Correlation between T_H1 response standard cytokines as biomarkers in patients with the delta virus in the western Brazilian Amazon

Larissa Deadame de Figueiredo Nicolete[1,2,3/+], Lourdes Maria Pinheiro Borzacov[2], Deusilene Souza Vieira[1,2,3], Roberto Nicolete[1,3], Juan Miguel Villalobos Salcedo[1,2,3]

[1]Universidade Federal de Rondônia, Programa de Pós-Graduação em Biologia Experimental, Porto Velho, RO, Brasil
[2]Centro de Pesquisa em Medicina Tropical, Porto Velho, RO, Brasil
[3]Fundação Oswaldo Cruz, Laboratório de Biotecnologia Aplicada à Saúde, Porto Velho, RO, Brasil

Hepatitis D virus (HDV) is endemic in the Amazon Region and its pathophysiology is the most severe among viral hepatitis. Treatment is performed with pegylated interferon and the immune response appears to be important for infection control. HDV patients were studied: untreated and polymerase chain reaction (PCR) positive (n = 9), anti-HDV positive and PCR negative (n = 8), and responders to treatment (n = 12). The cytokines, interleukin (IL)-2 (p = 0.0008) and IL-12 (p = 0.02) were differentially expressed among the groups and were also correlated (p = 0.0143). Future studies will be conducted with patients at different stages of treatment, associating the viral load with serum cytokines produced, thereby attempting to establish a prognostic indicator of the infection.

Key words: HDV - T_H1 cytokines - patients treated

Hepatitis delta is considered the most severe form of viral hepatitis and is caused by the hepatitis D virus (HDV) (Pascarella & Negro 2011). This virus is a RNA, hepatotropic virus, and is dependent on the hepatitis B virus (HBV), since HDV uses the HBV surface antigen (HBsAg) for the assembly of new viral particles (Karayiannis 1998). Currently, there are 240 million people positive for HBsAg worldwide (Ott et al. 2012), making the prevalence of HDV infection about 15 million carriers. In Brazil, the endemic areas correspond to states of the western Amazon Region (Acre, Amazonas, Rondônia, and Roraima) with a prevalence of 41.9% among carriers of HBsAg (Braga et al. 2012).

The most recent studied treatments consist of the association between pegylated interferon (PEG-IFN) and HBV reverse transcriptase inhibitors, as adefovir and tenofovir, for extremely long periods (Heidrich et al. 2013, 2014, Lunemann et al. 2015).

In order to reinforce the importance of the host immune response against viral infection, this study investigated whether serum cytokines could indicate some response in the antiviral therapy of patients who achieved a negative HDV RNA at week 24, consistent with a virological response against HDV. Therefore, the cytokines interleukin (IL)-2, IL-10, IL-12, IFN-γ, and tumour necrosis factor-alpha were quantified using the ELISA method (Opteia, USA). Nine untreated patients and polymerase chain reaction (PCR) positive for HDV RNA (HDV posi-

tive), eight anti-HDV positive and PCR negative for HDV patients (HDV negative), 12 patients with HDV who ended the specific antiviral treatment remained PCR RNA negative for the virus six months after the treatment protocol ended (HDV TTO) (Ethical Committee approval: 146.474 of 11/14/2012 CAAE 08485112.4.0000.5300).

After the quantification of cytokines in the patients' serum, the Kruskal-Wallis test was used followed by Dunn's post-test in order to compare the results obtained. A p-value < 0.05 was considered significant. Among all the cytokines tested, IL-2 and IL-12 were shown to be differentially expressed with values of p = 0.0008 (A in Figure) and p = 0.02 (B in Figure), respectively. The increase in IL-2 and IL-12 showed a significant positive correlation (p = 0.0143) after Spearman analysis (C in Figure).

