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Summary

HIV-HBV infected patients require optimal control of viral replication in order to prevent severe comorbidities, such as liver cirrhosis and hepatocellular carcinoma. The genetic diversity of HBV is a poorly investigated factor of such viral replication in HIV-infected hosts. HBV genome diversity can be differentiated in two major aspects: genotypic and phenotypic. Genotypic diversity is more related to the natural history of HBV infection and genotypes are mostly determined by geographical origin. Phenotypic diversity arises from attempts to escape from host immune surveillance (i.e. Precore, Core, and Basal Core Promoter mutants), selection due to the use of treatments with weak genetic barrier (i.e. Pol mutants), exposure to hepatitis B immunoglobulin (i.e. "immune-escape" S mutants), or treatment-induced mutations from overlapping genes (i.e. Pol mutants inducing "vaccine-escape" S mutants). Pol mutations typically lead to uncontrolled viral replication, whereas S gene mutations

can significantly alter HBsAg synthesis and reduce binding to antiHBs antibodies, which renders persons vaccinated or cured of HBV-infection susceptible to infection. During coinfection with HIV, treatment options must be seriously considered with the aim of reducing the risk of HBV mutations, thereby preventing their clinical consequences for which the public health implications are fairly unknown.

Introduction

The decrease in AIDS-related mortality and morbidity due to effective combined antiretroviral therapy (cART) post 1996 has ushered a new era of liver-related diseases associated with chronic hepatitis, which is now the second leading cause of death in HIV-infected patients [1]. About 7% to 10% of all HIV-infected patients are chronically co-infected with hepatitis B virus (HBV) and there is now strong evidence of higher AIDS-events and death rates in this population [2],[3]. In HBV/HIV-coinfected hosts, the occurrence of hepatocellular carcinoma as a consequence of long-lasting chronic hepatitis replication is already a reality, with chronic HBV being the cause of death among 11% of HCC cases, as reported in France in 2008 [4]. Evidence from the REVEAL study (conducted in a cohort of HBV-mono-infected patients) [5] suggests that one of the best ways to prevent liver fibrosis progression, and ultimately the emergence of liver cancer in chronic HBV carriers, is to optimally control viral replication with efficient antiviral therapy alongside careful clinical follow-up. However, all the determinants of HBV pathogenicity in HIV co-infection have not yet been fully elucidated – one of them being the genetic diversity of HBV itself. The role of HBV genetic diversity, regardless of its influence from long-term exposure to drugs with dual anti-HIV and anti-HBV activity, has been proposed as a possible cofactor in hepatitis B evolution as well as in HBV transmission.

Origins of HBV genetic diversity

HBV is the only human virus belonging to the *Hepadnaviridea* family whose genome is composed of four open reading frames (ORFs) [6]. The preS/S region encodes three proteins, one of which being HBsAg. The pre C/C region encodes the hepatitis B e antigen (HBeAg) and hepatitis B core antigen. The P ORF (*pol*) specifically encodes viral polymerase, the main target of all oral anti-HBV agents currently used to treat chronic hepatitis B. Lastly, the X ORF encodes a protein involved in host and viral gene expression [7]. The replication of the DNA genome occurs via reverse

transcription mediated by the pregenomic RNA, for which polymerase plays an essential role. A major characteristic of the HBV genome is its extensive overlapping of genes. A mutation on one nucleotide might have little effect on the expression of one protein, but can severely impact the expression of an overlapping gene. This effect explains why mutations on the P gene, mainly selected by drug exposure, may induce mutations on the S gene that results in an altered expression of HBsAg.

The diversity of HBV genome can be viewed from two perspectives [8]: Genotypic diversity, which results from the gradual evolution of the HBV genome in the absence of selective pressure; phenotypic diversity, which is a consequence of exerted selective pressures (e.g., antiviral drugs, etc.). Such genomic variability has been poorly addressed in the context of HIV coinfection, even though the prevalence of chronic HBs Ag carriers ranges between 7 to 15% among HIV infected patients who have been extensively treated with dual anti-HIV and anti-HBV drugs since the late 1980s.

Genotypic diversity

Serotypes and genotypes are the two principal outcomes of HBV genotypic variability. Serotypes are derived from the intrinsic nature of the viral strain and result from HBsAg reactivities to a standard panel of antisera. Classification is performed according to the amino-acid composition of two mutually exclusive epitopes located at positions 126 and 160 of HBsAg. Serotyping was mainly used for epidemiological purposes (outbreak investigation, etc.). So far, there is no evidence of the influence of HIV on HBV serotype expression.

