Real-time Fluorospot assay to detect antigen-specific, antibody-producing B cells in a live-cell culture setting

QingQing Wu, Xiaohu Fan, Rong Yin, Lori J. West

Department of Pediatrics, Surgery, and Medical Microbiology and Immunology, University of Alberta

Abstract

Antibody-mediated immunity plays a critical role in organ transplantation. Currently, the evaluation of this B cell response is achieved by the enzyme-linked immunosorbent assay (ELISA) and the enzyme-linked immunospot (ELISpot) assay, which only suffice for quantification purposes and have considerable limitations, such as lengthy development times and low sensitivity. Furthermore, these techniques stipulate the discarding of the antigen-specific B cells during their respective procedures, which prevents further analysis of the function-confirmed B cells. We have developed a fluorescent antibody immunospot assay (Fluorospot) to detect real-time B cell antibody production with defined antigen specificities via the high affinity streptavidin-biotin interaction. This novel method enables us to not only visually detect ABO antigen-specific B cells in a highly sensitive and low background manner, but also to conduct studies of these lymphocytes at cellular and molecular levels. We may then garner the resources and the knowledge to construct a database of genetic repertoire information regarding antigen-specific B cells vis-à-vis different disease statuses in humans. The significance of such a technology extends to fields such as autoimmune diseases and cancer.

Introduction

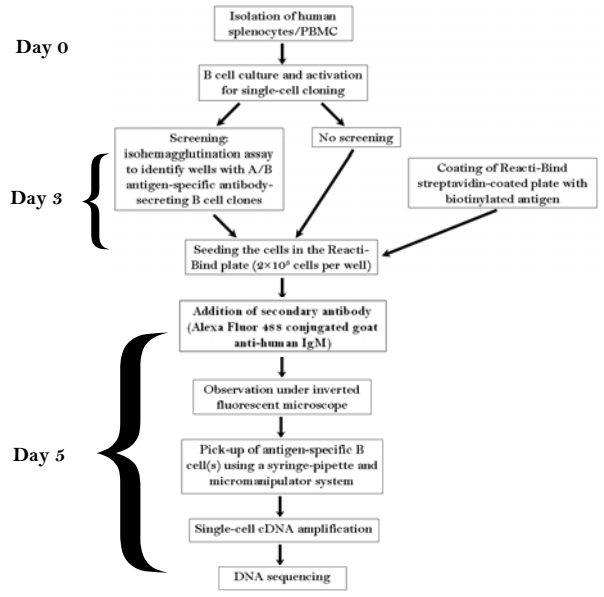
Premise: Many limitations exist with the ELISA and the ELISpot assay when used to evaluate behaviors and characteristics of antigen-specific B cells: lengthy development times, low sensitivity, low signal-background ratio, prevalent occurrence of "false-positive" spots, and the procedural stipulation to discard the B cells.

Need: We determined the possibility of conducting ABO-incompatible heart transplants in infants via donor-specific B cell tolerance in 2004. However, the clinical significance of this finding has been overshadowed by our imperfect understanding of the underlying molecular mechanisms by which donor-specific B cell tolerance occurs. We speculate that many clues lie within the complementarity-determining regions (CDRs) based repertoire study of the ABO antigen specific B cell population, which will be different between different B cell tolerant status. Through a genetic analysis of numerous antigen-specific B cell clones, we will be able to build a database of genetic repertoire information regarding ABO antigen-specific B cells for humans in different disease statuses and to study the molecular mechanism of the development of self or transplant ABO antigen specific B cell tolerance by association with of their mechanistic features, such as V(D)J recombination, usage frequency of immunoglobulin V_H and V_L gene families, and somatic hypermutation.

