Profiling antibody cross-reactivity amongst SIV isolates using a peptide microarray

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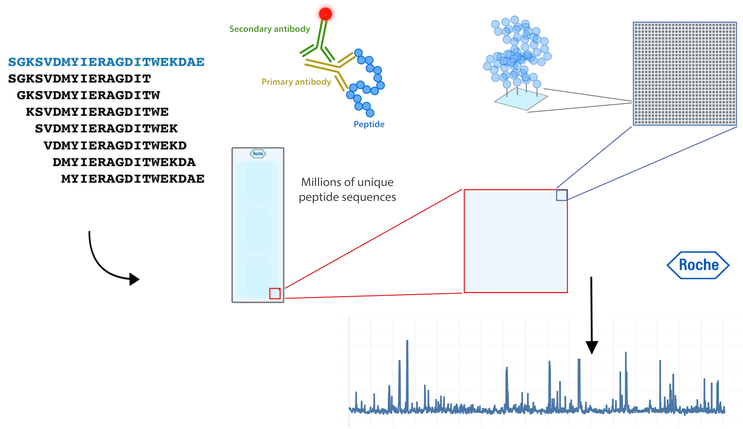
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BACKGROUND

- The high mutability of the human immunodeficiency virus (HIV) makes development of effective therapies and vaccines challenging. In the past decade, a number of broadly neutralizing anti-HIV antibodies have been identified; however, tools for assessing antibody cross-reactivity are still relatively low throughput.
- Here we prototyped the use of an ultradense peptide microarray to assess antibody cross-reactivity in macaques infected with SIVmac239 against a panel of simian immunodeficiency virus (SIV) species.

METHODS

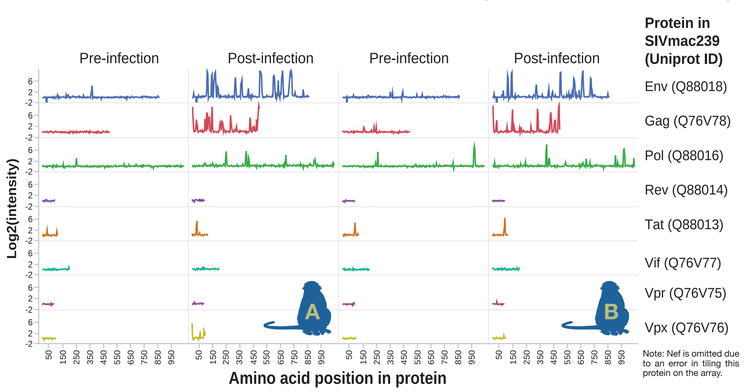
- We collected blood samples from Mauritian cynomolgous macaques infected with SIVmac239. Data from two macaques are presented here.
- Using recently developed Roche Nimblegen ultradense peptide array technology, we designed an array tiling the complete proteome of SIVmac239 and the complete Env proteins of 21 strains of SIV in overlapping contiguous 16-residue peptides.
- Macaque sera samples were assayed for IgG antibody binding.



RESULTS

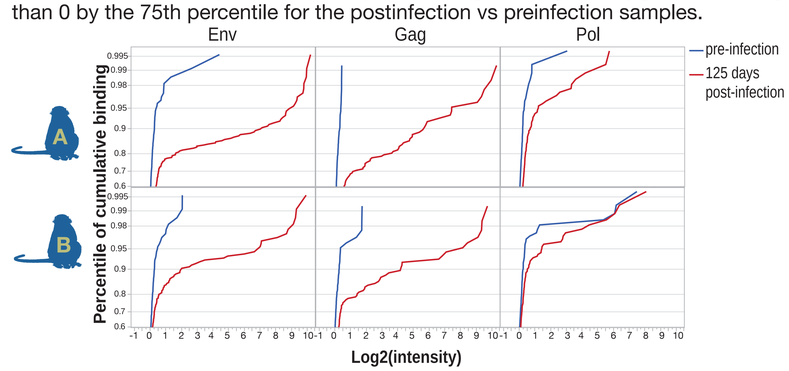
Anti-SIV antibodies bind throughout SIVmac239 genome

- At 125 dpi, antibodies consistently bind peptides representing the Env, Gag, and Pol proteins of SIVmac239, with the most frequent binding observed in Env and Gag.