Genotypes were until recently divided into 8 categories (A to H) which diverge from one to another by at least 8% of amino-acid sequences. Two new genotypes, designated I and J, have been discovered in 2008 in Laotian and Japanese patients [9],[10]. In HBV monoinfection, genotypes seem to be determined mostly by the patient’s geographical origin. Genotypes A and D are ubiquitous, whereas B and C are mostly found in Asia, with E predominantly in Africa, and F and H genotypes probably of Pacific and Latin American origin. Until now, the origin of genotype G remains a mystery and has been described only in Europe, USA and Japan. As in HBV monoinfection, the distribution of genotypes among HIV/HBV coinfected

patients is dependent upon the geographical origin of the patient. In three studies of HIV/HBV coinfected patients from France (n=206) [11], USA/Australia (n=53) [12] and Spain (n=62) [13], genotypes A and D were predominant (76%, 94% and 90%, respectively), and genotypes B and C were not observed. However, 11% of the French study sample was infected with genotype E, reflecting the high proportion of patients from Africa. Similarly, 3% of the study population harboured genotype F, reflecting patients originating from Latin America. Finally, the proportion of genotype G-infected patients varied: 5% from Spain, 6% from USA and Australia and 12.3% from France. In HBV monoinfection, the clinical and therapeutic consequences of genotypes are still doubtful because studies addressing the issue have been conducted in countries with a narrow genotypic range. Moreover, these studies made direct endpoint comparisons between at most 2 genotypes. In the context of HIV/HBV coinfection, only one study to date has addressed the impact of HBV genotypes on liver disease severity [11]. In this cohort study, a retrospective analysis found a strong association between the degree of liver fibrosis and the presence of genotype G, after adjustment on age, sex, anti-HBV treatment, the level of immunodeficiency and the presence of hepatitis C with or without hepatitis D infection. No association has been established between genotypes and response to treatment with nucleos(t)ide analogs [14],[15].

Phenotypic diversity

Phenotypic mutants can occur under selective pressures resulting from host-immune responses to natural HBV infection as well as from prophylactic or therapeutic interventions.

C gene mutations

Precore, core and basal core promoter (BCP) mutants are derived from aberrant coding of the preC/ C ORF. They mainly represent attempts to escape from host immune surveillance and emerge at later stages of chronicity, thereby reflecting long-term evolution of HBV disease [16]. PreC and BCP mutations affect HBeAg expression. Specific BCP mutations, such as T1753C, A1762T, G1764A and C1766T, have been associated with a decreased synthesis in mRNA and thus a down regulation of HBeAg expression [17],[18],[19]. The consequences of such mutations are a reduction of synthesized HBeAg and an enhanced viral replication,

which have been associated with fulminant hepatitis in HBV monoinfected patients [20],[21]. The prevalence of double A1762T/G1764A and triple T1753C/A1762T/G1764A mutations found in sequenced strains present in the USA/Australia study of 81 HIV/HBV coinfected patients was 15% and 6%, respectively [12], yet its impact on disease severity has not been studied due to the cross-sectional nature of the study.

PreC mutations completely abolish HBeAg synthesis by disruption of the pre C reading frame. The most common mutation is a nonsense mutation of codon 28 in the precore region. The expression of preC mutations is strongly linked to HBV genotype and is often associated with BCP mutations. In HIV/HBV coinfection, their prevalence varies between studies: 10% in the USA/Australia study [12], 25% in the French [11] and Spanish [13] studies. In the French study, the presence of the W28 mutation was associated with advanced liver fibrosis. However, this effect was abrogated by coinfection with genotype G, which exhibits two stop codons in the preC region, at positions 2 and 28, consequently preventing translation of the HBeAg precursor and leading to a predominance of preC mutants in HBV/G-infected patients [22],[23].

