

Cytokine Expression During Chronic Versus Occult Hepatitis B Virus Infection in HIV Coinfected Individuals

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Hepatitis B Virus



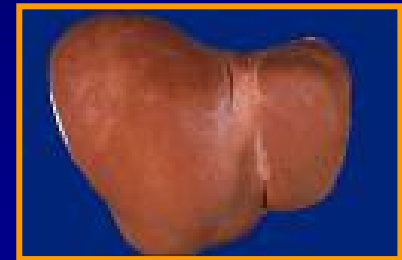
- 350 million chronic HBV infections worldwide
- Common coinfection with HIV and HCV
- Liver disease is a major cause of morbidity and mortality in HIV+ patients
- *Chronic HBV* is characterized by presence of hepatitis B surface antigen (HBsAg) and HBV DNA in the serum

Occult HBV Infection

- *Occult HBV* lacks detectable HBsAg in the serum although HBV DNA is still present
- Antibodies against HBV core protein (anti-HBc) are often the only detectable serologic marker
 - Serologically negative individuals have been identified¹
- Identified most often with HIV and/or HCV coinfection

What Do We Know?

- Prevalence of occult HBV infection ranges from 0% to 65%
- Occult HBV is transmissible through blood transfusion and liver transplantation¹⁻³
- Potentially influences the development of cirrhosis and hepatocellular carcinoma (HCC)^{4,5}



1. Hoofnagle JH, et al. *NEJM*. 1978;298:1379-83; 2. Liu CJ, et al. *J Hepatol*. 2006;44:39-46;
3. Thiers V, et al. *Lancet*. 1988;2:1273-6; 4. Koyama H, et al. *Osaka City Med J*. 1988;34:51-66;
5. Uchida T, et al. *Liver*. 1994;14:251-6.

What Do We Need To Know?

- Risk factors for occult HBV infection
- Clinical outcomes directly related to occult HBV infection
 - Longitudinal analysis yet to be performed
- Mechanisms linking occult HBV and HCC development
- Role of the immune response in occult HBV infection compared with chronic HBV infection

Potential Mechanisms

- HBsAg contained in immune complexes
- Interference of replication by other viruses
- Viral mutations that reduce HBsAg expression, secretion, and/or detection
- Altered host immunologic response

Experimental Design

- Retrospective study using previously identified HBV infections from HIV+ patients¹
 - 25 chronic and 12 occult HBV serum samples
 - PBMCs and liver biopsies not routinely available for HIV patient cohorts
- Multiplex immunoassay to measure serum levels of 10 cytokines as markers of immune response
 - Regulatory: IL-2, -4, -13, IFN- γ and IP-10
 - Apoptotic: sFas and sFasL
 - Fibrotic/anti-fibrotic: IL-8, -10 and TGF- β 1

Patient Demographics

HBV Infection n = 37	Chronics n = 25	Occults n = 12
Age¹	34.7 years (21.2 – 51.3)	32.9 years (25.2 – 47.1)
Race		
African American	14 (56%)	4 (42%)
Caucasian	11 (44%)	6 (50%)
Other	0	1 (8%)
Gender		
Male	25 (100%)	9 (75%)
Female	0	3 (25%)
ALT^{1, 2} n = 34	49.0 U L ⁻¹ (16 – 158)	26.0 U L ⁻¹ (7 – 87)
Detectable HCV² n = 20	0	1 (14%)
Detectable HIV² n = 26	17 (89%)	7 (100%)
CD4+ count^{1, 2} n = 36	306 cells mL ⁻¹ (7 – 665)	167 cells mL ⁻¹ (6 – 753)
HBV DNA¹	8.0×10^6 IU mL ⁻¹ (1.1×10^2 – 7.6×10^8)	8.2×10^3 IU mL ⁻¹ (1.1×10^2 – 7.6×10^5)

