Project X: Mechanisms underlying sex differences in HIV reservoir composition and size in people living with HIV-1 on antiretroviral therapy

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Background and preliminary data:
Manifestations of HIV-1 infection differ between females and males. Previous studies have shown that cis-gender women living with HIV-1 (WLWH) control viral replication better than cis-gender men living with HIV-1 (MLWH) in acute HIV-1 infection (Meditz et al., JID 2011). In contrast, WLWH experience faster loss of CD4+ T cells and faster progression to AIDS during untreated chronic HIV-1 infection after controlling for the level of viral replication (Sterling et al., NEJM 2001). Increasing data indicate that these sex differences in the manifestations of HIV-1 disease are mediated by sex-specific differences in antiviral immunity.

In previous studies, we demonstrated that pDCs derived from females produce more IFNα in response to HIV-1-mediated TLR7 stimulation than pDCs from males, potentially contributing to the better control of viremia in acute infection (Meier et al., Nat Med 2009; Ziegler et al., EHI 2017). We showed that this increased IFNα production in response to HIV-1-derived TLR7 ligands also led to significantly higher immune activation in WLWH during chronic untreated infection (Meier et al., Nat Med 2009), and that WLWH experienced higher expression levels of interferon stimulated genes (ISGs) compared to MLWH (Chung et al., JID 2013). More recent studies by us and others in PLWH on ART furthermore demonstrated that WLWH exhibit better control of residual viral replication and lower viral reservoirs that MLWH (Scully et al., JID 2019), as well as greater viral control after ART withdrawal (Le et al., AIDS 2019).

The Altfeld group has investigated the molecular mechanisms underlying sex differences in Type I IFN responses by pDCs in response to viruses over the past decade, and identified important contribution to these sex differences mediated by both signaling molecules within the TLR7 pathway that are regulated by sex hormones (Griesbeck et al., JI 2015) and genes that are encoded by the X chromosome and escape inactivation of the second X chromosome, resulting in higher gene dosage effects (Hagen et al, CellRep 2020). To better understand the potential relationship between Type I IFN responses and HIV-1 reservoir sizes, we furthermore performed preliminary studies in 20 cis-gender WLHW on stable suppressive ART, in which we quantified *in vitro* Type I IFN responses of pDCs following TLR7 stimulation, *ex vivo* ISG responses and HIV-1 reservoir sizes in sorted CD4+ T cells. These studies revealed an inverse correlation between the strength of the IFNα response of pDCs following TLR7 stimulation and the size of the intact proviral HIV-1 reservoir (figure 1).

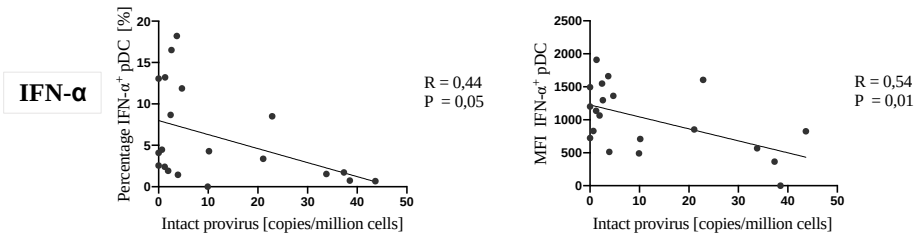

Figure 1:
Inverse correlation between levels of intact proviral HIV-1 DNA copies in CD4+ T cells and IFNα production by pDCs following TLR7 stimulation in the same WLWH on ART.

Taken together, these studies have identified critical mechanisms that underlie sex differences in Type I IFN responses, and their potential implication for the size and quality of the latent HIV-1 reservoir in PLWH. In the next step, we would like to investigate the precise mechanisms by which sex differences in Type IFN responses regulate the HIV-1 reservoir.