P gene (mutations

The spontaneous heterogeneity of the HBV genome generates a complex mixture of *pol* mutant quasi-species whose composition evolves over time according to selective pressures exerted by nucleos(t)ide analogs. When drugs are used with a weak genetic barrier or at incorrect dosing, mutants are progressively selected and a mutation can emerge as predominant, leading to viral breakthrough. Cross-resistance is common with lamivudine, emtricitabine, entecavir and telbivudine. Mutations in the B domain (V173L and L180M) and C domain (M204I/V/S) of the reverse transcriptase region of the P gene emerge in more than 90% of HIV/HBV coinfected patients after 4 years of lamivudine exposure [24]. Mutations selected by exposure to entecavir (I169T and T184G in the B domain, S202G/I in the C domain and M250V in the D domain) appear at a higher rate in patients already exposed to lamivudine, as observed in 5% of 68 HIV/HBV coinfected patients with lamivudine-resistant strains undergoing a clinical trial of entecavir [25]. No data are available on the emergence of resistance in coinfected patients treated with emtricitabine or

telbivudine so far, since the former is never prescribed alone for HIV-infected patients and the use of the latter in HIV infection has been constrained due to its possible anti-HIV activity [26], although yet to be corroborated by *in vitro* analysis [27].

In regard to adefovir and tenofovir, data on the acquisition of HBV resistance in the context of HIV/HBV confection are scarce. While an efficient antiviral activity of adefovir in patients harbouring lamivudine-resistant HBV strains has been previously reported [28], the appearance of an A181T mutation conferring resistance to adefovir has been described in an HIV/HBV coinfected patient experiencing a second viral breakthrough while on adefovir, after a previous breakthrough associated with takeover by a lamivudine-resistant mutant [29]. Finally, the A194T mutation in the B domain might confer partial resistance to tenofovir which can lead to an increased viral replication when associated with pre-existing preC and BCP mutations, as seen in HBeAg seronegative patients [30]. However, these data were obtained *in vitro* and have not thus far been confirmed *in vivo*. Indeed, this mutation was not found in patients with viral replication after a mean 13 months of tenofovir treatment [31]. Furthermore, an HIV/HBV coinfected patient from France [32] and several HBV-monoinfected patients from Canada [33] who were already harbouring an A194T strain at tenofovir initiation were still virologically suppressed after 36 and 18 months of follow-up, respectively.

S gene mutations

PreS and S mutants stem from aberrant encoding of the preS/S ORF. Two main preS mutations, preS1 and preS2, have been described, which seem to emerge during chronic infection as a consequence of host-immune response evasion [8]. Their transmission potential as well as pathogenicity are unclear. The frequency of preS2 mutation in the USA/Australia study of HIV/HBV coinfected patients was 16%, most of whom experienced lamivudine resistance with high levels of HBV replication [12].

HBsAg synthesis can be significantly altered due to S gene mutations. The selection of such mutants is triggered by exposure to hepatitis B immunoglobulin [34] or by pressure on *pol* that induces mutations on the overlapping portion of the S gene. The former may be referred to as “immune-escape” mutants, and the latter as “vaccine-escape” mutants. The immunodominant “a” determinant of HBsAg is the major target

for neutralizing antibodies. An insertion during its encoding can produce a replicative HBV strain whose HBsAg is undetectable by commonly used immunoassays. This phenomenon was observed in 23% of an HIV/HBV coinfected cohort starting antiretroviral treatment in South Africa [35], and might be associated with profound HIV-related immunosuppression. Most of the S gene mutations are due to missense mutations. Both P120T/S and G145R/K/A have been reported to decrease the antigenicity and immunogenicity of HBsAg [36],[37]. Such mutational patterns were found in 13% of 53 patients with ongoing HBV replication [11]. More alarming patterns of S gene mutations occur in the *pol*/S gene overlap selected during exposure to nucleos(t)ides analogs. In particular, the E164D, W196S, I195M, M198I and E164D/I195M emerge in connection with lamivudine resistance and are responsible for reduced binding to antiHBs antibodies [38]. Furthermore, a recent study of vaccinated chimpanzees has shown that the E164D/I195M mutation, associated with the 173/180/204 triple mutations acquired under lamivudine, emtricitabine or telbivudine therapy, could lead to infection despite the presence of high antiHBs in their blood [39]. Such mutations have been found in 17% of the USA/Australia study [12].

