Author/s:
Selva, KJ;Bavinton, BR;Grulich, AE;Pazgier, M;Kelleher, AD;Kent, SJ;Parsons, MS

Title:
Impact of HIV-1 viremia or sexually transmitted infection on semen-derived anti-HIV-1 antibodies and the immunosuppressive capacity of seminal plasma

Date:
2019-12-01

Citation:
Selva, K. J., Bavinton, B. R., Grulich, A. E., Pazgier, M., Kelleher, A. D., Kent, S. J. & Parsons, M. S. (2019). Impact of HIV-1 viremia or sexually transmitted infection on semen-derived anti-HIV-1 antibodies and the immunosuppressive capacity of seminal plasma. European Journal of Immunology, 49 (12), pp.2255-2258. https://doi.org/10.1002/eji.201848055.

# Impact of HIV-1 viremia or sexually transmitted infection on semen-derived anti-HIV-1 antibodies and the immunosuppressive capacity of seminal plasma

Kevin J Selva[1], Benjamin R Bavinton[2], Andrew E Grulich[2], Marzena Pazgier[3], Anthony D Kelleher[2], Stephen J Kent[*1,4,5] and Matthew S Parsons[*1,6,7]

[1]Department of Microbiology and Immunology, Peter Doherty Institute for Infection and Immunity, The University of Melbourne, Melbourne, Victoria, Australia.

[2]The Kirby Institute, UNSW Sydney, Sydney, New South Wales, Australia.

[3]Infectious Diseases Division, Uniformed Services University of the Health Sciences, Bethesda, MD, USA.

[4]Melbourne Sexual Health Centre and Infectious Disease Department, Alfred Health, Monash University Central Clinical School, Melbourne, Victoria, Australia.

[5]ARC Centre of Excellence in Convergent Bio-Nano Science and Technology, The University of Melbourne, Parkville, Victoria, Australia.

[6]Division of Microbiology and Immunology, Yerkes National Primate Research Center, Emory University, Atlanta, Georgia, USA.

[7]Department of Pathology and Laboratory Medicine, Emory University, Atlanta, Georgia, USA.

[*]Address correspondence to Dr. Stephen J Kent – skent@unimelb.edu.au or Dr. Matthew S Parsons – matthew.s.parsons@emory.edu

Keywords: HIV-1; NK cells; Semen; IgG; Fc-dependent functions

The peer review history for this article is available at https://publons.com/publon/10.1002/eji.201848055

HIV-1 infections commonly occur following exposure to semen at anogenital mucosae. Semen contains factors capable of both enhancing and diminishing the likelihood of HIV-1 transmission [1]. Understanding how semen contributes to the transmission of HIV-1 could assist with vaccine design.

Semen from HIV-1-infected men contains anti-HIV-1 IgG that trigger antibody-dependent NK cell activation [2, 3]. Anti-HIV-1 antibodies with ADCC capacity in breast milk associate with protection of infants from mother-to-child transmission [4]. The relevance of semen-derived anti-HIV-1 IgG with Fc-dependent functions to mucosal HIV-1 transmission remains unknown. A characteristic of semen that could contribute to mucosal HIV-1 transmission is its immunosuppressive capacity. Immunosuppressive factors within semen could quell anti-viral immune responses and promote HIV-1 transmission [5]. We previously demonstrated that seminal plasma (SP) from both HIV-1-uninfected and -infected donors inhibits both direct and antibody-dependent responses of NK cells [6].

Herein, we assessed: (1) if antiretroviral therapy (ART) resulted in a waning of the antibody-dependent NK cell activation potential of semen-derived IgG; (2) if seminal anti-HIV-1 IgG with the capacity to trigger antibody-dependent NK cell activation recognize CD4-induced epitopes, such as the epitope recognized by the A32 monoclonal antibody, as is the case for anti-HIV-1 antibodies in blood plasma from infected individuals or vaccinees [7, 8]; and (3) If SP from donors with viremic versus ART-suppressed HIV-1 infections, or with or without active bacterial sexually transmitted infections, differ in terms of immunosuppressive capacity.

