hayashi N, Mita E. Involvement of Fas system-mediated apoptosis in pathogenesis of viral hepatitis. *J Viral Hepat* 1999;6:357–65. [PubMed: 10607251]
19. Iio S, Hayashi N, Mita E, Ueda K, Mochizuki K, Hiramatsu N, et al. Serum levels of soluble Fas antigen in chronic hepatitis C patients. *J Hepatol* 1998;29:517–23. [PubMed: 9824259]
20. Jodo S, Kobayashi S, Nakajima Y, Matsunaga T, Nakayama N, Ogura N, et al. Elevated serum levels of soluble Fas/APO-1 (CD95) in patients with hepatocellular carcinoma. *Clin Exp Immunol* 1998;112:166–71. [PubMed: 9649177]
21. Khabar KSA, Al-Zoghaibi F, Al-Ahdal MN, Murayama T, Dhalla M, Mukaida N, et al. The alpha chemokine, interleukin 8, inhibits the antiviral action of interferon alpha. *J Exp Med* 1997;186:1077–85. [PubMed: 9314556]
22. Friedman SL. Liver fibrosis—from bench to bedside. *J Hepatol* 2003;38:38–53.

23. Friedman SL. Molecular regulation of hepatic fibrosis, an integrated cellular response to tissue injury. *J Biol Chem* 2000;275:2247–50. [PubMed: 10644669]
24. Matsuzaki K, Date M, Furukawa F, Tahashi Y, Matsushita M, Sakitani K, et al. Autocrine stimulatory mechanism by transforming growth factor beta in human hepatocellular carcinoma. *Cancer Res* 2000;60:1394–402. [PubMed: 10728705]
25. Shire NJ, Rouster SD, Stanford SD, Blackard JT, Martin CM, Fichtenbaum CJ, et al. The prevalence and significance of occult hepatitis B virus in a prospective cohort of HIV-infected patients. *JAIDS* 2007;44:309–14. [PubMed: 17159656]
26. Hu KQ. Occult hepatitis B virus infection and its clinical implications. *J Viral Hepat* 2002;9:243–57. [PubMed: 12081601]
27. Lyon DE, McCain NL, Walter J, Schubert C. Cytokine comparisons between women with breast cancer and women with a negative breast biopsy. *Nurs Res* 2008;57:51–8. [PubMed: 18091292]
28. Hoge EA, Brandstetter K, Moshier S, Pollack MH, Wong KK, Simon NM. Broad spectrum of cytokine abnormalities in panic disorder and posttraumatic stress disorder. *Depress Anxiety* 2009;26:447–55. [PubMed: 19319993]
29. Shimada A, Morimoto J, Kodama K, Suzuki R, Oikawa Y, Funae O, et al. Elevated serum IP-10 levels observed in type 1 diabetes. *Diabetes Care* 2001;24:510–5. [PubMed: 11289477]
30. Wetzig T, Petri JB, Mittag M, Hausteil UF. Serum levels of soluble Fas/APO-1 receptor are increased in systemic sclerosis. *Arch Dermatol Res* 1998;290:187–90. [PubMed: 9617437]
31. Hefler L, Mayerhofer K, Nardi A, Reinthaller A, Kainz C, Tempfer C. Serum soluble Fas levels in ovarian cancer. *Obstet Gynecol* 2000;96:65–9. [PubMed: 10862844]
32. Suzuki K, Endo R, Nakamura N, Yatomi T, Iwai M, Abe K, et al. Serum levels of soluble Fas ligand in patients with acute and fulminant hepatitis: relationship with soluble Fas, tumor necrosis factor [alpha] and TNF receptor-1. *Hepatol Res* 2000;17:19–30.
33. Scala E, Pallotta S, Frezzolini A, Abeni D, Barbieri C, Sampogna F, et al. Cytokine and chemokine levels in systemic sclerosis: relationship with cutaneous and internal organ involvement. *Clin Exp Immunol* 2004;138:540–6. [PubMed: 15544634]
34. Ayada M, Ishikawa T, Okumura A, Tanabe J, Ito H, Ohashi T, et al. Alteration of serum cytokine balances among different phases of chronic hepatitis B virus infection. *Hepatol Res* 2006;34:214–21. [PubMed: 16469532]
35. Breen EC. Pro- and anti-inflammatory cytokines in human immunodeficiency virus infection and acquired immunodeficiency syndrome. *Pharmacol Ther* 2002;95:295–304. [PubMed: 12243799]
36. Song LH, Binh VQ, Duy DN, Kun JFJ, Bock TC, Kremsner PG, et al. Serum cytokine profiles associated with clinical presentation in Vietnamese infected with hepatitis B virus. *J Clin Virol* 2003;28:93–103. [PubMed: 12927756]
37. Wang J, Zhao JH, Wang PP, Xiang GJ. Expression of CXC chemokine IP-10 in patients with chronic hepatitis B. *Hepatobiliary Pancreat Dis Int* 2008;7:45–50. [PubMed: 18234638]
38. Izmailova E, Bertley FMN, Huang Q, Makori N, Miller CJ, Young RA, et al. HIV-1 Tat reprograms immature dendritic cells to express chemoattractants for activated T cells and macrophages. *Nat Med* 2003;9:191–7. [PubMed: 12539042]
39. Wetzel MA, Steele AD, Henderson EE, Rogers TJ. The effect of X4 and R5 HIV-1 on C, C-C, and C-X-C chemokines during the early stages of infection in human PBMCs. *Virology* 2002;292:6–15. [PubMed: 11878903]
40. Lapinski TW, Kowalczyk O, Prokopowicz D, Chyczewski L. Serum concentration of sFas and sFasL in healthy HBsAg carriers, chronic viral hepatitis B and C patients. *World J Gastroenterol* 2004;10:3650–3. [PubMed: 15534924]

Fig. 1.

