Antibody Profiling Identifies Novel Biomarkers for Duration of HIV Infection

Oliver Laeyendecker[1,2,3], Kai Kammers[2], Athena Chen[2], Mariya V. Sivay[1], Brandon M. Sie[1], Tiezheng Yuan[1], Divya Mohan[1], Sanjay Kottapalli[1], Tomasz Kula[4], Charles Morrison[5], Stephen J. Elledge[4], Ron Brookmeyer[6], Ingo Ruczinski[2], H. Benjamin Larman[1], and Susan H. Eshleman[1]

[1] Johns Hopkins University School of Medicine, Baltimore, MD, USA; [2] Johns Hopkins University School of Public Health, Baltimore, MD, USA; [3] NIAID, Baltimore, MD, USA; [4] Harvard University, Boston, MA, USA; [5] FHI 360, Durham, NC, USA; [6] University of California Los Angeles, Los Angeles, CA, USA.

Poster 943
Oliver Laeyendecker
Staff Scientist, NIAID
Assistant Prof., JHU, JHSPH
855 North Wolfe St.
Rangos Building, room 538A
Baltimore MD, 21205
410-502-3268

BACKGROUND

Improved methods for cross-sectional HIV incidence estimation are needed for surveillance of the epidemic and evaluating the impact of HIV prevention interventions. We used a massively-multiplexed system, VirScan, to identify novel biomarkers for HIV incidence estimation. VirScan uses phage display immunoprecipitation sequencing to quantify antibody binding to >3,300 peptides spanning the HIV genome.

METHODS

Plasma samples were obtained from the Hormonal Contraception and Genital Shedding Study (HC-GS Study; Uganda, Zimbabwe; 2001-2009, Table).

Characteristic	Discovery Sample Set	Validation Sample Set		
Country of origin	Zimbabwe	Zimbabwe	Uganda	
HIV subtype	C	C	D	Total
Number of samples	403	44	28	72
Number of participants	57	25	7	32
Duration of infection (years)	0.04 to 8.7	0.24 to 8.8	0.23 to 9.1	0.23 to 9.1
Mean samples per participant, (range)	7 (5 - 8)	1 (1- 4)	4 (4-4)	2 (1-4)
Female sex, % of participants	100%	100%	100%	100%
# samples: duration of infection[a]				
≤3 months	48	1	1	2
>3-6 months	67	3	7	10
>6-12 months	95	4	7	11
>12-24 months	53	9	3	12
>2-4 years	47	5	6	11
>4-6 years	42	10	2	12
>6-8.7 years	51	12	2	14
# samples: CD4 cell count (cells/mm^3)				
>500	141	13	9	22
500-350	93	6	7	13
≤350	94	25	12	37
missing	75			
# samples: viral load (copies/mL)				
>10,000	154	27	18	45
10,000-1,000	62	3	7	10
≤1,000	44	13	3	16
missing	143	1		1
# participants who started ART	24	4	1	5
# samples from participants who started ART	48	9	3	12
Mean time to ART (range; years)	3.3 (1.2, 6.4)	1.9 (0.6, 5.5)	1.1 (1.1, 1.1)	1.6 (0.6, 5.5)

Antibody binding was quantified for HIV peptides in the VirScan library. Peptides were assessed for association between the duration of HIV infection and antibody binding (normalized z-scores). Analysis of duration of infection used generalized estimating equations (GEEs) to account for inclusion of multiple samples from the same individual; P-values were adjusted using the Bonferroni correction.

METHODS

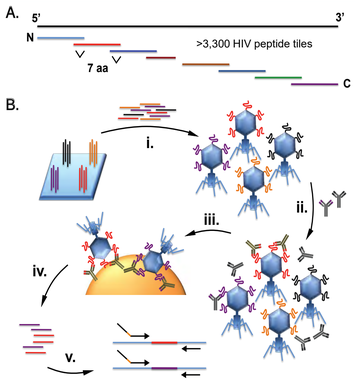


Figure 1. VirScan assay.

Synthesize large libraries of overlapping DNA tiles

Express encoded peptides on bacteriophage

Quantify antibody reactivity to each peptide
→ phage immunoprecipitation
→ DNA sequencing

RESULTS

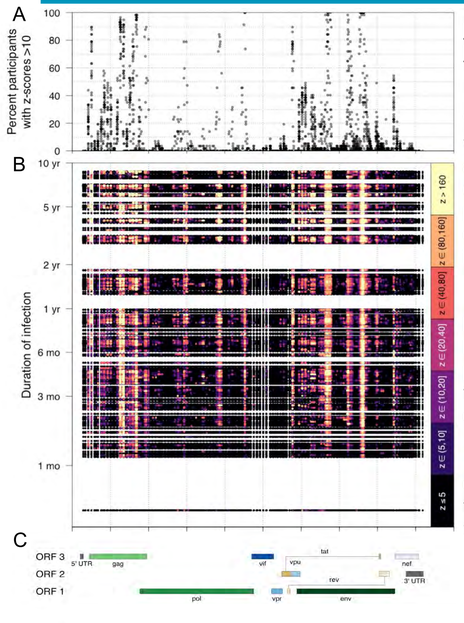


Figure 2. Antibody binding data.