We obtained paired SP and sera samples from dates prior to and following ART initiation from 11 HIV-1-infected participants in the Opposites Attract study [9] (donor characteristics are listed in

Table S1 within the Supporting Material). Anti-HIV-1 antibody-dependent NK cell activation was measured using a previously described intracellular cytokine staining (ICS) assay (Figure 1A; Methodology is within Supporting Material) [6, 10]. No significant differences in anti-HIV-1 antibody-dependent NK cell activation between samples from dates prior to and following initiation of ART were observed for a 1:1000 dilution of blood sera [median: 3.93% (0.26%–17.84%) vs 3.90% (0.20%–21.65%), $p$=0.88] or SP-derived IgG utilized at the equivalent of a 1:10 dilution [2.83% (0.16%–14.80%) vs 4.47% (0.23%–14.20%), $p$=0.46] (Figure 1B). As represented in Figure 1C, a positive correlation (r=0.86, $p$=0.002) was noted between the capacity of paired blood sera (1:1000 dilution) and SP-derived IgG (1:10 dilution) samples to trigger anti-HIV-1 antibody-dependent NK cell activation.

We next assessed if SP-derived anti-HIV-1 IgG capable of activating NK cells recognize similar CD4-induced epitopes as blood sera IgG. For this purpose, we performed a series of anti-HIV-1 antibody-dependent NK cell activation ICS assays using Fab fragments of the A32 monoclonal antibody to block its CD4-induced epitope on cell surface-bound gp120. As shown in Figure 1D, the presence of A32 Fab significantly inhibited anti-HIV-1 antibody-dependent NK cell activation triggered by IgG derived from SP samples (used at equivalent of a 1:10 dilution) from nine HIV-1-infected donors [70.90% inhibition (14.03%–87.13%), $p$=0.004]. Similarly, A32 Fab fragments significantly inhibited anti-HIV-1 antibody-dependent NK cell activation by 1:10,000 dilutions of blood sera samples from nine HIV-1-infected donors [75.17% inhibition (28.18%–84.67%), $p$=0.004 ; Figure 1E].

Next, we assessed if viremic HIV-1 or active bacterial sexually transmitted infections altered the capacity of SP to suppress direct and antibody-dependent NK cell activation. We studied direct NK cell activation through intracellular IFNγ production following stimulation with MHC I deficient 721.221 target cells, as previously described [6]. Antibody-dependent NK cell activation was measured as intracellular IFNγ production following stimulation with Rituximab-coated 721.221

target cells, as previously described [6]. The gating strategy used to analyze the capacity of SP to inhibit direct and antibody-dependent NK cell activation is depicted in Figure 2A. No significant difference was observed in the inhibition of direct [94.66% inhibition (82.3%–98.0%) vs 95.33% inhibition (56.26%–98.33%), *p*=0.62; Figure 2B] or antibody-dependent [89.53% inhibition (65.85%–92.03%) vs 87.86% inhibition (85.01%–92.62%), *p*=0.76; Figure 2C] NK cell activation by 1:100 dilutions of SP samples from 11 donors obtained at dates prior to or during ART. Similarly, no significant difference was observed in inhibition of direct [96.70% inhibition (90.51%–98.72%) vs 95.63% inhibition (89.15%–97.98%), *p*=0.73; Figure 2D] or antibody-dependent [85.94% inhibition (70.72%–90.22%) vs 84.43% inhibition (65.99%–89.49%), *p*=0.25; Figure 2E] NK cell activation for 1:100 dilutions of SP samples from nine additional Opposites Attract participants collected during urethral or rectal bacterial sexually transmitted infection(s) (*Neisseria gonorrhoeae* and/or *Chlamydia trachomatis*) or times when no infection was present.

We demonstrate that anti-HIV-1 antibodies within semen overlap with antibodies present in blood plasma. Indeed, antibodies within paired SP and blood sera samples similarly recognize the CD4-induced A32 epitope within the Cluster A region of HIV-1 envelope. Furthermore, the capacity of SP-derived IgG to trigger anti-HIV-1 antibody-dependent NK cell activation correlates with the capacity of blood sera antibodies from the same donor to trigger NK cell activation. Lastly, we note that active HIV-1 infection, or bacterial infection(s), does not influence the capacity of SP to inhibit direct or antibody-dependent NK cell activation. Future research is needed to determine if seminal anti-HIV-1 antibodies contribute to mucosal HIV-1 transmission efficiency. Furthermore, the impact of SP-mediated immunosuppression on vaccine-induced responses within mucosal tissues requires investigation.

**Acknowledgements**

The current study was funded by grants from the National Health and Medical Research Council (NHMRC) to Matthew S Parsons and Stephen J Kent.

Funding for the Opposites Attract Study was received from the NHMRC, National Health and Medical Research Council, Australia; amfAR, Foundation for AIDS Research, USA; ViiV Healthcare, UK; and Gilead Sciences, USA.

