In vitro class switching staining for flow cytometry

Ewen Gallagher (e.gallagher@ucl.ac.uk)
UCL
Thomas Enzler (tenzler@ucsd.edu)
UCSD
Atsushi Matsuzawa
UCSD
Michael Karin
UCSD

Method Article

Keywords: cytometry

Posted Date: June 26th, 2006

DOI: https://doi.org/10.1038/nprot.2007.36

Abstract

This protocol was used in our Nature Immunology paper.

Reagents

Frosted microscope slides \(Fisher Scientific, Pittsburgh, PA) RPMI-1640 medium \(Cellgro) ACK Lysing Buffer \(Cambrex) Cell strainers, 70 µm nylon \(Becton Dickinson) CD43 \(Ly-48) magnetic beads and separation columns \(Miltenyi) B cell complete medium: RPMI-1640 medium \(Cellgro) plus 2 mM glutamine \(Gibco) and 100 U/ml of penicillin g/ml and 100 µg/ml of of streptomycin \(Gibco), 10% fetal calf serum \(heat inactivated; Sigma), and 5×10-5 M 2-mercaptoethanol \(Sigma). 24-well flat bottom culture plates \(Nunc) Anti-CD40 \(clone 3/23; Pharmingen) Recombinant mouse IL-4 \(Peprotech) Fc blocker \(anti-CD16/CD32; Pharmingen) Biotinylated anti-IgG1, anti-IgG2a-2b \(both Pharmingen) Streptavidin conjugate \(Pharmingen) FACS buffer: PBS + 0.5% fetal calf serum + 5mM EDTA

Procedure

Perform the following steps at 4 ^{o}C unless indicated otherwise. 1 Harvest splenocytes by grinding of spleens in RPMI-1640 medium between two frosted microscope slides. Spin cells at 1000 RPM for 5 min. Remove erythrocytes by adding one equivalent volume of ACK Lysing Buffer to the remaining pellet. Gently resuspend, swirl tube for 1 min. Fill tube with 10 ml of RPMI-1640, centrifuge at 1000 RPM for 5 min. Resuspend in 10 ml of RPMI-1640. Filter through 70 µm nylon cell strainer. Centrifuge at 1000 RPM for 5 min. Resuspend 1 × 107 cells in 500 µl of FACS buffer. Obtain B cells by CD43 negative purification using anti-CD43 magnetic beads. 2 Dilute CD43- B cells to a concentration of 5×105 cells/ml in B cell complete medium. Use 24-well plates. Incubate 5×105 B cells in presence of 10 µg/ml of anti-CD40 \(clone 3/23), plus 10 ng/ml of recombinant mouse IL-4 for IgG1 analysis \(6). Incubate for 4 d in a humidified 37 ^{o}C, 5% CO2 incubator. 3 Wash cells with FACS buffer. Preincubate cells on ice for 10 min in presence of Fc blocker \(anti-CD16/CD32). We usually use 0.5 µl of Fc blocker/ 5×105 cells in a total volume of 100 µl. CRITICAL STEP: Good Fc blockade. 4 Stain cells with biotinylated anti-IgG1, anti-IgG2a-2b \(we used the antibodies at a dilution of 1:200 in a total volume of 100 µl). Incubate on ice for 20 min. Wash 2x with FACS buffer. 5 Stain cells with streptavidin conjugate of your choosing. Wash 2x with FACS buffer. 6 Analyze by flow cytometry.

References

1. Anzelon, A. N., Wu, H. & Rickert, R. C. Pten inactivation alters peripheral B lymphocyte fate and reconstitutes CD19 function. _Nat Immunol_ **4**, 287-294 \(2003). 2. Sanchez, Y. _et al._ Humoral and cellular immunity to hepatitis B virus-derived antigens: comparative activity of Freund complete adjuvant alum, and liposomes. _Infect Immun_ **30**, 728-733 \(1980). 3. Engel, P. _et al._ Abnormal B lymphocyte development, activation, and differentiation in mice that lack or overexpress the CD19 signal transduction molecule. _Immunity_ **3**, 39-50 \(1995). 4. Gallagher, E. D., Xu, S., Moomaw, C., Slaughter,

C. A. & Cobb, M. H. Binding of JNK/SAPK to MEKK1 is regulated by phosphorylation. _J Biol Chem_ **277**, 45785-45792 \(2002). 5. Gallagher, E. D., Gutowski, S., Sternweis, P. C. & Cobb, M. H. RhoA binds to the amino terminus of MEKK1 and regulates its kinase activity. _J Biol Chem_ **279**, 1872-1877 \(2004). 6. Rush, J. S., Hasbold, J. & Hodgkin, P. D. Cross-linking surface Ig delays CD40 ligand- and IL-4-induced B cell Ig class switching and reveals evidence for independent regulation of B cell proliferation and differentiation. _J Immunol_ **168**, 2676-2682 \(2002).

Acknowledgements

Supported by the National Institutes of Health \(ES04151 and ES06376 to M.K.), the American Cancer Society \(M.K.) and the Leukemia and Lymphoma Society of America \(T.E.; SCOR grant 7332-06).