Fluorescent-bait labelling for the ex vivo detection of HBV antigen-specific B cells

Immunology Assays

Authors Information

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Introduction

Fluorescently conjugated antigen-bait systems have been extensively used to identify antigen-specific B cells and probe humoral immunity across different settings. Using this principle, HBV antigens are used to bind the B cell receptor (BCR), permitting antigen-specific B cell detection by flow cytometry[1,2]. Fluorochromes can either be attached covalently via chemical conjugation to the antigen or attached non-covalently by biotinylating the antigen. Dual-staining antigen-baits (where an antigen is directly conjugated to two distinct fluorochromes) have now been used to identify HBsAg- and HBcAg-specific B cells with a high degree of reliability and specificity[3]. This system can be used to detect and characterise cells *ex vivo* or adapted to isolate antigen-specific cells using fluorescence-activated cell sorting.

Materials and Reagents

- HBV antigen baits:
  - HBcAg-Dy550 and HBcAg-Dy650
  - HBsAg-Dy550 and HBsAg-Dy650

NB:// Antigen baits not commercially available but gained via courtesy of Gilead Sciences Inc.

- Fixable Live/Dead™ stain (Invitrogen™)
- FcR blocking reagent (Miltenyi Biotec)
- Brilliant Stain buffer (BD Biosciences™)
- BD Cytofix™ (BD Biosciences™)
- 96-well U-bottomed plates OR 5ml polypropylene tubes

Experimental Procedures

Prepare 1 x 10^6 – 3 x 10^6 PMBCs, from freshly isolated PBMCs or thawed from frozen, per stain. All staining should be performed on a single-cell suspension either in 96-well U-bottomed plates or in 5ml polypropylene tubes.

1. Centrifuge cells (300g, 4 min at 4ºC)
2. Stain cell pellet with a fixable cell viability dye diluted in PBS (Live/Dead™ stain, Invitrogen™) for 15 min at 4ºC
3. Wash cells using 1x PBS and centrifugation (300g, 4 min at 4ºC)

4. Block non-specific antibody binding using FcR blocking reagent (Miltenyi Biotec) for 15 min at 4ºC
5. Stain cells with 50ml of anti-human monoclonal antibodies of interest, in combination with Dy550 and Dy650 antigen baits at the following concentrations:

HBsAg -Dy550 and -Dy650: 10-20ug/ml HBcAg -Dy550 and -Dy650: 300-600ng/ml Dilute antibodies in 50%-Brilliant Stain buffer (BD Biosciences™) and 50%-PBS supplemented with 0.5%-FBS and 2mM EDTA to minimize interactions between multiple fluorescent dyes. Incubate cells with monoclonal antibodies and antigen-baits for 30 min in the dark on ice.

6. Wash cells with PBS supplemented with 0.5%-FBS and 2mM EDTA and centrifugation (300g, 4 min at 4ºC)
7. Fix cells (BD Cytofix™, BD Biosciences™) prior to acquisition

TIP: Run the cells at a low threshold rate and make sure to acquire all of the cells Stringent gating criteria should be applied during analysis to exclude doublet, dead and CD19-negative cells. Cells stained with an identical panel minus antigen-bait staining can be used to control for non-specific binding and guide gating. All reagents used should be stored at 4ºC; monoclonal antibodies and antigen-baits should be stored in the dark.

References

1. Salimzadeh, L. *et al.* PD-1 blockade partially recovers dysfunctional virus-specific B cells in chronic hepatitis B infection. *J. Clin. Invest.* (2018) doi:10.1172/JCI121957.
2. Burton, A. R. *et al.* Circulating and intrahepatic antiviral B cells are defective in hepatitis B. *J Clin Invest* **128**, 4588–4603 (2018). DOI: 10.1172/JCI121960
3. Bert, N. L. *et al.* Comparative characterization of B cells specific for HBV nucleocapsid and envelope proteins in patients with chronic hepatitis B. *Journal of Hepatology* **72**, 34–44 (2020). DOI: 10.1016/j.jhep.2019.07.015