Serum cytokine detection rates. Cytokine levels above detectable limits in serum of chronic and occult HBV infections. Black bars indicate percent of patients with detectable limits out of 25 chronic HBV infections. White bars indicate percent of patients with detectable limits out of 12 occult HBV infections. Lower limit of detection = 16 pg/mL for IL-2, IL-4, IL-8, IL-10, IL-13, IFN- γ , and IP-10. Lower limit of detection = 48 pg/mL for sFas and sFasL. Lower limit of detection = 320 pg/mL for TGF- β 1.

Fig. 2. sFas levels. sFas cytokine levels in chronic HBV infections ($n = 25$) and occult HBV infections ($n = 12$). (○) Represents individual sFas levels for each patient. (●) Represents the geometric mean sFas level for each infection group and the vertical line represents the 95% confidence interval. The horizontal dashed line represents the average of previously reported normal sFas levels in healthy controls [20,30–32]. sFas levels were significantly decreased during occult HBV infection compared to chronic HBV infection ($p = 0.01$).

Table 1

Patient demographics. Serum samples from 37 patients—25 with chronic HBV infection and 12 with occult HBV infection—were used for cytokine analysis. The median HBV DNA level is significantly decreased in occult HBV infections compared to chronic HBV infections ($p = 0.019$) indicated in bold, using the Wilcoxon rank-sum test.

HBV infection $n = 37$	Chronic $n = 25$	Occult $n = 12$
<i>Age^a</i>	34.7 years (21.2–51.3)	32.9 years (25.2–47.1)
<i>Race</i>		
African-American	14 (56%)	5 (42%)
Caucasian	11 (44%)	6 (50%)
Other	0	1 (8%)
<i>Gender</i>		
Male	25 (100%)	9 (75%)
Female	0	3 (25%)
<i>ALT^{a,b} $n = 34$</i>	49.0 U/L (16–158)	26.0 U/L (7–87)
<i>HCV Co-Infection^b $n = 20$</i>	0	1 (14%)
<i>HIV^b</i>		
Detectable $n = 26$	17 (89%)	7 (100%)
Viral load ^a $n = 13$	6.3 $\times 10^4$ copies/mL (7.2 $\times 10^2$ –2.0 $\times 10^5$)	1.7 $\times 10^5$ copies/mL (1.0 $\times 10^4$ –3.7 $\times 10^5$)
<i>ART</i>		
On therapy	4 (16%)	3 (25%)
With HBV activity	3 (12%)	0
<i>CD4⁺ count^{a,b} $n = 36$</i>	306 cells/mL (7–665)	167 cells/mL (6–753)
<i>HBV DNA^a</i>	8.0 $\times 10^6$ IU/mL (1.1 $\times 10^2$ –7.6 $\times 10^8$)	8.2 $\times 10^3$ IU/mL (1.1 $\times 10^2$–7.6 $\times 10^5$)

^a Median values are given with the range in parentheses.

^b Information was not available for all individuals (number of patients with available information are listed next to the heading); therefore, the median ALT and CD4⁺ count and percent detectable HCV and HIV are given for the individuals tested.

Table 2

Normal serum cytokine levels. Average serum cytokine levels from healthy individuals as previously published (in pg/mL).

Cytokine	Normal level (pg/mL)
IL-2 ^a	28.1
IL-4 ^a	144.6
IL-8 ^a	59.9
IL-10 ^a	40.9
IL-13 ^a	19.6
IFN- γ ^a	19.8
IP-10 ^b	82.6
sFas ^c	1049.0
sFasL ^d	130.0
TGF- β 1 ^e	3542.0

^aRefs. [27,28].

^bRefs. [28,29].

^cRefs. [20,30–32].

^dRef. [32].

^eRef. [33].

Table 3

Measured serum cytokine levels. Number of patients and percentages in parentheses are given for cytokines with detectable levels above 50% in occult and chronic HBV patients. Geometric mean values in pg/mL (with 95% confidence intervals in parentheses) are given for each cytokine during occult and chronic HBV infections. *p* Values were determined using Wilcoxon rank-sum tests. The comparison for sFas was statistically significant (<0.05) and is indicated in bold.

Cytokine	Infection status	Detection	Geometric mean (95% CI)	<i>p</i> Value
IL-8	Occult	8 (67%)	13.7 pg/mL (6.1–30.4)	0.31
	Chronic	18 (72%)	39.9 pg/mL (23.9–66.4)	
IL-10	Occult	7 (58%)	26.0 pg/mL (12.2–55.6)	0.47
	Chronic	21 (84%)	49.6 pg/mL (29.1–84.6)	
IP-10	Occult	12 (100%)	381.7 pg/mL (247.5–558.8)	0.20
	Chronic	25 (100%)	515.8 pg/mL (430.7–617.8)	
sFas	Occult	12 (100%)	7089.0 pg/mL (5644.1–8903.8)	0.01
	Chronic	25 (100%)	10242.3 pg/mL (9054.4–11586.0)	
sFasL	Occult	11 (92%)	126.3 pg/mL (85.6–186.4)	0.24
	Chronic	25 (100%)	168.9 pg/mL (130.6–218.4)	
TGF- β 1	Occult	10 (83%)	10300 pg/mL (2900–36800)	0.72
	Chronic	19 (76%)	4400 pg/mL (1200–16900)	