Implications

The entangling of HIV and HBV epidemics and the scaling up of antiretroviral use at a worldwide level underlie the emergence of new constellations of HBV mutants. Furthermore, the degree of immunosuppression may influence the extent of HBV genetic variability in HIV/HBV coinfected patients. Recent data presented at the Conference on Retroviruses and Opportunisic Infections (CROI) in 2009 showed that HIV-infected patients with mild immunosuppression (defined as a CD4+ cell count above 600/mm3) are similar to patients without HIV infection regarding plasma-circulating HBV immune-escape mutants, yet have a higher prevalence of such mutants when compared to heavily immunosuppressed HIV-HBV co-infected patients [40]. The prevalent use of antiretrovirals with a consequent immunorestoration might therefore predispose the emergence of HBV immune-escape mutants in the absence of potent anti-HBV drugs. Lastly, the widespread use of antiretroviral agents with anti-HBV activity that have a low genetic barrier, such as lamivudine, may favour the emergence of vaccine-escape mutants in regions of high HIV-HBV endemicity. In

these areas, more potent drugs, such as tenofovir, are not easily accessible. Longitudinal studies are strongly needed to examine the incidence of such mutations and their impact in terms of HBV-related morbidity in the HIV-infected population as well as their transmission potential.

References

1. Lewden C, May T, Rosenthal E, Burty C, Bonnet F, Costagliola D, Jougla E, Semaille C, Morlat P, Salmon D, Cacoub P & Chene G. Changes in causes of death among adults infected by HIV between 2000 and 2005: The "Mortalite 2000 and 2005" surveys (ANRS EN19 and Mortavic). *J Acquir Immune Defic Syndr* 2008; **48:**590-598.
2. Thio CL, Seaberg EC, Skolasky R, Jr., Phair J, Visscher B, Munoz A & Thomas DL. HIV-1, hepatitis B virus, and risk of liver-related mortality in the Multicenter Cohort Study (MACS). *Lancet* 2002; **360:**1921-1926.
3. Nikolopoulos GK, Paraskevis D, Hatzitheodorou E, Moschidis Z, Sypsa V, Zavitsanos X, Kalapothaki V & Hatzakis A. Impact of hepatitis B virus infection on the progression of AIDS and mortality in HIV-infected individuals: a cohort study and meta-analysis. *Clin Infect Dis* 2009; **48:**1763-1771.
4. Salmon-Ceron D, Rosenthal E, Lewden C, Bouteloup V, May T, Burty C, Bonnet F, Costagliola D, Jougla E, Semaille C, Morlat P, Cacoub P & Chene G. Emerging role of hepatocellular carcinoma among liver-related causes of deaths in HIV-infected patients: The French national Mortalite 2005 study. *J Hepatol* 2009; **50:**736-745.
5. Iloeje UH, Yang HI, Su J, Jen CL, You SL & Chen CJ. Predicting cirrhosis risk based on the level of circulating hepatitis B viral load. *Gastroenterology* 2006; **130:**678-686.
6. Lee W. Hepatitis B virus infection. *N Engl J Med* 1997; **11:**1733-1745.
7. Ganem D & Prince AM. Hepatitis B Virus Infection -- Natural History and Clinical Consequences. *N Engl J Med* 2004; **350:**1118-1129.
8. Kay A & Zoulim F. Hepatitis B virus genetic variability and evolution. *Virus Res* 2007; **127:**164-176.
9. Olinger C, Jutavijittum P, Hübschen J, Yousukh A, Samountry B, Thammavong T, Toriyama K & Muller C. Possible New Hepatitis B Virus Genotype, Southeast Asia. *Emerg Infect Dis* 2008; **14:**1777-1780.
10. Tatematsu K, Tanaka Y, Kurbanov F, Sugauchi F, Mano S, Maeshiro T, Nakayoshi T, Wakuta M, Miyakawa M & Mizokami M. A Genetic Variant of Hepatitis B Virus Divergent from Known Human and Ape Genotypes Isolated from a Japanese Patient and Provisionally Assigned to New Genotype J. *J Virol* 2009; **83:**10538-10547.
11. Lacombe K, Massari V, Girard PM, Serfaty L, Gozlan J, Pialoux G, Mialhes P, Molina JM, Lascoux-Combe C, Wendum D, Carrat F & Zoulim F. Major role of hepatitis B genotypes in liver fibrosis during coinfection with HIV. *AIDS* 2006; **20:**419-427.
12. Matthews GV, Bartholomeusz A, Locarnini S, Ayres A, Sasaduesz J, Seaberg E, Cooper DA, Lewin S, Dore GJ & Thio CL. Characteristics of drug resistant HBV in