The authors would like to acknowledge the Opposites Attract Study site investigators and sites participating in the Semen Sub-Study: Nittaya Phanuphak (Thai Red Cross AIDS Research Centre, Bangkok, Thailand); Beatriz Grinsztejn (Evandro Chagas Institute of Clinical Research, Rio de Janeiro, Brazil); Catherine Pell (Taylor Square Private Clinic, Sydney, Australia); Mark Bloch (Holdsworth House Medical Practice, Sydney, Australia); David Baker (East Sydney Doctors, Sydney, Australia); David J Templeton (RPA Sexual Health, Sydney, Australia); Anna M McNulty (Sydney Sexual Health Centre, Sydney, Australia); and David A Cooper (St Vincent's Hospital, Sydney, Australia).

We would like to thank Dr. W.D. Tollbert from the Institute of Human Virology of the University of Maryland for mAb A32 production.

**DISCLAIMER**

The views expressed in this presentation are those of the authors and do not reflect the official policy or position of the Uniformed Services University, US Army, the Department of Defense, or the US Government.

Conflict of interest:

The authors declare no commercial or financial conflict of interest.

1 **Doncel, G. F. et al.,** *Am J Reprod Immunol* 2014. **71**: 564-574.

2 **Wolff, H. et al.,** *J Acquir Immune Defic Syndr* 1992. **5**: 65-69.

3 **Parsons, M. S. et al.,** *J Acquir Immune Defic Syndr* 2016. **71**: 17-23.

4 **Mabuka, J. et al.,** *PLoS Pathog* 2012. **8**: e1002739.

5 **Kelly, R. W.** *Prostaglandins Leukot Essent Fatty Acids* 1997. **57**: 113-118.

6 **Selva, K. J. et al.,** *AIDS* 2017. **31**: 333-342.

7 **Bonsignori, M. et al.,** *J Virol* 2012. **86**: 11521-11532.

8 **Ferrari, G. et al.,** *J Virol* 2011. **85**: 7029-7036.

9 **Bavinton, B. R. et al.,** *Lancet HIV* 2018. **5**: e438-e447.

10 **Kristensen, A. B. et al.,** *J Virol* 2018 92: e02146-17.

**Figure Legends:**

**Figure 1. Anti-HIV-1 antibody-dependent NK cell activation by seminal plasma (SP) and blood antibodies.** Anti-HIV-1 antibodies, derived from seminal plasma (SP) (n=11) or within paired blood sera samples (n=11) from HIV infected donors, were assessed for anti-HIV-1 antibody-dependent NK cell activation capacity. (A) Gating implemented to assess NK cell activation, identifying lymphocytes, single cells, $CD56^{dim}CD3^{-}$ NK cells and revealing the proportion of NK cells expressing IFNγ in the presence of HIV-$1^{-}$ serum (top), presence of HIV-$1^{+}$ serum (middle) or presence of SP-derived IgG from an HIV-$1^{+}$ donor (bottom). (B) The relative anti-HIV-1 antibody-dependent NK cell activation triggered by 1:1,000 dilutions of paired blood sera samples (left) or by SP-derived IgG utilized at the equivalent of a 1:10 dilution (right) from samples collected prior to and following ART. Data were analyzed with Wilcoxon matched pairs tests. $p<0.05$ was considered significant. (C) The relationship between anti-HIV-1 antibody-dependent NK cell activation triggered by anti-HIV-1 antibodies within paired pre-ART blood sera and SP. Data were analyzed by Spearman correlation. $p<0.05$ was considered significant. (D-E) Percent inhibition of anti-HIV-1 antibody-dependent NK cell activation triggered by (D) SP-derived IgG or (E) blood sera against gp120-coated CEM.NKr-CCR5 target cells pre-treated or not with A32 Fab. Data were analyzed with Wilcoxon matched pairs tests. $p<0.05$ was considered significant. Lines on graphs depict the medians. The data represented within (A-C) are single measurements collected within a single experiment. The data represented in (D-E) are single measurements collected in two independent experiments.

