Author's response to reviews

Title: Hepatitis B virus variants in an HIV-HBV co-infected patient at different periods of antiretroviral treatment with and without lamivudine.

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LIST OF REVISIONS

Reviewer Stephan Schaefer

Minor Essential Revisions

1- Serological data of the patient. The serological data were given in the first paragraph of the results section (line 3): "He became an HBV chronic carrier, with all blood samples collected between 1996 and 2002 being positive for HBsAg and HBeAg". To emphasize that the samples analysed by sequencing were also HBsAg and HBeAg, the first sentence of the second paragraph (HBV variants) was modified as follows: "Nucleotide sequences of HBVs (pre-S/S region) derived from HBsAg/HBeAg positive samples collected in March 1999, August 2001 and November 2002 were determined (three clones each)".

2- Phylogenetic tree. According to the observations of both referees, the legend of figure 2 was considerably modified. In particular one reference and bootstrap values were added.

3- Nomenclature of HBV subgenotypes. As mentioned by Dr. Schaefer, there is not yet a consensus about the nomenclature of genotype A subgroups. Dr. Schaefer prefers A1 and A2 to designate the two subgroups, as proposed by Kimbi et al. (J. Gen. Virol. 2004, 85:1211-1220). Dr. Pujol asked us to adopt the nomenclature, Aa and Ae proposed by Sugauch et al. (J. Gen. Virol. 2004, 85:811-820). Recently, we used the original nomenclature A' and A-A', to characterize Brazilian isolates from genotype A (Araujo et al., Arch Virol. 2004, 149:1383-1395). To circumvent difficulties with this nomenclature, we propose to maintain the original A' and A-A' nomenclature and include in the Results section (HBV variants) the three references cited above: "This genotype has been subdivided into two subgenomic groups, designated A-A' (genotype A excluding A') and A' [20]. Recently, subgroups A-A' and A' were designated respectively as A1 and A2 [21] or Ae and Aa [22]. Isolates belonging to subgroup A' have been first identified in South Africa and circulate in a high proportion among HBV Brazilian isolates [23]".

Reviewer Flor Pujol

Major Compulsory Revisions

1- Viral load. It was added in the Methods section (Quantification of HBV DNA) that "Serial dilutions and PCR assays for HBV DNA quantification were performed in triplicate".

2- HBV serological markers. See comments to Dr. Stephan Schaefer item 1.

3- Stop codons. It was added in the Discussion section (third paragraph) that "Although two out three clones of this population possessed stop codon mutations in S gene, HBV load (109 copies/ml) was moderately higher during the lamivudine interruption period than that found during lamivudine periods".

4- Direct sequencing. Detection by automated sequencer of nucleotide mixtures has been previously published (Pas et al., J. Clin Virol 2002, 25:63-71). The following sentence was added in the Results section
(HBV variants, second paragraph):“However, by direct sequencing of PCR products of this last sample, the electropherogram could detect two nucleotides (A and G) at the same sequence position, indicating a mixture of V519 and L519 residues”.

Minor Essential Revisions

1- Number of references: The number of references was reduced to 37.

2- YSDD: It was added in the Discussion (second sentence): "More rarely, HBV variant presenting M550S replacement may be selected during lamivudine treatment [24]"

3- Bootstrap value: See comments to referee 1, item 2

4- Abstract and 5-Materials and Method. The suggested corrections were done.