Characterization of the Neutralizing Antibody Response in a Linked HIV Superinfection

Deogratius Ssemwanga[1]*, Nicole A. Doria-Rose[2]*, Andrew D. Redd[3,4]*, Andrea R. Shiakolas[2], Andrew F. Longosz[3], Rebecca N. Nsubuga[1], Gershim Asiki[1], Janet Seeley[1,5], Amy Ransier[2], Sam Darko[2], Daniel Douek[2], Stephen F. Porcella[6], Thomas C. Quinn[3,4], John R. Mascola[2], and Pontiano Kaleebu[1,5]

1- Medical Research Council/Uganda Virus Research Institute (MRC/UVRI) Uganda Research Unit on AIDS, Entebbe, Uganda; 2- Vaccine Research Center, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, Maryland, USA; 3-Laboratory of Immunoregulation, Division of Intramural Research, NIAID, NIH, Bethesda, USA; 4-Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, USA; 5-London School of Hygiene and Tropical Medicine, Department of Clinical Research; 6- Genomics Unit, Research Technologies Branch, Rocky Mountain Laboratories, Division of Intramural Research, NIAID, NIH, Hamilton MT, USA. *These authors contributed equally

Poster 288
Andrew Redd; Staff Scientist, NIAID, NIH
855 North Wolfe St.
Rangos Building, room 540
Baltimore MD, 21205
Phone: 410-614-0813
Email: aredd2@jhmi.edu

Background

- HIV-superinfection (HIV-SI) occurs when an HIV-infected individual acquires a new HIV strain that is phylogenetically distinct from their existing viral population.
- Screening for cases of HIV-SI in large population cohorts has allowed for the examination of the potential role of pre-existing HIV-specific neutralizing antibodies (NAb) in protecting against HIV-SI.
- Two matched case control studies observed that individuals who became superinfected appear to have lower NAb responses compared to controls, but a larger study of female bar workers in Kenya found no association between pre-existing NAb and protection from HIV-SI.
- An alternative approach for exploring HIV-SI risk is to examine HIV-infected couples who acquired their viruses from different sources, thereby making their viral populations phylogenetically unlinked

Methods

- Participants in this study were enrolled in a General Population Cohort (GPC); established in 1989 in rural Southwest Uganda. Individuals in monogamous (n=15) and polygamous relationships (n=6) from the Rural Clinical Cohort previously identified as being HIV-infected, but virally unlinked by bulk HIV sequence analysis, were tested for occurrence of HIV-SI by next-generation sequencing (NGS) of three viral genomic regions (gag, pol, gp41).
- For the linked HIV-SI case, serum samples from before and after the time of HIV-SI for both the female, and her male husband, were subjected to single-genome amplification (SGA) to generate full-envelope sequences.
- For male samples prior to the female's HIV-SI event, full-length SGA was unsuccessful, therefore total RNA was amplified using universal primers and sequenced using a shotgun sequencing method.
- Full-length Env amplicons from SGA were subcloned or synthesized and used to generate Env-pseudoviruses. Env-pseudoviruses were tested for their neutralization susceptibility to their homologous serum, as well as their partner's heterologous serum from before and after HIV-SI

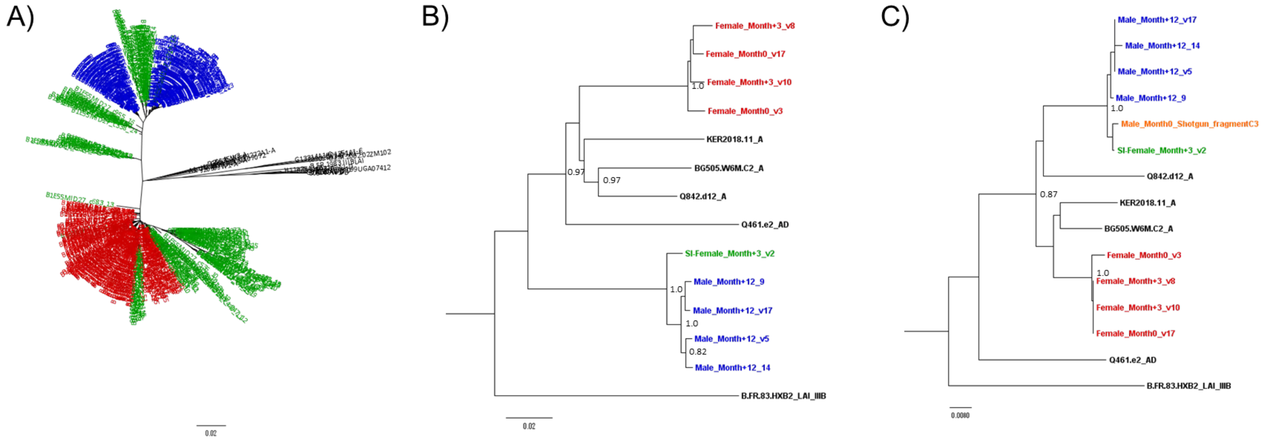


Figure 1: Neighbor-joining phylogenetic trees of A) NGS data of the female (Red) and male's (Blue) initial time point, and the female's post HIV-SI time point (Month +3; Green) B) Full SGA derived viral envelopes used for pseudotyped viruses and C) the 230 bp portion of the of the 5' end of Env from the pseudotyped viral isolates aligned with the NGS shotgun-sequencing fragment from husband's sample prior to HIV-SI (Orange).