**P* = 0.019

Healthy Control Serum Cytokine Levels

- Previously published normal serum levels¹⁻⁷

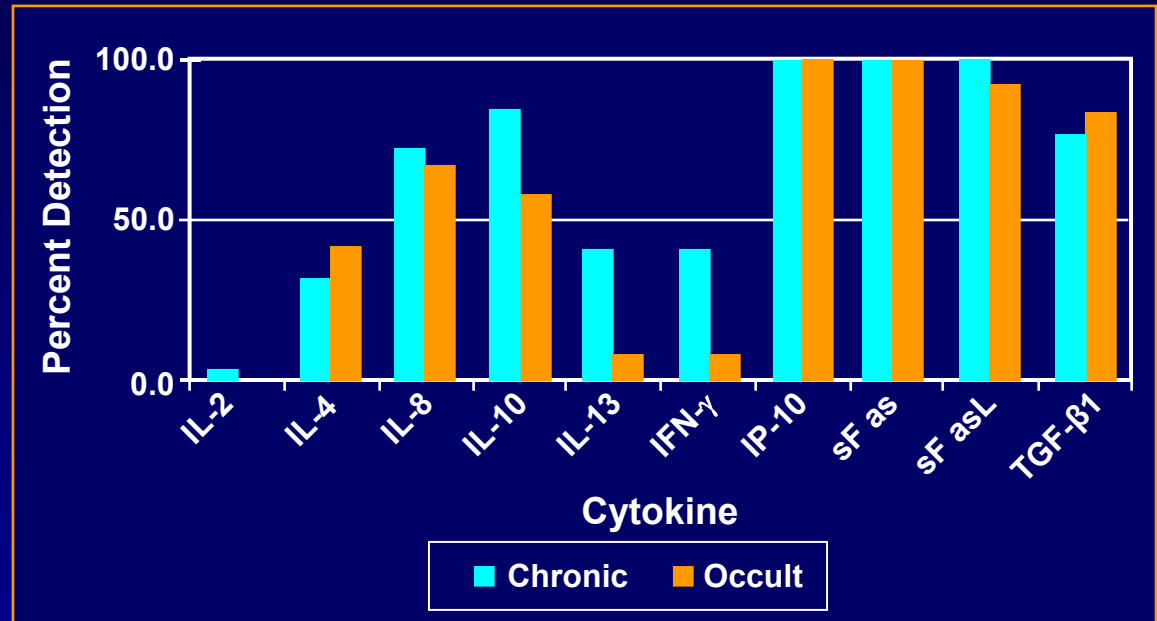
1. Lyon DE, et al. *Nurs Res.* 2008;57:51-8.
2. Shimada A, et al. *Diabetes Care.* 2001;24:510-5.
3. Jodo S, et al. *Clin Exp Immunol.* 1998;112:166-71.
4. Wetzig T, et al. *Arch Dermatol Res.* 1998;290:187-90.
5. Hefler L, et al. *Obstet Gynecol.* 2000;96:65-9.
6. Suzuki K, et al. *Hepato Res.* 2000;17:19-30.
7. Scala E, et al. *Clin Exp Immunol.* 2004;138:540-6.

- All normal levels are within the detectable limits of the multiplex assay (16-320 pg/mL)

Cytokine	Normal Level (pg/mL)	Reference(s)
IL-2	36.8	Lyon
IL-4	236.8	Lyon
IL-8	108.7	Lyon
IL-10	76.5	Lyon
IL-13	34.5	Lyon
IFN- γ	34.0	Lyon
IP-10	41.5	Shimada
sFas	1049.0	Jodo, Wetzig, Hefler, Suzuki
sFasL	130.0	Suzuki
TGF- β 1	3542.0	Scala