Figure 1

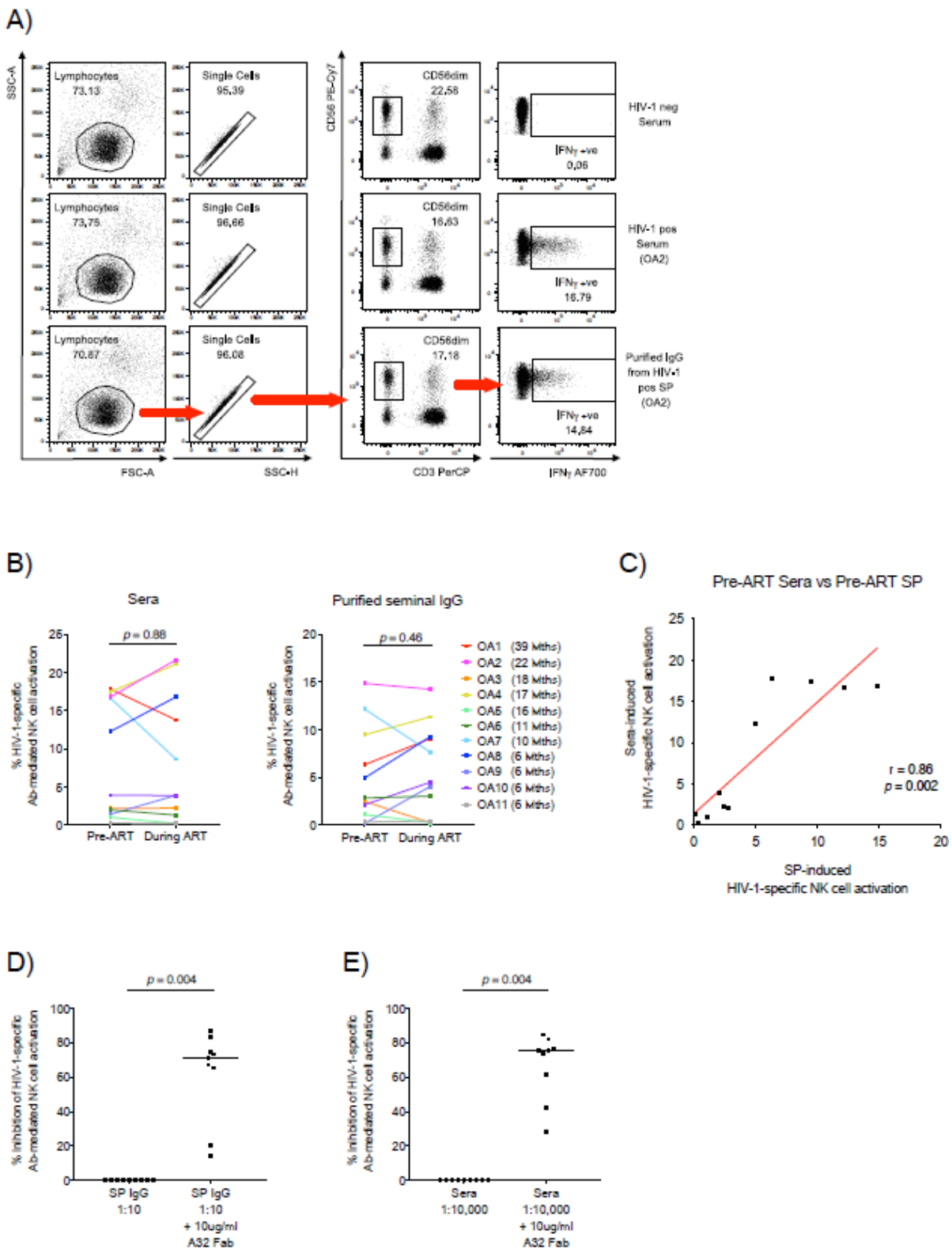
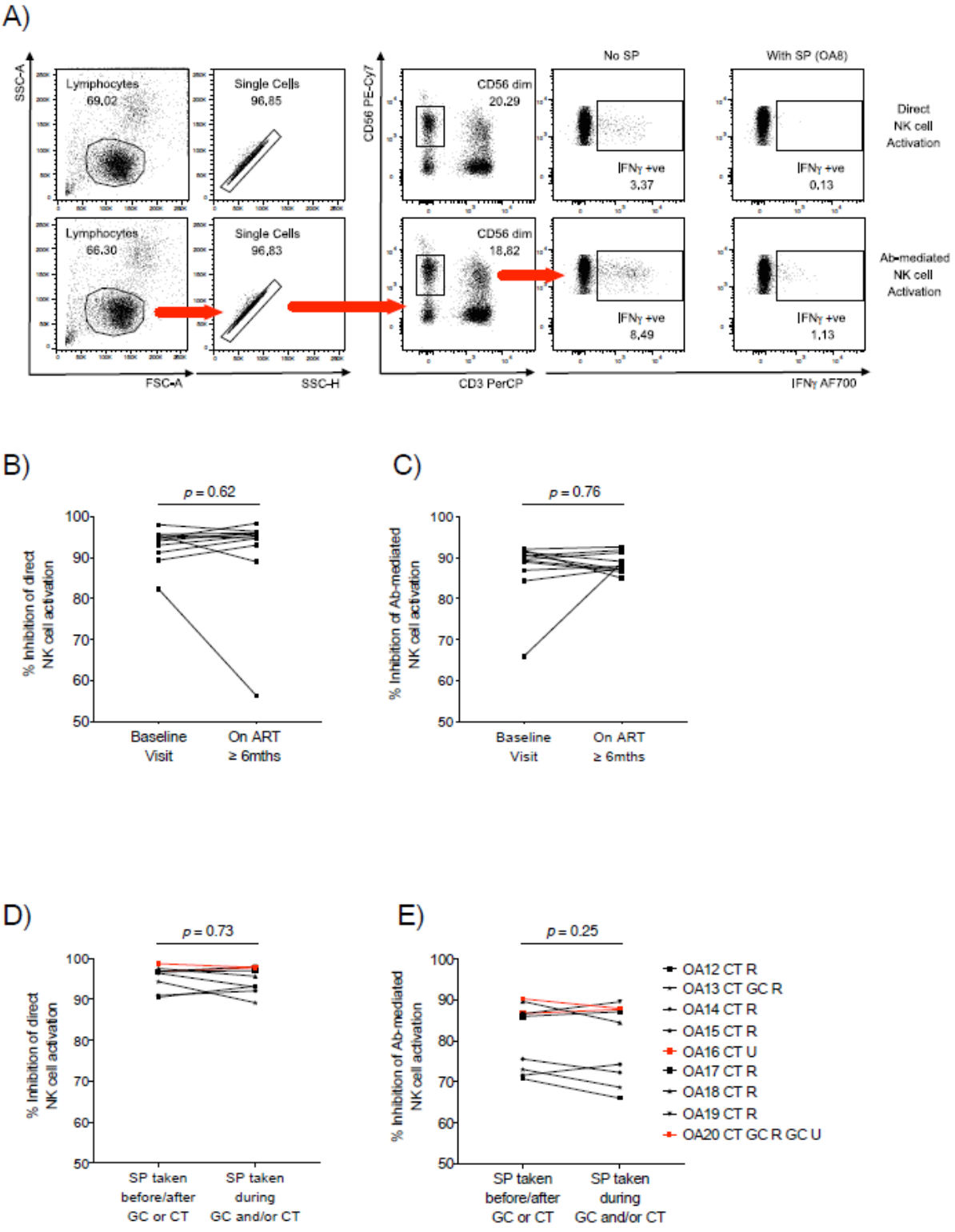