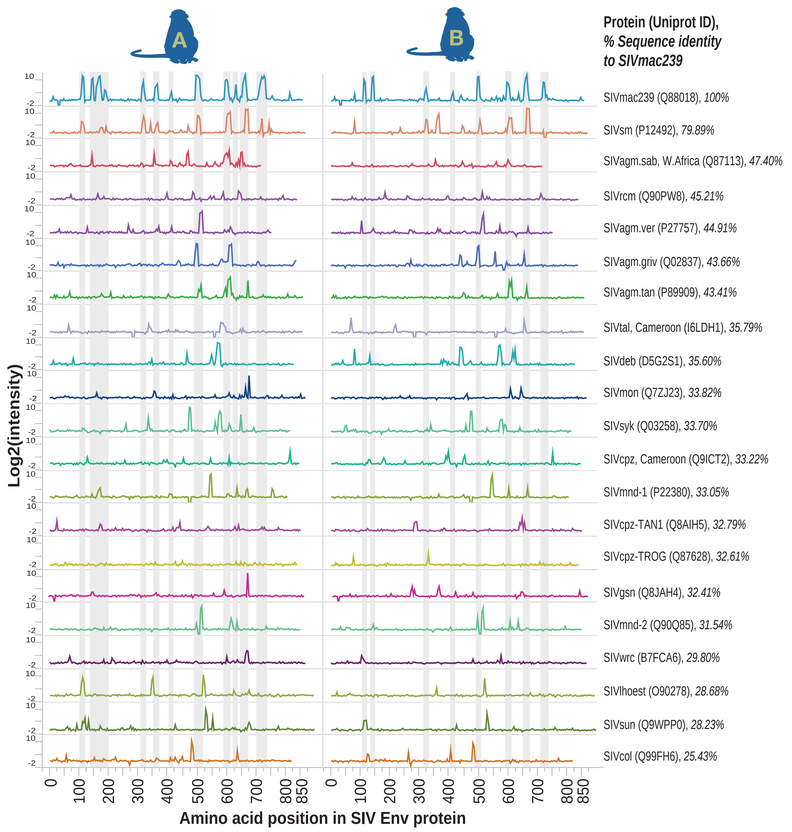
Cumulative binding differentiates post-infection samples

- Cumulative distribution function (CDF) plots of Env, Gag, and Pol demonstrate the differences in preinfection (blue) and 125 dpi (red) binding.
- Strains with more (higher percentage of) peptides bound by antibodies will branch from 0 earlier; for example, SIVmac239 peptides begin having notably greater intensity than 0 by the 75th percentile for the postinfection vs preinfection samples.



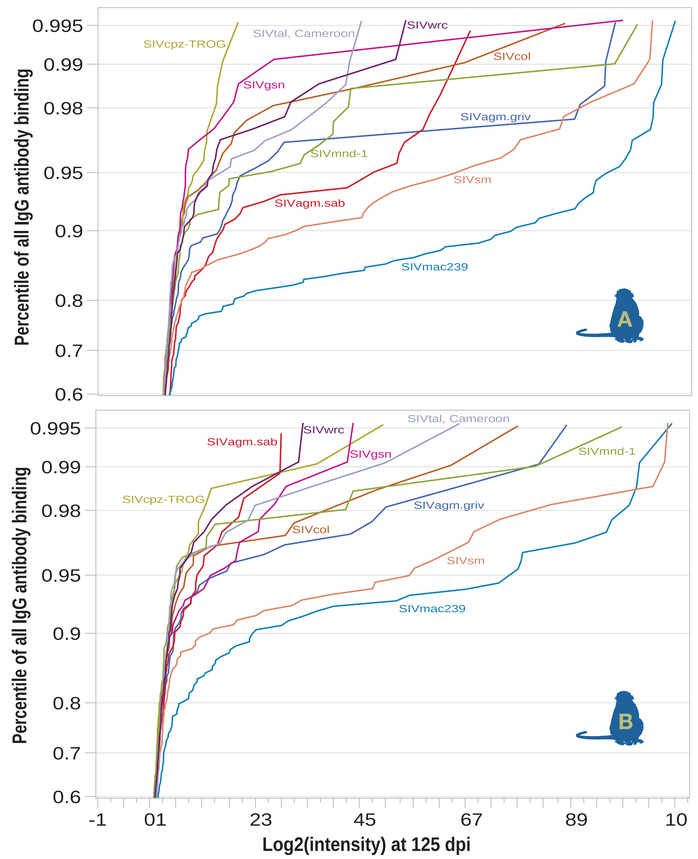
Cross-reactive binding is observed across SIV Env variants

- Antibody binding in samples taken at 125 dpi is strongest against the Env protein of SIVmac239. Cross-reactive binding to other SIV Env proteins generally parallels percent sequence identity of the variants to SIVmac239, with more frequenct binding to strains with greater identity to SIVmac239.
- Shading indicates regions corresponding to each animal's SIVmac239 epitopes.



Cumulative binding varies by similarity to infecting strain

- CDF plots show proportionally greater cumulative binding throughout Env proteins of SIV strains with greater sequence identity to SIVmac239. Strain coloration matches that in the previous graphic; selected strains are shown.



CONCLUSIONS

- Serum IgG antibodies bind proteins throughout the SIVmac239 proteome to varying degrees, with most pronounced binding in the Env and Gag proteins.
- Profiling of IgG antibody binding to the Env proteins of multiple SIV strains reveals cross-reactive antibody activity and shows antibodies to SIVmac239 are capable of recognizing and binding to Env proteins of a large variety of SIV strains.
- Future directions:
  - Expand this technology to survey binding capabilities of antibodies against a large number of HIV mutants, allowing for efficient, high-throughput screening for antibodies with broad recognition capabilities.
  - Adapt this method for use in directing diagnostic and therapeutic development for other rapidly mutating pathogens.

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