Retrovirology

BioMed Central

Poster presentation

P04-01. Simultaneous enumeration of HIV-1 gp41 Env-specific IgG and IgM antibody-secreting cells with a multiplex B-cell fluorospot assay

M Bonsignori*, K Hwang, N Vandergrift, MA Moody and BF Haynes

Address: Department of Medicine, Duke University Medical Center – Duke Human Vaccine Institute, Durham, NC, USA
* Corresponding author

from AIDS Vaccine 2009
Paris, France. 19–22 October 2009

Published: 22 October 2009

Retrovirology 2009, **6**(Suppl 3):P29 doi:10.1186/1742-4690-6-S3-P29

This abstract is available from: http://www.retrovirology.com/content/6/S3/P29

Background

Understanding antibody generation and maintenance in the context of HIV-1 infection and vaccination are key for the development of an AIDS vaccine. Antigen-specific B-cells can be enumerated with the ELISpot assay but B-cell availability is generally limited. Therefore, a multiplex detection system is needed to study multiple antibody types. We report a multiplex B-cell fluorospot assay in which the chromogenic reaction was replaced by a fluorescent detection system for the simultaneous enumeration of total and Env-specific IgG and IgM antibody-secreting cells (ASCs).

Methods

Anti-Human IgG and IgM antibodies were conjugated with quantum dot nanocrystals and isotype-specific ASCs were identified by spots visible at different wavelengths. ASC counts from single-color ELISpot and fluorospot assays were compared using serial dilutions of IgG- and IgM-secreting HIV-1 gp41-specific human hybridoma cells (5000 to 100 cells/well). In multiplex experiments, IgG and IgM ASCs were detected using different cell ratios (1:0, 1:0.5 and 1:1). Gp41-specific responses were evaluated using the cognate gp41 antigen as capture reagent.

Results

Both assays detected equivalent numbers of ASCs at each cell concentration ($r = 0.999$, for IgG ASCs and $r = 0.998$ for IgM ASCs; $p < 0.0001$) within their linear range. Isotype-mismatched nanocrystal-conjugated antibodies did not interfere with spot development when only one cell line was used. In multiplex experiments, the percentage of observed spots was within 3.0% and 2.4% of the expected spot counts for IgG and IgM, respectively, both of which were well less than one standard deviation from the expected value. No statistically significant differences in the counts of gp41-specific vs total IgG (313 ± 13 vs 335 ± 4 ASCs; $p = 0.55$. Mean ± SD) and IgM (174 ± 9.9 vs 160 ± 21.2 ASCs; $p = 0.49$) were found.

Conclusion

The fluorospot assay allows simultaneous detection of anti-HIV-1 Env-specific IgG and IgM ASCs in a multiplex format. This feature is critical when specimen availability is limited and multiplexing can be expanded to include additional specificities.