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HIV-Specific Antibodies Enhance Type I Interferon Production from Plasmacytoid DCs

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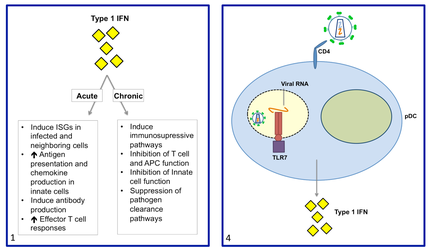
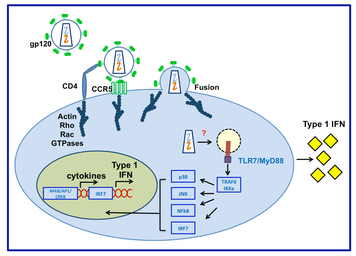
INTRODUCTION

Type I interferon (IFN) production is essential in innate control of acute viral infection, but prolonged IFN production is associated with chronic immune activation in HIV [1,2]. The mechanisms that maintain high-level IFN production following the acute phase are unknown. Plasmacytoid dendritic cells (pDCs) are the primary producers of type I IFN in viral infections[3]. HIV is sensed by pDCs through endosomal Toll like receptor (TLR) 7 recognition of RNA[2,4].

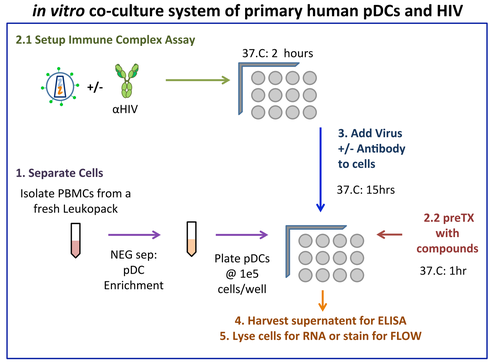


In this study, we further define the mechanism through which HIV activates pDC to produce IFN and demonstrate that antibodies (Abs) generated in persistent HIV infection enhance IFN production by pDCs. This mechanism for enhancing IFN production after the acute phase of infection when the humoral response has developed may explain persistent IFN elevation and immune activation characteristic of chronic HIV infection.

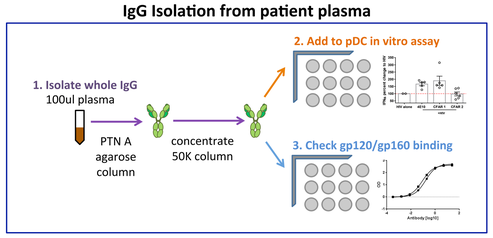


METHODS

***in vitro* co-culture system of primary human pDCs and HIV**



IgG Isolation from patient plasma



RESULTS

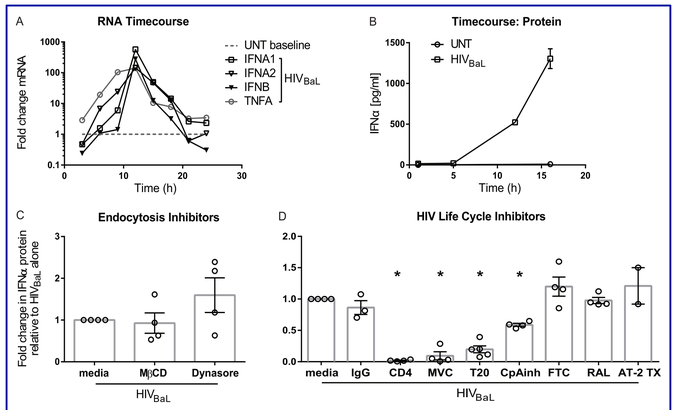


Fig 1. Type I IFN induction by HIV requires receptor mediated entry; not endocytosis or productive infection. Human pDCs were cultured with HIV_{BaL} and cells were harvested and assessed for type I IFN and TNFα mRNA expression (A) or supernatants were harvests and assessed for IFNα protein production (B). Human pDCs were cultured with endocytosis (C) or HIV life cycle inhibitors (D) for 1h followed by the addition of HIV_{BaL} for 15h. Supernatants were harvested and assessed for IFNα protein production. Conditions were compared using a one way ANOVA with correction for multiple comparisons *p<0.01.