One study analysed the profile of cytokines in HDV patients during treatment with PEG-IFN and associated the virological response of subjects who were responders with those who produced IL-2, IFN-γ, and inducible protein-10 (Grabowski & Wedemeyer 2010). Our results also showed that the production of IFN-γ by patients presented medians of 0.69, 2.77, and 1.27 pg/mL (data not shown) in the groups HDV positive, HDV negative, and HDV TTO, respectively, suggesting a decrease in production in the groups in which HDV is replicating.

With respect to IL-2, the same above mentioned authors suggest that, despite the effects of treatment with PEG-IFN, patients who responded and who present decreased HDV viral load must have an antigen-specific T-cell dependent cellular immune response. Our study also suggested that the exacerbation of IL-2 is not observed in the other groups and is important in the virological response after the end of treatment. Perhaps this is so because of the importance of this cytokine in the clonal expansion of specific cells that fights the virus effectively (Nijkamp & Parnham 2011, Boyman & Sprent 2012).

Patients who responded to treatment also presented an elevated quantification of IL-12. Although it is usu-
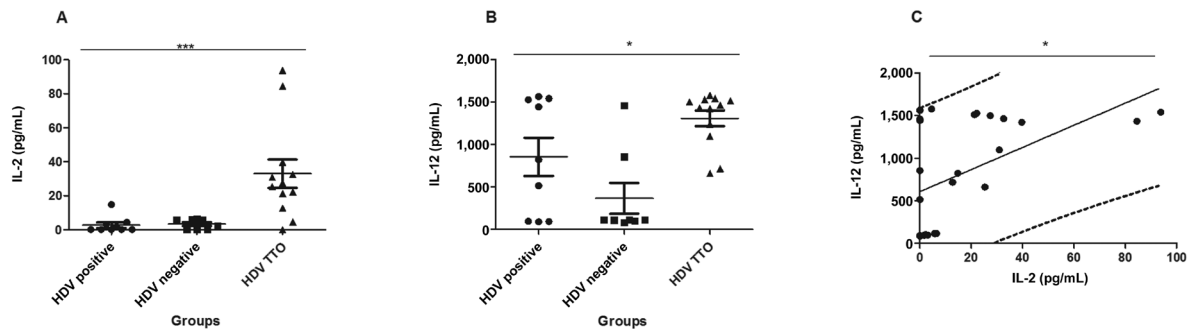
A: the significant difference of the interleukin (IL)-2 cytokine (p = 0.0008) in patients who completed treatment. The statistical tests used were the Krusal-Wallis test followed by the Dunn post-test; B: the same statistical test for the cytokine IL-12. The p-value was significant (p = 0.002); C: Spearman analysis. The values of IL-2 vs. IL-12 were positively correlated (p = 0.0143); HDV: hepatitis D virus.

ally not a cytokine analysed in patients with HDV, some authors have correlated the increase of this cytokine in HBV patients, when treatment of this disease was performed with IFN-α (Cavanaugh et al. 1997, Ozkan et al. 2010). Our results suggest that IL-12 may be important in those patients in whom the HDV virus is replicating represented by the HDV positive group.

By analysing the correlation obtained between IL-12 and IL-2, the polarisation of a T_H1 standard cell response is strongly suggested in patients who completed treatment (Boyman & Sprent 2012). Although there are no reports with HDV, the participation of peripheral cells in HBV infection is well established (Jung & Pape 2002). Human leukocyte antigen class II restricted CD4^{+} helper T-cells are responsible for the recognition of innumerable viral antigens and also for the mechanisms which lead to vaccine protection against the virus (Caillat-Zucman et al. 1998). Thus, our results support the T-cell homing hypothesis (Grabowski et al. 2011) in order for individuals to remain HDV RNA negative after the end of the treatment protocol, since the HBV envelope is essential for delta virus replication and can support assembly and release of new infectious HDV particles (Freitas et al. 2014). Thus, the results obtained in this study suggest that IL-2 is a potential biomarker for the indication of a good prognosis for the patient. Regarding the importance of these cytokines for the maintenance of an effective viral response, we believe that the different levels of the produced cytokines measured may occur due to the dynamics of the treatment protocol. Future studies with IL-2, IL-12, and IFN-γ will be conducted with patients at different stages of treatment in order to better understand how the response to treatment may possibly be dependent on the status of the host immune system. Therefore, its cellular and humoral parameters should be further studied and taken into account when improving the current treatment protocol.