an international collaborative study of HIV-HBV-infected individuals on extended lamivudine therapy. *AIDS* 2006; **20:**863-870.
13. Ramos B, Nunez M, Martin-Carbonero L, Sheldon J, Rios P, Labarga P, Romero M, Barreiro P, Garcia-Samaniego J & Soriano V. Hepatitis B virus genotypes and lamivudine resistance mutations in HIV/hepatitis B virus-coinfected patients. *J Acquir Immune Defic Syndr* 2007; **44:**557-561.
14. Peters MG, Andersen J, Lynch P, Liu T, Alston-Smith B, Brosgart CL, Jacobson JM, Johnson VA, Pollard RB, Rooney JF, Sherman KE, Swindells S & Polsky B. Randomized controlled study of tenofovir and adefovir in chronic hepatitis B virus and HIV infection: ACTG A5127. *Hepatology* 2006; **44:**1110-1116.
15. Matthews GV, Avihingsanon A, Lewin SR, Amin J, Rerknimitr R, Petcharapirat P, Marks P, Sasadeusz J, Cooper DA, Bowden S, Locarnini S, Ruxrungtham K & Dore GJ. A randomized trial of combination hepatitis B therapy in HIV/HBV coinfected antiretroviral naive individuals in Thailand. *Hepatology* 2008; **48:**1062-1069.
16. Thomas H. The hepatitis B virus and the host response. *J Hepatol* 1990; **11:**S83-89.
17. Baumert TF, Rogers SA, Hasegawa K & Liang TJ. Two core promotor mutations identified in a hepatitis B virus strain associated with fulminant hepatitis result in enhanced viral replication. *J Clin Invest* 1996; **98:**2268-2276.
18. Parekh S, Zoulim F, Ahn SH, Tsai A, Li J, Kawai S, Khan N, Trepo C, Wands J & Tong S. Genome replication, virion secretion, and e antigen expression of naturally occurring hepatitis B virus core promoter mutants. *J Virol* 2003; **77:**6601-6612.
19. Jammeh S, Tavner F, Watson R, Thomas HC & Karayiannis P. Effect of basal core promoter and pre-core mutations on hepatitis B virus replication. *J Gen Virol* 2008; **89:**901-909.
20. Hasegawa K, Huang J, Rogers SA, Blum HE & Liang TJ. Enhanced replication of a hepatitis B virus mutant associated with an epidemic of fulminant hepatitis. *J Virol* 1994; **68:**1651-1659.
21. Liang TJ, Hasegawa K, Rimon N, Wands JR & Ben-Porath E. A hepatitis B virus mutant associated with an epidemic of fulminant hepatitis. *N Engl J Med* 1991; **324:**1705-1709.
22. Stuyver L, De Gendt S, Van Geyt C, Zoulim F, Fried M, Schinazi RF & Rossau R. A new genotype of hepatitis B virus: complete genome and phylogenetic relatedness. *J Gen Virol* 2000; **81:**67-74.
23. Kato H, Orito E, Gish RG, Sugauchi F, Suzuki S, Ueda R, Miyakawa Y & Mizokami M. Characteristics of Hepatitis B Virus Isolates of Genotype G and Their Phylogenetic Differences from the Other Six Genotypes (A through F). *J. Virol.* 2002; **76:**6131-6137.
24. Benhamou Y, Bochet M, Thibault V, Di Martino V, Caumes E, Bricaire F, Opolon P, Katlama C & Poynard T. Long-term incidence of hepatitis B virus resistance to lamivudine in human immunodeficiency virus-infected patients. *Hepatology* 1999; **30:**1302-1306.
25. Pessoa MG, Gazzard B, Huang AK, Brandao-Mello CE, Cassetti I, Mendes-Correa MC, Soriano V, Phiri P, Hall A & Brett-Smith H. Efficacy and safety of entecavir for chronic HBV in HIV/HBV coinfected patients receiving lamivudine as part of antiretroviral therapy. *AIDS* 2008; **22:**1779-1787.
26. Low E, Cox A, Atkins M & Nelson M. Telbivudine has activity against HIV-1. *AIDS* 2009; **23:**546-547.