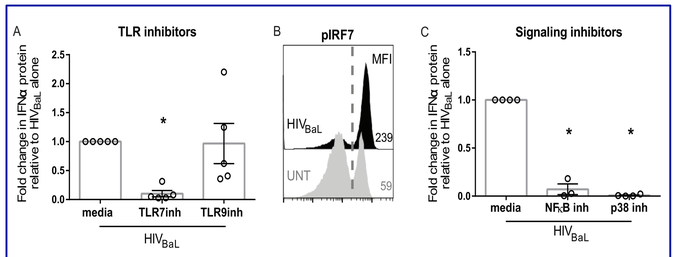


Fig 2. pDCs utilizes TLR7, IRF7, NFκB and CREB signaling to produce IFN in response to HIV. Human pDCs were cultured with TLR inhibitors (A) for 1h followed by the addition of HIV_{BaL} for 15h. Supernatants were harvested and assessed for IFNα protein production. (B) Human pDCs were cultured with HIV_{BaL} (black histogram) or without HIV_{BaL} (gray histogram) for 15h cell were then permeabilized and stained for phosphorylated IRF7. Human pDCs were cultured with signaling inhibitors (C) for 1h followed by the addition of HIV_{BaL} for 15h. Supernatants were harvested and assessed for IFNα protein production. Error bars and gray box represent the standard error of the mean and mean, respectively. *p<0.05.

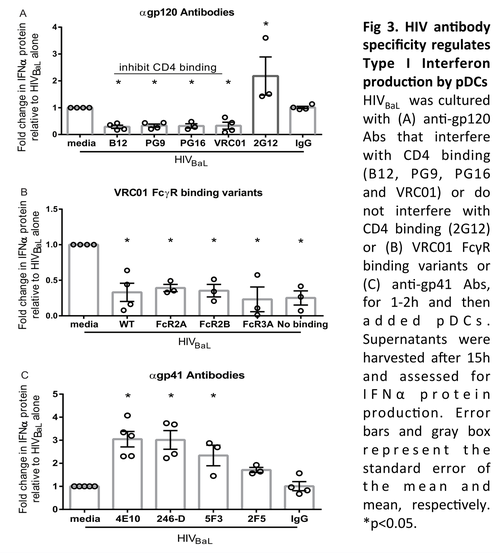


Fig 3. HIV antibody specificity regulates Type I Interferon production by pDCs HIV_{BaL} was cultured with (A) anti-gp120 Abs that interfere with CD4 binding (B12, PG9, PG16 and VRC01) or do not interfere with CD4 binding (2G12) or (B) VRC01 FcγR binding variants or (C) anti-gp41 Abs, for 1-2h and then added pDCs. Supernatants were harvested after 15h and assessed for IFNα protein production. Error bars and gray box represent the standard error of the mean and mean, respectively. *p<0.05.

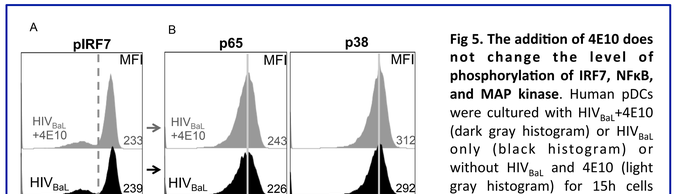


Fig 5. The addition of 4E10 does not change the level of phosphorylation of IRF7, NFκB, and MAP kinase. Human pDCs were cultured with HIV_{BaL}+4E10 (dark gray histogram) or HIV_{BaL} only (black histogram) or without HIV_{BaL} and 4E10 (light gray histogram) for 15h cells were then permeabilized and stained for phosphorylated IRF7, NFκB p65, and p38.

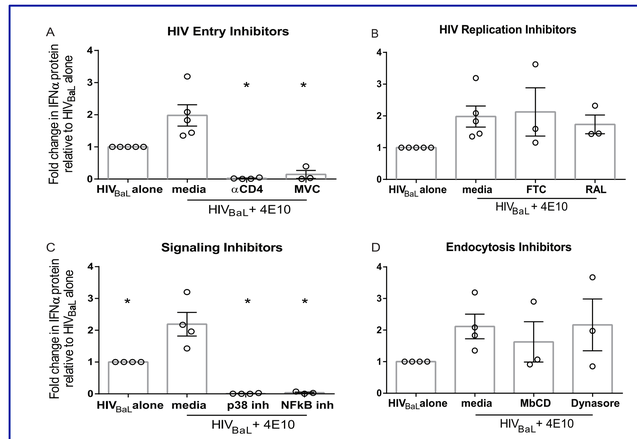


Fig 4. IFN enhancement by HIV specific Abs requires gp120 binding and NFκB signaling; not endocytosis or infection.
Human pDCs were cultured with HIV entry inhibitors (A) or HIV replication inhibitors (B) or signaling inhibitors (C) or endocytosis inhibitors (D) for 1h followed by the addition of HIV_{BaL}+/- 4E10 for 15h. Supernatants were harvested and assessed for IFNα protein production. Error bars and gray box represent the standard error of the mean and mean, respectively. *p<0.01.

