# *Title: IgG from HIV-1-exposed seronegative and HIV-1-infected subjects differently modulate IFN-γ production by thymic T and B cells*

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**Conflicts of interest**

The authors declare that they have no relevant conflicts of interest.

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**To the editors:**

Interferon-gamma (IFN-γ), a pleiotropic cytokine, which is mainly produced by activated lymphocytes, has long been considered to play a pivotal role in mediating host-pathogen interactions. Understanding how cells produce IFN-γ is thus important key to eventually elucidate the mechanisms involved in the pathogenesis and therapy1. Specifically, in HIV-1 infection, increased plasma levels of IFN-γ has been linked to lower CD4+ cell count recovery during antiretroviral therapy (ART)2, a parameter of great clinical importance for such patients.

Our group has investigated the effect of IgG molecules in mediating the modulation of lymphocyte function3-11. For instance, our previous studies in humans have demonstrated that IgG molecules can exert their regulatory and/or modulatory function by interacting with lymphocytes during their maturation process in primary lymphoid organs and that their effective immunological profile is dependent on the donor's immune status. More precisely, we have shown that IgG antibodies from atopic donors and atopic dermatitis patients are associated with inhibition of IFN-γ production and induction of IL-10 and IL-17 production, respectively, by the thymic TCD4 and TCD8 cells12,13. In our previous studies, we found that IgG could exert a regulatory function not only on the production of IL-17 by thymic murine and human γδT cells14 but also on the production of both IFN-γ and IL-10 by the thymic human γδT cells15. The recent study by Oliveira et al 14 has demonstrated the capability of IgG to interact with the membrane of thymic murine and human γδT cells that generally do not express FcγRs (IgG receptors). This finding reinforces the hypothesis that IgG can interact with the immature cells by its variable sites and, thus, would possibly recognize the clonal receptors of these cells and consequently modulate their functional properties according to its IgG repertoire16.

Taken together, these pieces of evidence would strongly suggest that murine and human IgG might be a mediator of modulatory effects in several disorders. Here, we sought to investigate this possibility further in other diseases characterized by immunological modulation.

There is scarce evidence in the literature to explain the biological properties in some individuals who escape HIV infection despite being relatedly exposed and who have no genetic traits to justify this resistance (HIV-1-exposed seronegative individuals - ESN)17-19. Although these properties are certainly related to the immunological characteristics of the exposed individuals20, there is exists as yet little evidence in the literature that requires more evaluations.

Here, we used a brief and unprecedented approach to evaluate whether the IgG from ESN individuals can exert some modulatory effect on the production of IFN-γ, which is widely known as an extremely important cytokine for CTL- and NK-cell-mediated immune responses. To this end, we performed a well-standardized *in vitro* protocol of human thymocytes maturation in the presence of IgG12-15 to investigate whether the major human lymphocytes populations that produce IFN-γ (TCD4, TCD8, γδT and B cells) could be modulated according to the IgG donor's immune status in HIV infection or exposition.

**METHODS**

To obtain purified IgG from our analyses groups, HIV-1-serodiscordant couples were recruited from the outpatient clinic at the Emílio Ribas Infectious Diseases Institute in São Paulo, from the Ambulatory Service (ADEE/3002) of the Department of Secondary Immunodeficiency Clinic of the Clinical Hospital, University of São Paulo Medical School (HC/FMUSP). ESN individuals (n = 20), HIV-1-infected partners of ESN individuals (n = 20), and healthy non- HIV exposed and non-infected individuals (n = 20) were enrolled in this study. This study was approved by the São Paulo University Institutional Use Committee (CAPPesq n° 0683/09), and informed consent was obtained from all subjects. All experimental protocols were performed in accordance with relevant guidelines and regulations approved by the Ethics Committee of this institution.

Additional information of the study population is listed in supplementary table 1. Couples reported mean relationship duration of 13 years with a single partner and participation in 5 episodes of unprotected sexual intercourse at a frequency of 3–4 times per month. The ESN subjects were seronegative at the studied time point and were genetically evaluated to confirm that they do not carry the most relevant genetic mutation of HIV-1 resistance (CCR5-Delta 32 homozygous genotype). All HIV-1 infected individuals included in this analysis were on the virally effective ART soon after their diagnosis was made. Two blood samples were obtained from each individual via venipuncture and placed in tubes without anticoagulants. After the blood samples were centrifuged, the serum was fractionated, pooled and stored at -80°C until IgG purification. As an additional control, we used the commercially purified IgG (IVIg - Endobulin, Baxter, AT). Additional methods description can be found online in the Supplementary Material.

**RESULTS**

First, we provided evidence that the frequency of IgG subclasses in all purified IgG pools was similar between groups (Figure S1). Next, we evaluated if the culture conditions used in this study could influence the frequency and viability of the thymic lymphocytes populations. As depicted in Figure 1, no alterations were found in the frequency and viability of γδT cells after 7 days of culture (Figure 1A), B cells (Figure 1B), TCD4 cells (Figure 1C), and TCD8 cells (Figure 1D). Then, cultures were performed to evaluate the intracellular production of IFN-γ in the studied populations. These results strongly suggest that IgG molecules from ESN individuals have the potential to induce the production of IFN-γ in γδT (Figure 1E) and B cells (Figure 1F) compared to all other culture conditions. As presented in Figure 1G and 1H, we observed a similar effect mediated by IgG from HIV-1 infected individuals with the induction of IFN-γ in TCD4 and TCD8 cells compared to all culture conditions.