Panel A shows the % of participants who had strong antibody binding (z-score >10) for peptides at different positions in the HIV genome.

Panel B shows the level of antibody binding to HIV peptides as a function of duration of HIV infection.

Panel C shows the open reading frames (ORFs) of the HIV genome.

RESULTS

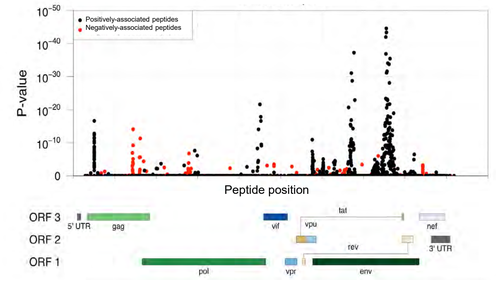


Figure 3. Informative peptides. The figure shows the position of peptides where antibody binding was significantly associated with the duration of HIV infection (adjusted P-value <0.05). Black dots: 266 peptides where binding increased over time; red dots: 43 peptides where binding decreased over time. ORF: open reading frame.

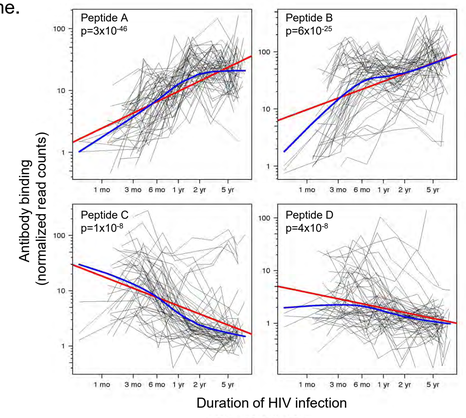


Figure 4. Longitudinal data for the top four peptides. The figure shows data for the four peptides that had the strongest independent association between antibody binding and the duration of HIV infection. Two peptides had increased antibody binding over time (A in gp41, B in gp120), and two had decreased antibody binding over time (C in gag, D in pol). Black lines: longitudinal data from individual study participants. Blue lines: locally-weighted regression curves (all participants); red lines: least squares regressions lines (all participants).

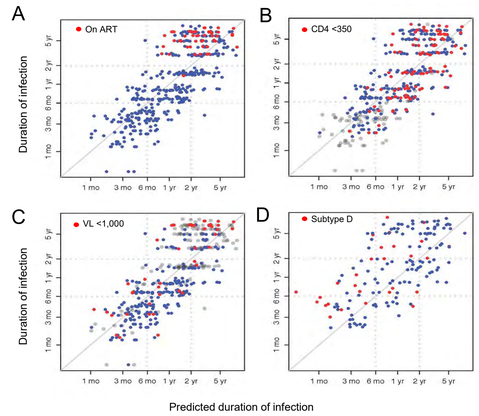


Figure 5. Use of a 4-peptide model to predict duration of HIV infection. Antibody binding data for the four top peptides were used to generate a simple, unweighted, composite measure (A+B-C-D). Panels A-C show predicted duration of infection vs. observed duration of infection for the Discovery sample set. These two factors were highly correlated (GEE p<1x100^{-100}). Red dots show data for samples with low CD4 cell count, low viral load, and those on antiretroviral treatment (ART). None of the "challenge" samples (low CD4 cell count, low viral load, on ART) from those with infections >1 year were misclassified as "recent" (<6 months) by the 4-peptide model. Panel D shows data from the independent Validation sample set (GEE p<3x10^{-36}); red dots show data for subtype D samples.

CONCLUSIONS

- This study provides proof-of-principle that the VirScan assay can be used to identify peptides for applications such as cross-sectional HIV incidence estimation.
- A simple, unweighted, 4-peptide model generated using data from the VirScan assay can be used to predict duration of HIV infection.
- We are optimizing a high-throughput multi-peptide assay for quantifying antibody binding to peptides of interest for applications such as cross-sectional HIV incidence estimation.

This work was funded by R01-095068 (Eshleman). Additional funding was provided by the HIV Prevention Trials Network (UM1AI068613) and additional support was provided by the Division of Intramural Research, NIAID.
We would like to thank the participants and staff of the HC-GS study.
The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Presented at the 2019 CROI Conference
Seattle, WA, USA, March 4-7, 2019