

LETTER TO THE EDITOR

## Study design may explain discrepancies in GB virus C effects on interferon- $\gamma$ and interleukin-2 production and CD38 expression in T lymphocytes

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Recently, Baggio-Zappia et al. (2011) reported the effects of GB virus C (GBV-C) on interleukin (IL)-2 and interferon (IFN)- $\gamma$  in CD4 and CD8 T cells. GBV-C coinfection was not associated with lower immune activation in human immunodeficiency virus (HIV)-positive individuals as measured by CD38 expression per cell and IL-2 and IFN- $\gamma$  expressing cells in their study. We agree with Baggio-Zappia et al. (2011) that "several factors, such as differences in the study populations, the stage of HIV infection and the time of GBV-C acquisition, may account for the discrepant results reported by different research groups". However, two major factors may confound interpretation of the results reported by Baggio-Zappia et al. and provide the most likely explanation for the discrepant results observed between this study and previous reports (Maidana-Giret et al. 2009, Baggio-Zappia et al. 2011).

First, approximately 85% of the subjects studied were on potent combination antiretroviral therapy (cART) and had nondetectable HIV RNA levels in their plasma. cART lowers T cell activation, raises CD4 counts, decreases HIV VL and thus interferes significantly with other factors associated with HIV disease progression (Autran et al. 1997, Carcelain et al. 2001, Lederman 2001). Since the vast majority of subjects in the study of Baggio-Zappia et al. (2011) were effectively treated by cART, it is not surprising that GBV-C did not have an observed effect on the clinical variables studied (CD4, HIV VL etc.).

Secondly, Baggio-Zappia et al. (2011) froze the peripheral blood mononuclear cells (PBMCs) prior to conducting in vitro studies of activation, IL-2 and IFN- $\gamma$  cytokine production. Freeze-thawing PBMCs can alter detection of both surface marker expression and T cell function (Kvarnstrom et al. 2004, Mallone et al. 2011). By comparison, several groups found a reduction in CD38 on CD4 and/or CD8 T cells in HIV-infected subjects using fresh PBMCs (Bhattarai et al. 2011, Maidana-Giret et al. 2009). In addition, HIV entry (chemokine) receptors CCR5 and CXCR4

are upregulated on CD4 and CD8 T cells following T cell activation (Smith et al. 2002). Three independent groups found reduced levels of CCR5 on CD4 cells using fresh, non-frozen PBMCs and one of these studies also found reduced levels of CXCR4 on CD4 cells (Nattermann et al. 2003, Maidana-Giret et al. 2009, Schwarze-Zander et al. 2010). Finally, IL-2 interactions with GBV-C have been demonstrated in vitro (George et al. 2003, Bhattarai 2011) and in vivo (Stapleton et al. 2009) using fresh, non-frozen cells for flow cytometry studies.

Since cART will lower detection of cell surface markers of T cell activation, making detection of a diminution by GBV-C more difficult to detect, and since frozen PBMCs can alter levels of T cell activation markers, we believe that the study of Baggio-Zappia et al. (2011) does not negate the prior studies that used fresh PBMCs to evaluate T cell activation. However, the results illustrate the need for more direct and thorough studies of GBV-C on T cell function in individuals with HIV-infection.

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## REPLY

### Comments in regards to Bhattarai and Stapleton's letter

Recently, our group reported the findings of a study that evaluated the effect of GBV-C viremia on IL-2 and IFN- $\gamma$  production in CD4, CD8 and  $\gamma\delta$  T cells and CD38 expression in CD4 and CD8 T cells. Our results did not find an association of GBV-C viremia with lower immune activation in human immunodeficiency virus (HIV)-positive or GBV-C-hepatitis C virus (HCV) co-infected individuals (Baggio-Zappia et al. 2011). In agreement, other studies conducted with different cohorts of HIV-infected patients did not find a beneficial effect of GBV-C on the course of HIV disease (Brumme et al. 2002, Quiros-Roldan et al. 2002, Bjorkman et al. 2004). N Bhattarai and JT Stapleton point that the results found in the study are a result of the ART and to the use of frozen PBMCs to conduct the in vitro experiments.

We agree with N Bhattarai and JT Stapleton that ART lowers T cell activation, raises CD4 T cell counts, decreases HIV VL, and interferes significantly with other factors associated with HIV disease progression, as numerous studies previously demonstrated (Autran et al. 1997, Carcelain et al. 2001, Lederman et al. 2001), however, in this study, all the patients presented a considering degree of immune activation, as demonstrated by the evaluation of CD38 activation marker, independent of the use of ART. In addition, in order to address if the lack of statistical significance was the use of ART, we performed two analyses, considering HIV-treated and HIV-non treated patients and we found the same results (data not shown). Interestingly, Antonucci et al. evaluated the relationship between GBV-C infection and response to antiretroviral therapy in a cohort of 400 HIV-infected patients found that those patients who were GBV-C infected presented a lower risk of HIV rebound when compared with those who were GBV-C negative, showing that an effect of the virus was observed in spite of the ART therapy. In the same study, the authors found that the probability of achieving initial virological success or CD4 T cell count response after HAART did not differ between GBV-C negative and GBV-C positive subjects.

In our study (Baggio-Zappia et al. 2011) frozen PBMCs was used to conduct the in vitro studies of activation, IL-2 and IFN- $\gamma$  cytokine. As an experienced group in the methodology, we have already conducted experiments with fresh PBMCs and the same results were obtained. In addition to our experience, other studies demonstrated that cytokine release in vitro is not significantly different in fresh or cryopreserved PBMCs (Friberg et al. 1994, Venet et al. 2010). Another point to be considered regarding CD38 is that in the present study, we evaluated the quantitative expression of the immune activation marker, not only if

the cells were expressing it, as performed in previous studies (Maidana-Giret et al. 2009), and this could explain the differences of the results, besides the differences between chronically and early infected patients.

Our study has never had the intention to negate the prior studies; instead it adds new data that corroborate the need to conduct more detailed studies that consider the stages of the HIV infection and also, the role of the co-infection with other viruses such as HCV in the context of the HIV disease.

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