P53 Enveloped viruses and immune cells exposed to seminal plasma adsorb surface CD52g and bind to Human Contraception Antibody (HCA), potentially mediating physical entrapment of pathogens

Sarah Dohadwala, Jai Marathe, Gabriela Baldeon Vaca, Kevin Whaley, Deborah Anderson

INTRODUCTION

There is a need for multipurpose prevention technology (MPT) products that protect sexually active women and men from both unintended pregnancy and sexually transmitted infections. Currently, the only clinically approved MPT is the condom. One potential novel MPT product is the human contraception antibody (HCA), a monoclonal antibody that targets a carbohydrate epitope, CD52g, on sperm and induces sperm agglutination and immobilization[1]. CD52g is produced by male reproductive tract (MRT) epithelial cells and binds to lipid rafts in the sperm membrane via a GPI anchor[2]. We hypothesize that leukocytes and enveloped viruses in the MRT also adsorb CD52g, and that HCA potentially mediates physical entrapment of infected cells and pathogens in sperm agglutinates. Thus HCA has the potential to not only agglutinate sperm for contraception but also trap STI pathogens as a new MPT mechanism.

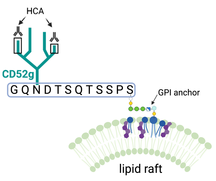

Figure 1: CD52g is an *N*-glycan on a GPI anchored peptide that nonspecifically adsorbs onto lipid rafts

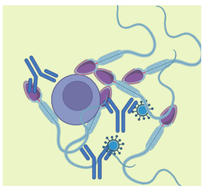

Figure 2: Schematic of physical entrapment of leukocytes and enveloped virus in sperm agglutinates

METHODS

Negative Stain Electron Microscopy was used to determine whether CD52g from seminal plasma binds to HIV. 10^5 infectious particles were incubated in seminal plasma for 24 hrs at 37°C, fixed in 4% PFA for 1 hr at RT, then concentrated via ultracentrifugation. Samples were quenched with glycine, then adsorbed for 1 min to a carbon coated grid. The grid was blocked, incubated with HCA for 30 min, washed with PBS, treated with 15 nm Protein A gold, then washed with PBS again. In order to positively identify HIV, the samples were fixed in 2.5% glutaraldehyde, incubated with VRC01, then 10nm Protein A gold, and finally stained with 1% Uranyl Acetate for 30 sec.

Resin Embedded TEM Immunogold was used to determine whether CD52g binds to HIV-infected seminal leukocytes and nascent HIV. Cells were washed in 0.1M cacodylate buffer and postfixed with 1% Osmium tetroxide (OsO4)/1.5% Potassium ferrocyanide (KFeCN6), incubated in 1% uranyl acetate for 1 hr followed by dehydration in grades of alcohol. The samples were then put in propylene oxide for 1 hr and infiltrated O/N in a 1:1 mixture of propylene oxide and TAAB Epon. The following day the samples were embedded in TAAB Epon and polymerized at 60°C for 48 hrs. Ultrathin sections (~80 nm) were stained with lead citrate and examined.

PBMC Infection: PBMCs were used due to the poor viability of seminal leukocytes. PBMCs were activated with PHA and IL-2, infected with TRJO HIV, incubated for 72 hrs, and then treated with seminal plasma for 4 hrs. Cells were washed, fixed with PFA, stained with HCA for 1 hr, then Protein A gold, and then prepared for resin embedded TEM.

Leukocyte-Sperm Coagglutination: Monocyte-derived macrophages were incubated with seminal plasma for 2 hours, stained with CMFDA viability dye, mixed with sperm, then treated with 100 µg/mL HCA to assess co-agglutination.

Virus-Sperm Coagglutination: HIV-1 was incubated in seminal plasma for 24 hrs. Freshly isolated sperm were added to the seminal plasma-virus mixture at 35 million/mL, followed by treatment with HCA at 100 µg/mL for 5 min. Samples were fixed in 4% PFA, then spun down and prepared for resin embedded TEM.

RESULTS

Both cell-free virions treated with seminal plasma, and nascent virions that had budded from CD52g positive HIV infected cells, were shown to have CD52g in their membranes (Figure 3 and Figure 5). CD52g positive PBMCs that were incubated with seminal plasma can be used to model seminal leukocytes (Figure 4 and 5). CD52g-positive PBMCs were physically trapped in sperm agglutinates upon HCA treatment (Figure 6). Preliminary evidence suggests that CD52g-positive viruses may be similarly trapped in sperm agglutinates (Figure 7).

