Most vaccines elicit B cells that undergo affinity maturation to produce high-affinity memory B cells. These cells are re-elicited by exposure to the relevant pathogen and provide protection against it. Many vaccines require multiple immunizations to afford adequate protection, though it is not clear what changes are induced in the responding B-cell populations by repeated vaccination. Our sought to determine the key components of intraclonal evolution and interclonal competition underlying protection in such vaccines.

We enrolled six volunteers with no previous exposure to anthrax or Anthrax Vaccine Adsorbed (AVA) to receive the standard AVA protocol of 5 injections over 18 months. We performed single-cell paired heavy-chain/light-chain immunoglobulin variable region gene (IgVRG) sequencing on IgG+ plasmablasts isolated from pre-immune and one-week post-immunization samples. We analyzed these sequences, partitioning over 10,000 IgVRG pairs into clones using phylogenetic methods and carrying out statistical analyses to elucidate diversification and selection. We measured kinetic constants via surface plasmon resonance for several antibodies synthesized recombinantly.

Immunogen-reactive IgG+ plasmablast clones were first observed after the second immunization. In spite of intense affinity maturation and strong genetic evidence of selection, both intra- and inter-clonal diversity remained high through the 18 months of observation. At each immunization, approximately half or more of the IgVRG recovered belonged to clones that had not been observed previously. Each clone exhibited a pattern of growth and decline; at no time did any clone rise to dominance. Patterns of selection pressure within clones changed regularly over the lifetime of the clone.