



POSTER PRESENTATION

Open Access

Isolation of broadly neutralizing HIV-1 antibodies from high-throughput single B cell culture

NS Longo*, N Doria-Rose, K McKee, S O'Dell, MK Louder, Z Yang, R Bailer, JR Mascola

From AIDS Vaccine 2012

Boston, MA, USA. 9-12 September 2012

Background

The isolation of broadly neutralizing HIV-1 antibodies that arise during infection has provided insights into the design of vaccine immunogens capable of eliciting similar antibody response. The use of HIV-specific sorting probes resulted in the isolation of antibodies to vulnerable viral epitopes, such as the CD4-binding site, but the use of such probes does not explore the subject's immunoglobulin repertoire breadth.

Methods

To identify new HIV-1 monoclonal antibodies (mAbs), we developed a B-cell culture system to isolate and screen thousands of B cells. Memory B cells were isolated by negative selection and individually cultured in 384 well plates with irradiated feeder cells expressing CD40 ligand. The addition of cytokines IL-2 and IL-21 stimulated proliferation and immunoglobulin secretion.

Results

After 14 days in culture, approximately 35% of the B cell clones secreted >100 ng/ul of IgG which met the sensitivity threshold to screen each clone in an automated micro-neutralization assay. B cell clonal expansion allowed recovery of the immunoglobulin heavy and light chains by RT-PCR, with subsequent cloning into expression vectors and mAb testing against a large panel of viruses. In one experiment screening approximately 8600 B cells, 9 clones were identified as potential contributors to the neutralization detected in the patient's serum. One of the isolated clones produced mAb VRC22 with 30% neutralization breadth and moderate potency. Further studies revealed that VRC22 was sensitive to JRCSF glycan mutants N332A and N301A but not N160K. VRC22 utilizes VH4-34 with a single amino acid CDR1 deletion

and a mutation frequency of 7% which is a lower level of affinity maturation than observed for most known HIV-1 neutralizing antibodies.

Conclusion

This B-cell culture system allows efficient screening of thousands of individual B cells and the recovery of antigen specific mAbs. This approach can be used to isolate human mAbs to diverse pathogens.

Published: 13 September 2012

doi:10.1186/1742-4690-9-S2-P91

Cite this article as: Longo *et al.*: Isolation of broadly neutralizing HIV-1 antibodies from high-throughput single B cell culture. *Retrovirology* 2012 9(Suppl 2):P91.

Submit your next manuscript to BioMed Central and take full advantage of:

- Convenient online submission
- Thorough peer review
- No space constraints or color figure charges
- Immediate publication on acceptance
- Inclusion in PubMed, CAS, Scopus and Google Scholar
- Research which is freely available for redistribution

Submit your manuscript at
www.biomedcentral.com/submit



NIH, Vaccine Research Center, Bethesda, MD, USA



© 2012 Longo *et al.*; licensee BioMed Central Ltd. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/2.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.