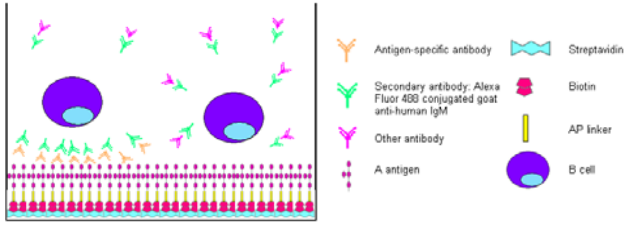
Objective: We aim to develop a technique that would enable us not only to visually detect antigen-specific B cells in a sensitive and timely manner, but also to examine their characteristics at cellular and molecular levels.

Further applications: The use of a live-cell assay to detect antigen-specific B cells such as this one is expandable to the research fields of autoimmune disease and cancer. Furthermore, this protocol may be adapted to hybridoma technology and could facilitate the identification of hybridomas with the desired antigen-specificity.

Methods



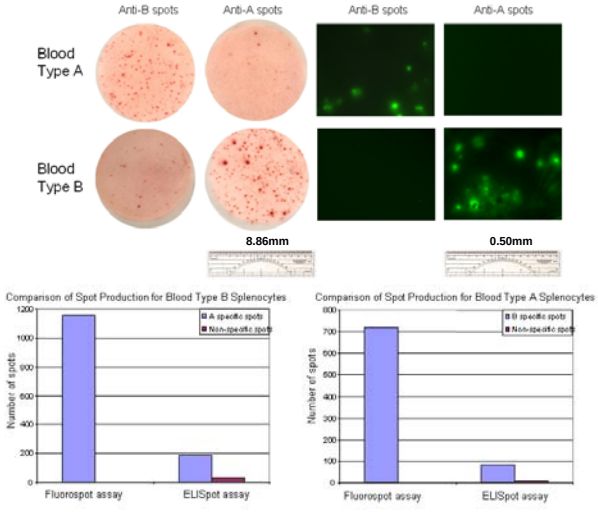
Results



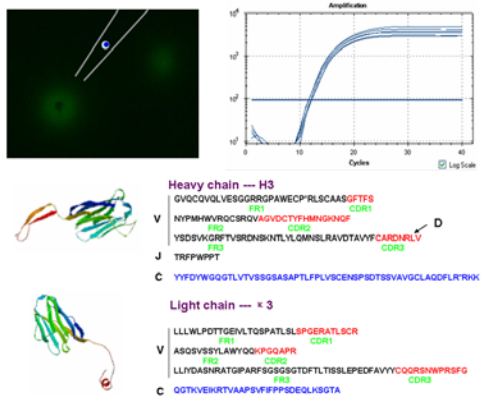
The mechanism of the Fluorospot assay is depicted. The streptavidin coating of the well forms a strong, non-covalent bond with the biotin molecule of the biotinylated antigen. The antibodies secreted by the B cells will be absorbed to local A antigen around the B cells during culture period. By adding the secondary antibodies, which are associated with fluorochromes, a fluorescent spot will formed around the secreting B cell.

As viewed under the inverted fluorescent microscope, the progression of the development of Fluorospots is shown, 6 to 96 hours after the addition of the secondary antibody. It can be observed that the optimal time to identify antigen-specific B cells is at 48 h.

Results (Continued)



A side-by-side comparison of the ELISpot and Fluorospot assays (not at same magnifications) clearly show the advantages of using the Fluorospot to identify antigen-specific B cells: higher signal-background ratio, higher sensitivity, smaller and more specific spots. The size of the formed Fluorospots is 30-40 µm in diameter, whereas that of the formed ELISpots is about 100-500 µm in diameter. There are typically around 50 times more Fluorospots than ELISpots in one well. "False-positive" spots are also absent in the Fluorospot.



The further applications of the Fluorospot assay is portrayed, as the antigen-specific B cell can be picked up with a micropipette system, single cell RT-PCR can be performed to amplify the desired cDNA, and the sequence of the B cell antibody CDRs can be obtained.

Acknowledgements

Thank you to Dr. Frank Fan for your mentorship; thank you to West Lab members for your technical and moral support!