27. Avila C, Karwowska S, Lai C & Evans T. Telbivudine Has No in vitro Activity against Laboratory and Clinical HIV-1, including 5 Clades and Drug-resistant Clinical Isolates. CROI 2009, Montréal, Canada. Abstract 142.
28. Benhamou Y, Thibault V, Vig P, Calvez V, Marcelin A-G, Fievet M-H, Currie G, Chang CG, Biao L & Xiong S. Safety and efficacy of adefovir dipivoxil in patients infected with lamivudine-resistant hepatitis B and HIV-1. *Journal of Hepatology* 2006; **44:**62-67.
29. Lacombe K, Ollivet A, Gozlan J, Durantel S, Tran N, Girard PM & Zoulim F. A novel hepatitis B virus mutation with resistance to adefovir but not to tenofovir in an HIV-hepatitis B virus-co-infected patient. *AIDS* 2006; **20:**2229-2231.
30. Amini-Bavil-Olyaee S, Herbers U, Sheldon J, Luedde T, Trautwein C & Tacke F. The rtA194T polymerase mutation impacts viral replication and susceptibility to tenofovir in hepatitis B e antigen-positive and hepatitis B e antigen-negative hepatitis B virus strains. *Hepatology* 2009; **49:**1158-1165.
31. Audsley J, Arrifin N, Yuen LK, Ayres A, Crowe SM, Bartholomeusz A, Locarnini SA, Mijch A, Lewin SR & Sasadeusz J. Prolonged use of tenofovir in HIV/hepatitis B virus (HBV)-coinfected individuals does not lead to HBV polymerase mutations and is associated with persistence of lamivudine HBV polymerase mutations. *HIV Med* 2009; **10:**229-235.
32. Lacombe K, Boyd A, Lavocat F, Gozlan J, Miailhes P, Bonnard P, Lascoux-combe C, Vernet G, Girard P & Zoulim F. Treament-induced and vaccine escape HBV mutants in HIV-HBV co-infected patients: a longitudinal analysis. AASLD 2009, Boston - USA. Abstract 218.
33. Fung S, Marzzuli T, Sherman M & Popovic V. Tenofovir (TDF) is effective in lamivudine (LAM)- resistant chronic hepatitis B patients who harbor rtA194T as baseline. AALSD 2009, Boston - USA. Abstract 399.
34. Protzer-Knolle U, Naumann U, Bartenschlager R, Berg T, Hopf U, Meyer zum Buschenfelde KH, Neuhaus P & Gerken G. Hepatitis B virus with antigenically altered hepatitis B surface antigen is selected by high-dose hepatitis B immune globulin after liver transplantation. *Hepatology* 1998; **27:**254-263.
35. Lukhwareni A, Burnett RJ, Selabe SG, Mzileni MO & Mphahlele MJ. Increased detection of HBV DNA in HBsAg-positive and HBsAg-negative South African HIV/AIDS patients enrolling for highly active antiretroviral therapy at a Tertiary Hospital. *J Med Virol* 2009; **81:**406-412.
36. Carman WF, Zanetti AR, Karayiannis P, Waters J, Manzillo G, Tanzi E, Zuckerman AJ & Thomas HC. Vaccine-induced escape mutant of hepatitis B virus. *Lancet* 1990; **336:**325-329.
37. Tian Y, Xu Y, Zhang Z, Meng Z, Qin L, Lu M & Yang D. The amino Acid residues at positions 120 to 123 are crucial for the antigenicity of hepatitis B surface antigen. *J Clin Microbiol* 2007; **45:**2971-2978.
38. Torresi J. The virological and clinical significance of mutations in the overlapping envelope and polymerase genes of hepatitis B virus. *J Clin Virol* 2002; **25:**97-106.
39. Kamili S, Sozzi V, Thompson G, Campbell K, Walker CM, Locarnini S & Krawczynski K. Efficacy of hepatitis B vaccine against antiviral drug-resistant hepatitis B virus mutants in the chimpanzee model. *Hepatology* 2009; **49:**1483-1491.
40. Svicher V, Forbici F, Alteri C, Ceccherini-Silberstein F, Boumis E, De Longis P, Stuyver L, Narciso P, Antinori A & Perno C. HIV-driven Immunosuppression Regulates HBV Evolution in HBV/HIV-co-infected Patients. *CROI Montréal. 8 - 11 February 2009. Abstract 100.*