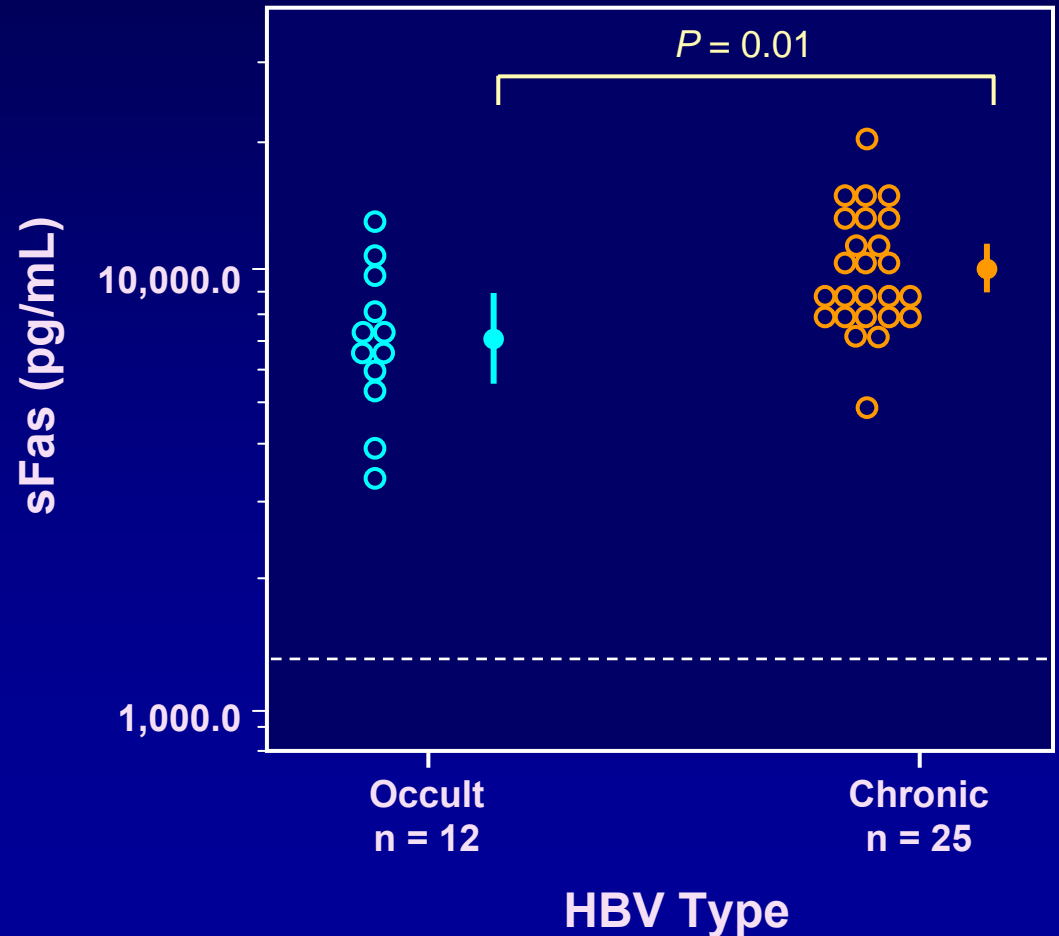
Serum Cytokine Detection Rates in Chronic Versus Occult HBV

- Several patients without detectable cytokine levels
- Differences in detection rates between chronic and occult HBV infections were not statistically significant
- 6 cytokines with at least 50% detection in both groups subjected to additional statistical analysis
 - Significant difference between chronic and occult HBV infections for sFas only



sFas Levels in Chronic Versus Occult HBV

- Significantly increased in both chronic and occult HBV infections compared with published healthy controls
- Significantly decreased sFas levels in occult HBV infections compared with chronic



Conclusions

- Similar to chronic HBV infection, several cytokines have limited detection during occult HBV infection and their absence may be important in viral persistence
- Increased sFas levels in occult HBV infection compared with healthy control levels also support viral persistence through apoptotic inhibition
- Significantly lower sFas levels in occult HBV infection compared with chronic HBV infection suggests increased levels of apoptosis possibly indicating similar or slightly increased pathogenesis in occult HBV

Future Directions

- Longitudinal analysis of occult HBV infection
 - Consistent lack of detectable HBsAg?
 - Clinical outcomes?
 - Persistent HBV mutations?
 - Immune responses?
- In vitro studies
 - Impact of mutants on HBsAg expression, secretion and/or detection

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