			Female Sera				Male Sera		Clade A/AD Sera Controls								
Virus from Sample	Pseudovirus	Subtype	-3 Months	0 Months	+3 Months	+10 Months	+0.3 Months	+ 12 Months	CHAVI219 2008	POC36633 1995	POC44951 1996	APOS101 2004	Uganda 357139	Uganda 191520	Uganda 119601	Uganda 355472	Uganda 171950
Female 0 Months	Female_Month0_v3	A	101	87	ND	681	<20	<20	102	<20	44	<20	69	72	112	62	78
	Female_Month0_v17	A	124	135	ND	680	<20	<20	101	164	158	<20	124	112	180	121	108
Female +3 Months	Female Month+3_v8	A	110	122	ND	263	<20	<20	72	68	199	<20	105	80	129	90	85
	Female Month+3_v10	A	150	184	ND	181	<20	<20	51	43	84	<20	91	79	100	95	62
	SI-Female Month+3_v2	AD	<20	<20	<20	112	34	870	410	<20	<20	<20	<20	22	28	<20	<20
Male +12 Months	Male Month+12_v5	AD	60	60	54	62	60	125	782	<20	<20	35	<20	104	102	<20	<20
	Male Month+12_v9	AD	30	39	ND	46	26	44	904	<20	<20	33	<20	123	153	<20	<20
	Male Month+12_v14	AD	<20	<20	<20	21	<20	44	478	<20	<20	36	<20	35	52	<20	<20
	Male Month+12_v17	AD	27	24	ND	29	21	53	950	<20	<20	24	<20	143	271	<20	<20
Heterologous Virus Panel	BG505.W6M.C2	A	62	35	ND	29	ND	29	721	<20	<20	62	30	<20	71	<20	<20
	KER2018.11	A	143	230	ND	294	ND	24	351	<20	<20	55	26	<20	23	<20	20
	Q842.d12	A	344	68	ND	344	ND	29	1240	79	116	36	151	109	211	101	137
	Q461.e2	AD	<20	<20	ND	<20	ND	<20	99	<20	<20	<20	<20	<20	<20	<20	<20
	TRO.11	B	31	29	ND	34	ND	96	218	<20	<20	140	<20	33	58	<20	<20
	DU422.01	C	166	27	ND	274	ND	25	77	111	116	45	121	74	151	76	87

Figure 2: Values in table indicate the dilution of the heat-inactivated serum required to block fifty percent of a standard infectious dose (ID50): weak (green), moderate (yellow), and strong (orange) neutralization values are highlighted. Along the top of the table are indicated time points of female and male sera, as well as a collection of sera from HIV-subtype A and A/D infected individuals.

Legend
>1000
100-1000
20-100
<20

Results

- A case of a linked HIV-SI was identified in a polygamous relationship in which an HIV-infected male had four wives who were also HIV-positive. One of his wives became superinfected, with a virus that was phylogenetically linked to her husband's viral strain.
- All Env-pseudoviruses from the couple were not unusually sensitive to neutralization, and had a tier-2 like phenotype.
- The female's serum samples prior to HIV-SI displayed moderate NAb activity against her homologous virus.
- Her serum prior to HIV-SI and immediately post HIV-SI contained no detectable NAb activity to the superinfecting strain.
- Ten months post HIV-SI the female had developed a moderate response to the superinfecting strain (Figure 2). In contrast, there was no increase in NAb response to the other male viruses from Month +12 (Figure 2).
- The male's serum at the time of HIV-SI had no detectable neutralizing activity against his wife's strains, and his NAb responses to those strains did not improve a year post HIV-SI.

Discussion

This identification and characterization of a genetically confirmed case of a linked heterosexual HIV-SI event provides a unique opportunity to examine HIV-SI in an individual where her infecting partner is known. In this case, HIV-SI occurred in a chronically infected female who had moderately potent and broad anti-HIV NAb responses. Despite this, she possessed no detectable NAb response to the superinfecting strain during the estimated HIV-SI window, which potentially could have protected her. This unique case highlights the exciting amount of potential information that even a small number of these linked HIV-SI events could provide

Acknowledgements

We would like to thank all the study physicians and staff, as well as a special thanks to all the study participants. This study was supported in part by the UK Medical Research Council (MRC) and the UK Department for International Development (DFID) under the MRC/DFID Concordat agreement; and the VRC and DIR, NIAID, NIH. This study was approved by the Science and Ethics Committee of the Uganda Virus Research Institute and by the Uganda National Council for Science and Technology (IRB#00001693). Clinical, epidemiological data and blood samples were obtained following informed written consent.