Altogether, our results demonstrate that IgG from ESN individuals induces IFN-γ production in thymic γδT and B cells, while IgG from HIV-1 infected individuals induces IFN-γ production in thymic TCD4 and TCD8 cells.

**DISCUSSION**

Since 1994, The IFN-γ production has been linked to the activation of TCD8 cells and the control of HIV infection in 199421, however, no comparative study to determine the importance of each lymphoid populations as a main source of IFN-γ has been made since. Thus, it is difficult to discuss the role of the differential modulation observed in response to IgG from ESN or HIV-1-infected individuals in terms of possible immunomodulatory consequences.

The previous study by Yen *et al* reported that γδ T cells predominantly produce IFN-γ upon activation, even in the presence of IL-4 or high GATA-3 expression22. Our current study provided evidence that the IgG molecules from ESN individuals have the potential to induce the production of IFN-γ in thymic γδT compared to the effect mediated by IgG from HIV-1 infected individuals and controls. More recently, it has been demonstrated that γδT cells have the potential to target and clear autologous HIV reservoirs upon latency reversal, thus indicating that they can effectively be used as immunotherapeutic agents in future strategies that involve elimination of HIV-123. Therefore, our observations suggest that IgG from ESN individuals can favor the activation of thymic γδT cells, thus hindering HIV infection, however, this is still a hypothetical possibility, with a need for well designed future study to confirm.

Our results also indicate that the capacity of the IgG from ESN individuals to induce IFN-γ production in thymic B cells compared to IgG from HIV-1 infected individuals. Concerning this analysis, we did not evaluate further functional alterations, which may have been induced in thymic B cells. However, as these cells are responsible for the production of antibodies that exert inhibitory or even protective functions against HIV-1-infection24, additional evaluations need to be undertaken to elucidate this issue. Furthermore, we did not observe any difference in the frequency of IgG subclasses between the evaluated groups. This finding is particularly important since B cells express FcγRs that can differentially interact with IgG subclasses and suggests that the differential effect of each IgG is mediated by its variable region.

The observation that IgG from HIV-1-infected individuals induces IFN-γ in TCD4 cells results in an interesting corroboration with the literature. For example, the study by Teixeira *et al*25 revealed that the increase of the poor TCD4 cell  observed in some HIV-1 infected patients can be caused by the failure of thymic TCD4 cell production. These results were further supported in a later study by Gazzola and collaborators26. Consistent with the prior findings, we believe that the augmentation of IFN-γ production in thymic TCD4 may indicate an exacerbated activation of these cells, thereby favoring apoptosis and consequently failing thymic TCD4 cells production.

 Finally, we observed that IgG from HIV-1-infected individuals also induces IFN-γ in TCD8 cells. As discussed above, it is well known that the TCD8 cells play a pivotal role in controlling HIV infection21 and we showed in the current study that the IgG produced in response to HIV-1 infection could mediate the activation of these cells in thymic tissue as observed by the augmented IFN-γ production. Of note, the induction of IFN-γ production in TCD8 cells has been associated with increased suppressive effect in viral replication27. Based on this fact, we assumed that the augmented IFN-γ production observed in this study might be related to the control of HIV dissemination in infected individuals.

 Transposing these in vitro results to the in vivo conditions, we suggest that the IgG repertoire naturally produced by the ESN group differs from the HIV-1-infected individuals and this could result in differential patterns of IFN-γ production. This difference can exert some role in the exposed uninfected individuals but it is important to highlight that the low exposition to HIV-1in term of low viral load is a feature of our ESN individuals group.

Although we have not elucidated the mechanisms underlying our observations, we assume that the IgG might directly interact with the clonal receptors expressed by lymphocytes during its maturation in thymus16. Also, because of technical limitations, we were unable to elucidate the precise mechanism of the IgG effect; we demonstrate that IgG antibodies produced by ESN or HIV-1-infected individuals have potential to exert divergent modulatory effects on the production of IFN-γ by thymic lymphocytes. We hope that our observations will contribute to the generation of future hypothesis and open field to assess the role of IgG in resistance or response to HIV-1 infection.

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**Figure Legend**

**Figure 1: Frequency, viability and the effect of purified HIV-1 infected and ESN IgG on thymic γδT, B, TDC4 and TCD8 cells.** Thymocytes (n=14) were evaluated after 7 days in culture in RPMI medium supplemented with FCS in the absence (mock) or presence of 100 µg/mL of IVIg or IgG purified from healthy individuals (HI), HIV-1infected individuals (HIV-1) or HIV-1-exposed seronegative individuals. The frequencies and viability of γδT (A), B (B), TCD4 (C) and TCD8 (D) were demonstrated. Intracellular IFN-γ production is demonstrated in γδT (E), B (F), TCD4 (G) and TCD8 (H) cells. The results are illustrated by box-and-whisker plots with 25th percentiles, and the Tukey’s method was used to plot outliers; \*p≤0.05 when compared to mock, IVIg, HI and HIV-1 conditions; \*\*p≤0.05 when compared to mock, IVIg, HI and ESN conditions.