REFERENCES

Boyman O, Sprent J 2012. The role of interleukin-2 during homeostasis and activation of the immune system. *Nat Rev Immunol 12*: 180-190.

Braga WSM, Castilho MC, Borges FG, Leão JRDT, Martinho ACS, Rodrigues IS, de Azevedo EP, de Barros Jr GM, Paraná R 2012. Hepatitis D virus infection in the western Brazilian Amazon - far from a vanishing disease. *Rev Soc Bras Med Trop 45*: 691-695.

Caillat-Zucman S, Gimenez JJ, Wambergue F, Albouze G, Lebkiri B, Naret C, Moynot A, Jungers P, Bach JF 1998. Distinct HLA class II alleles determine antibody response to vaccination with hepatitis B surface antigen. *Kidney Int 53*: 1626-1630.

Cavanaugh VJ, Guidotti LG, Chisari FV 1997. Interleukin-12 inhibits hepatitis B virus replication in transgenic mice. *J Virol 71*: 3236-3243.

Freitas N, Abe K, Cunha C, Menne S, Gudima SO 2014. Support of the infectivity of hepatitis delta virus particles by the envelope proteins of different genotypes of hepatitis B virus. *J Virol 88*: 5742-5754.

Grabowski J, Wedemeyer H 2010. Hepatitis delta: immunopathogenesis and clinical challenges. *Dig Dis 28*: 133-138.

Grabowski J, Yurdaydìn C, Zachou K, Buggisch P, Hofmann WP, Jaroszewicz J, Schlaphoff V, Manns MP, Cornberg M, Wedemeyer H 2011. Hepatitis D virus-specific cytokine responses in patients with chronic hepatitis delta before and during interferon alfa-treatment. *Liver Int 31*: 1395-1405.

Heidrich B, Manns MP, Wedemeyer H 2013. Treatment options for hepatitis delta virus infection. *Curr Infect Dis Rep 15*: 31-38.

Heidrich B, Yurdaydìn C, Kabaçam G, Ratsch BA, Zachou K, Bremer B, Dalekos GN, Erhardt A, Tabak F, Yalcin K, Gürel S, Zeuzem S, Cornberg M, Bock C-T, Manns MP, Wedemeyer H 2014. Late HDV RNA relapse after peg-interferon alpha-based therapy of chronic hepatitis delta. *Hepatology 60*: 87-97.

Jung M-C, Pape GR 2002. Immunology of hepatitis B infection. *Lancet Infect Dis 2*: 43-50.

Karayiannis P 1998. Hepatitis D virus. *Rev Med Virol 8*: 13-24.

Lunemann S, Malone DFG, Grabowski J, Port K, Beziat V, Bremer B, Malmberg K-J, Manns MP, Sandberg JK, Cornberg M, Ljunggren H-G, Wedemeyer H, Bjorkstrom NK 2015. Effects of HDV infection and pegylated interferon treatment on the natural killer cell compartment in chronically infected individuals. *Gut 64*: 469-482.

Nijkamp FP, Parnham MJ 2011. *Principles of immunopharmacology*, 2nd ed., Birkhäuser Verlag, Basel, 651 pp.

Ott JJ, Stevens GA, Groeger J, Wiersma ST 2012. Global epidemiology of hepatitis B virus infection: new estimates of age-specific HBsAg seroprevalence and endemicity. *Vaccine 30*: 2212-2219.

Ozkan TB, Budak F, Erdemir G, Ozgur T, Aker S 2010. Do liver IL-12 levels predict sustained response to IFN-alpha therapy in children with chronic hepatitis B? *J Interferon Cytokine Res 30*: 433-438.

Pascarella S, Negro F 2011. Hepatitis D virus: an update. *Liver Int 31*: 7-21.