Hypothesis:
Sex differences in Type I IFN responses to HIV-1 result in differences in HIV-1 reservoir characteristics in PLWH on ART

Aims and Work Programme:

1. To determine the relationship between biological sex, age, Type I IFN responses and HIV-1 reservoir characteristics in PLWH on ART
2. To identify the mechanisms by which sex differences in Type IFN responses and ISG expression control HIV-1 reservoir size and composition

In Aim #1, we will investigate the relationship between biological sex, age, Type I IFN responses and HIV-1 reservoir characteristics in cis-gender PLWH. For these studies, we will use peripheral blood samples (PBMC and Plasma) collected from 60 PLWH on stable ART with suppressed HIV-1 viremia for at least 24 months. These will include 30 WLWH and 30 MLWH, half of them under the age of 40 and half over the age of 50. The majority of these samples are already collected and available for the project, including samples received through the ACTG network. TLR7-induced Type I IFN responses will be quantified in cryopreserved PBMC using multiparameter flow cytometry, as described[5], and ISG expression in PBMCs will be quantified using an established qRT-PCR panel including over 25 primers. HIV-1 reservoir sizes and characteristics (total, intact) will be quantified on sorted CD4+ T cells using the Intact Proviral DNA Assay (IPDA). Linear regression analyses using Pearson correlation tests will be performed to identify associations between Type I IFN responses and HIV-1 reservoir characteristics, and we will assess the role of sex and age as biological variables modulating these associations.

In Aim #2, we will determine the mechanisms by which sex differences in Type IFN responses and ISG expression control HIV-1 reservoir size and composition. Previous studies by our group have identified biological factors that impact the strength of the Type I IFN responses, including escape from inactivation of the second X chromosome in X-chromosomal genes involved in the TLR signaling pathway[2], such as TLR7 and TASL, and regulation of gene expression of critical signaling molecules, such as IRF5, by sex hormones[4]. We have furthermore identified SNPs in these genes associated with increased or decreased Type I IFN induction following TLR7 stimulation. We will determine sex hormone levels in the 60 study participants and correlate these to the strength of the Type I IFN response and HIV-1 reservoir characteristics. mRNA and protein expression levels of critical signaling molecules, as well as potential SNPs in these genes, will furthermore be associated with the Type I IFN responses and HIV-1 reservoir sizes. Finally, we will use co-culture systems of HIV-1-infected CD4+ T cells and Type I IFN-producing cells to investigate the direct impact of Type I IFNs on HIV-1 reservoirs in CD4+ T cells, and use blocking antibodies and targeted KO of critical genes to validate the role of target gene candidates.

Project-related publications: (max. 5)

1. Pujantell M, Altfeld M. Consequences of sex differences in Type I IFN responses for the regulation of antiviral immunity. Front Immunol. 2022;13:986840.
2. Hagen SH, Henseling F, Hennesen J, Savel H, Delahaye S, Richert L, Ziegler SM, Altfeld M. Heterogeneous Escape from X Chromosome Inactivation Results in Sex Differences in Type I IFN Responses at the Single Human pDC Level. Cell Rep. 2020;33(10):108485.
3. Ziegler SM, Beisel C, Sutter K, Griesbeck M, Hildebrandt H, Hagen SH, Dittmer U, Altfeld M. Human pDCs display sex-specific differences in type I interferon subtypes and interferon α/β receptor expression. Eur J Immunol. 2017;47(2):251-256.
4. Griesbeck M, Ziegler S, Laffont S, Smith N, Chauveau L, Tomezsko P, Sharei A, Kourjian G, Porichis F, Hart M, Palmer CD, Sirignano M, Beisel C, Hildebrandt H, Cénac C, Villani AC, Diefenbach TJ, Le Gall S, Schwartz O, Herbeuval JP, Autran B, Guéry JC, Chang JJ, Altfeld M. Sex Differences in Plasmacytoid Dendritic Cell Levels of IRF5 Drive Higher IFN-α Production in Women. J Immunol. 2015;195(11):5327-36.
5. Meier A, Chang JJ, Chan ES, Pollard RB, Sidhu HK, Kulkarni S, Wen TF, Lindsay RJ, Orellana L, Mildvan D, Bazner S, Streeck H, Alter G, Lifson JD, Carrington M, Bosch RJ, Robbins GK, Altfeld M. Sex differences in the Toll-like receptor-mediated response of plasmacytoid dendritic cells to HIV-1. Nat Med. 2009;15(8):955-9.