**Figure 2. Capacity of seminal plasma (SP) from viremic or suppressed HIV-1 infection or from sexually transmitted bacterial infections to inhibit NK cell activation.** NK cells within PBMC were stimulated with 721.221 target cells uncoated or coated with the Rituximab (RTX) anti-CD20 monoclonal antibody. NK cell activation was measured as intracellular IFNγ by flow cytometry. Experiments were conducted in the presence or absence of paired SP samples from HIV-1-infected donors, collected prior to (n=11) or following (n=11) initiation of ART or paired SP samples collected during (n=9) or before/after (n=9) *Chlamydia trachomatis* (CT) and/or *Neisseria gonorrhoeae* (GC) infection, to determine the relative ability of the SP samples to inhibit NK cell activation. (A) Gating implemented to assess NK cell activation, identifying lymphocytes, single cells, $CD56^{dim}CD3^{-}$ NK cells and revealing the proportion of NK cells expressing IFNγ following direct (top) and antibody-dependent stimulation (bottom) in the absence (No SP) or presence (With SP) of SP. (B) Graph depicts relative inhibition of direct activation of NK cells by 721.221 target cells by paired SP samples collected prior to and following initiation of ART. (C) The graph depicts the relative inhibition of the antibody-dependent activation of NK cells by RTX-coated 721.221 target cells by paired SP samples collected prior to or following initiation of ART. (D) The relative inhibition of direct NK cell activation by paired SP samples collected during or before/after CT and/or GC infection. (E) The relative inhibition of antibody-dependent NK cell activation by paired SP samples collected during or before/after CT and/or GC infection. Red lines in (D) and (E) indicate samples with urethral infections and black lines indicate samples with rectal infections. Data were analyzed with Wilcoxon matched pairs tests, and $p<0.05$ was considered significant. The data represented in (A-C) are single measurements collected across two experiments. The data represented in (D-E) are single measurements collected across two experiments.

Figure 2



Semen from HIV-1-infected men contains anti-HIV-1 antibodies and immunosuppressive factor(s). We assessed if suppression of viremia with antiretroviral therapy (ART) impacted seminal plasma immunosuppressive capacity or the Fc-dependent functions of seminal anti-HIV-1 antibodies. We also tested if active bacterial sexually transmitted infections (STIs) altered the immunosuppressive capacity of SP.