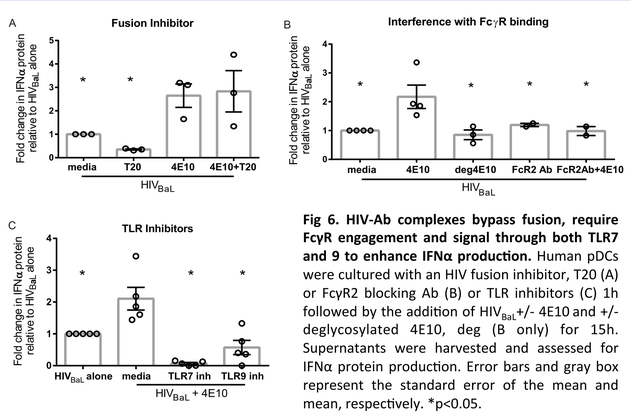


Fig 6. HIV-Ab complexes bypass fusion, require FcγR engagement and signal through both TLR7 and 9 to enhance IFNα production. Human pDCs were cultured with an HIV fusion inhibitor, T20 (A) or FcγR2 blocking Ab (B) or TLR inhibitors (C) 1h followed by the addition of HIV_{BaL}+/- 4E10 and +/- deglycosylated 4E10, deg (B only) for 15h. Supernatants were harvested and assessed for IFNα protein production. Error bars and gray box represent the standard error of the mean and mean, respectively. *p<0.05.

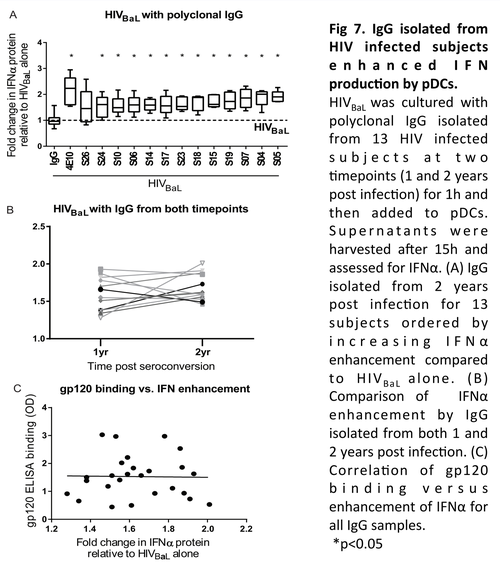


Fig 7. IgG isolated from HIV infected subjects enhanced IFN production by pDCs.
HIV_{BaL} was cultured with polyclonal IgG isolated from 13 HIV infected subjects at two timepoints (1 and 2 years post infection) for 1h and then added to pDCs. Supernatants were harvested after 15h and assessed for IFNα. (A) IgG isolated from 2 years post infection for 13 subjects ordered by increasing IFNα enhancement compared to HIV_{BaL} alone. (B) Comparison of IFNα enhancement by IgG isolated from both 1 and 2 years post infection. (C) Correlation of gp120 binding versus enhancement of IFNα for all IgG samples.
*p<0.05

SUMMARY

- HIV-driven activation of pDCs to produce IFN required TLR7 signaling, receptor-mediated entry, fusion, and viral uncoating, but not endocytosis or life cycle stages after uncoating
- Abs directed against the HIV envelope that do not interfere with CD4 binding significantly enhanced the IFN response irrespective of capacity to neutralize CD4 T cell infection
- Ab-mediated enhancement of IFN production required pDC Fc gamma receptor engagement, bypassed fusion, and initiated signaling through both TLR7 and TLR9, which was not utilized without Ab present
- Polyclonal Ab isolated from HIV-infected subjects universally enhanced pDC production of IFN in response to HIV
- Our data suggest that Abs produced *in vivo* in untreated HIV infection contributes to persistent high-level IFN responses during chronic HIV infection, representing a novel mechanism of immune activation

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