AN HIV-1 NEUTRALIZING ANTIBODY ESCAPE PATHWAY CONSTRAINED BY ANTIBODY-DEPENDENT CELLULAR CYTOTOXICITY (ADCC)

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Background

Both neutralization and antibody-dependent cellular cytotoxicity (ADCC) may be required for effective protection against HIV-1 infection. While there is extensive information on the targets of early neutralizing antibody (nAb) responses, much less is known about the targets of ADCC responses, which are more difficult to unravel due to overlap among nAb and ADCC epitopes.

Methods

To examine the potential role of ADCC immunity in controlling virus replication *in vivo*, we mapped the first targets of these responses and determined how they influenced viral evolution relative to nAb responses. We investigated the kinetics of early neutralizing and ADCC responses in four HIV-1 subtype C infected individuals. ADCC activity was detected using a flow-cytometry based ADCC-GranToxiLux (GTL) assay using recombinant gp120 coated CEM_NKR$_{CCR5}$ as target cells and cryopreserved PBMC from a HIV-seronegative donor as effector cells.

In one individual (CAP63), single genome amplification and next-generation sequencing were used to generate a detailed map of viral quasispecies over time in CAP63. We mapped Env targets of ADCC-mediating antibodies using HIV-1 infectious molecular clone (IMC)-infected cells and mapped nAb responses, using pseudovirus assays. The antibody response was further characterised using an HIV-1 binding antibody multiplex assay for IgG subclasses IgG1, IgG2, IgG3 and IgG4. The kinetics of viral replication was determined by p24 ELISA after growth in human PBMC.

Results

In four individuals recruited within 6 weeks of HIV-infection, ADCC responses were detected early and 3 to 7 weeks prior to nAb responses. In CAP63, who showed the highest nAbs and ADCC responses, we identified a V4 epitope recognised by both nAbs and ADCC antibodies, but with some differences in epitope recognition. We identified accelerated viral evolution in this region concurrent with emergence of nAb activity, but not ADCC activity. However, one nAb escape mutation rendered the virus highly susceptible to both autologous ADCC responses, as well as other ADCC-mediating antibodies. Deep sequencing of viral populations revealed that this escape mutation, which did not affect viral replicative fitness, was strongly suppressed, suggesting nAb escape pathways can be constrained by ADCC responses. In conclusion, ADCC responses and nAbs recognised overlapping but unique epitopes, with the ADCC activity present prior to

nAb responses but not impacting viral evolution. However, our data indicate ADCC activity may constrain nAb escape pathways that expose common ADCC antibody epitopes.

Conclusion

These data suggest that ADCC responses play a role in maintaining an ADCC resistant phenotype even among nAb escape variants. Together, these findings have implications for antibody-based vaccine design and antibody-based studies that aim to eliminate the reservoir in acute infection.