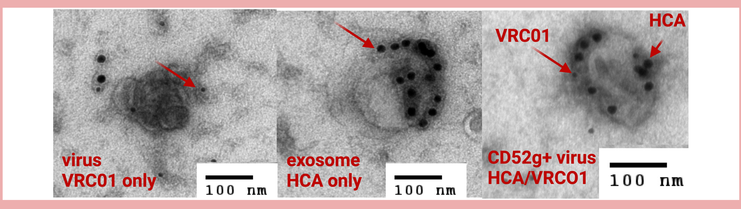

Figure 3: Viruses with surface CD52g after 24 hr incubation with seminal plasma. TEM negative stain images of single and double labeled particles. 10 nm beads are VRC01 labeling HIV Env and 15 nm are HCA labeling CD52g. Atypical morphology of virus may be due to adsorption onto carbon grid for imaging.

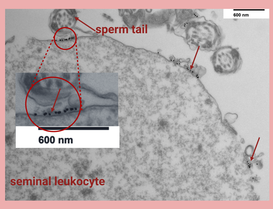

Figure 4: Seminal Leukocytes adsorb CD52g.

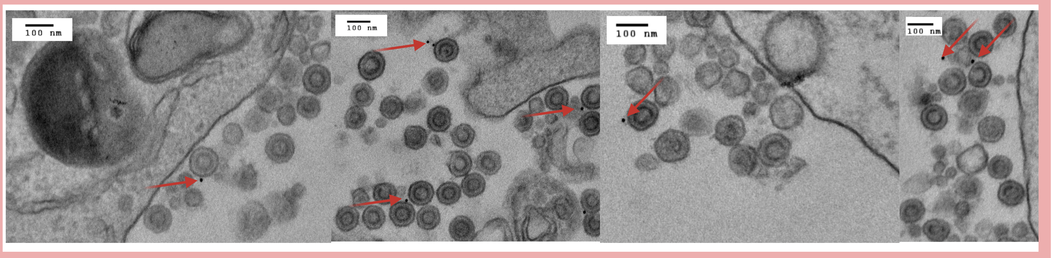

Figure 5: PBMCs treated with seminal plasma and infected with HIV show budded virus with surface CD52g. Arrows show gold beads labeling HCA (CD52g)

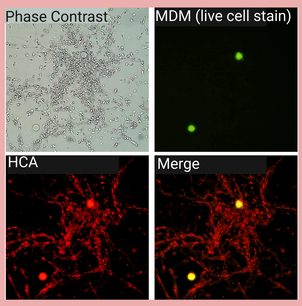

Figure 6: Physical entrapment of MDMs treated with seminal plasma in sperm-HCA agglutinates.

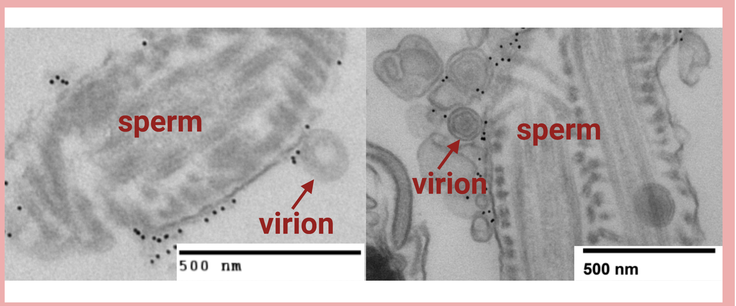

Figure 7: Preliminary evidence indicates CD52g+ virus may be physically entrapped in sperm-HCA agglutinates.

CONCLUSION

We demonstrated the presence of a GPI-anchored MRT peptide, CD52g, on leukocytes and enveloped viruses exposed to seminal plasma, and that HCA, which targets this peptide, entraps cells in sperm agglutinates. Preliminary data suggest that viruses are also entrapped after exposure to HCA in sperm co-cultures. This opens a new pathway to STI prevention coupled with contraception with physical entrapment as a primary mechanism to exclude pathogens from mucosal sites. Future studies will further characterize the potential of HCA to reduce infection in more physiologic contexts such as tissue explants and animal models and characterize other Fab and Fc mediated functions of HCA that may affect HIV and other pathogens in semen. In addition, future studies will use different isotypes of HCA, such as IgA, IgM, and other synthetic constructs containing additional Fab regions that agglutinate sperm more potently and quantify the physical entrapment of pathogens to understand downstream reduction in infection rates.

REFERENCES AND AUTHOR INFORMATION

References

1. Baldeon-Vaca G, Marathe JG, Politch JA, et al. (2021). Production and characterization of a human antisperm monoclonal antibody against CD52g for topical contraception in women. *EBioMedicine*.
2. Diekman et al. (2006). Evidence for a Unique N-Linked Glycan Associated with Human Infertility on Sperm CD52. *Immunological reviews*.

Figures generated in biorender.com

Author Information

SD, JM, DJA: Boston University Chobanian & Avedisian School of Medicine
https://www.linkedin.com/in/sarahdohadwala/
GBV: Moderna Inc, Cambridge, MA
KW: ZabBio, Inc., San Diego, CA

This study was supported by a grant from the NICHD (P50-HD098957).

BOSTON UNIVERSITY

Contraception Research Center