RESEARCH CASE STUDY

Revealing rare and transient cell states:

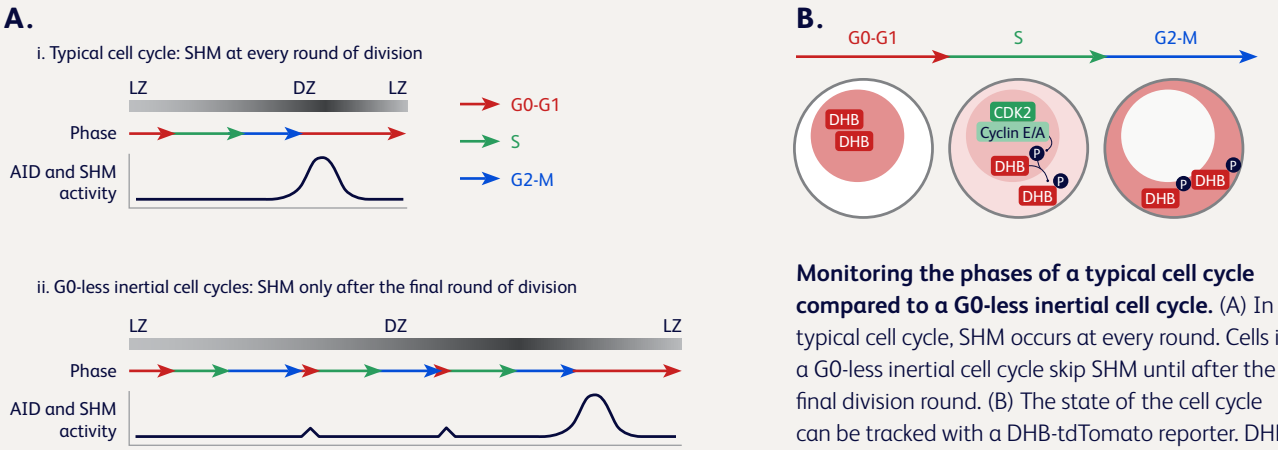
See how BD CellView™ Image Technology uncovers new mechanisms in antibody development

SYNOPSIS: Germinal centers in the lymph nodes are a crucial site of B cell maturation during an immune response. There, antibodies are refined through rapid mutations, called somatic hypermutation (SHM), to diversify the B cell repertoire followed by expansion to amplify high-affinity B cells. However, the number of deleterious mutations generated by this process usually outnumber affinity-enhancing mutations. Since the 1990s, scientists believed that this process occurs through a phase of rapid mutation followed by a selection phase that eliminates cells with harmful mutations, allowing cells producing high-affinity antibodies to continue to multiply. Yet, evidence from Gabriel Victora's lab contradicts this long-held hypothesis by finding that a single B cell clone can multiply so rapidly that it takes over the entire germinal center.[1] This phenomenon is known as "clonal burst" and these bursts happen without selection in what is called "inertia cell cycling." Without selection, however, the cells would presumably acquire many deleterious mutations.

IN A RECENT STUDY, the team used image-based cell sorting to investigate how these B cells undergo clonal bursts without harmful effects.[2] The researchers first noticed that clonally bursting cells have low SHM rates. Because the enzyme driving SHM (AID) acts primarily in the G0/G1 phases of the cell cycle, Pae et al hypothesized that clonally bursting cells spend less time in this phase. To study this, the researchers attached a fluorescent reporter to DNA helicase B (DHB), a protein that is phosphorylated by cyclin dependent kinase 2 (CDK2). CDK2 has low activity in the G0 and G1 phases of the cell cycle and without phosphorylation, DHB remains in the nucleus. When phosphorylated by CDK2, DHB moves into the cytoplasm indicating cell cycle progression through the G1, S and G2 phases.

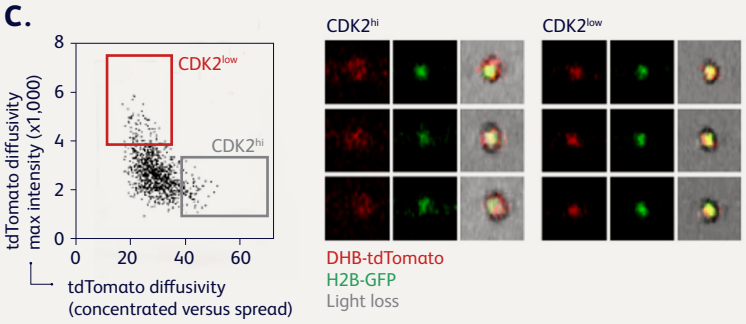
Using image-based cell sorting with BD CellView™ Image Technology in the BD FACSDiscover™ S8 Cell Sorter, the researchers separated cells based on DHB localization and thus cell cycle phase. They then sequenced the immunoglobulin heavy and light chain variable genes from these cells, as these regions normally mutate during SHM. Cells in the G0 phase had more mutations than cells in the G2/M phase. Based on the reporter's location, they found that cells that are undergoing clonal bursts lack the G0/G1 phase of the cell cycle, which explained why they have fewer mutations. This type of cell sorting based on protein localization and subsequent sequencing of the different populations would otherwise be impossible using traditional flow cytometry methods.

> "What we achieved with image sorting would not be doable without it."



Monitoring the phases of a typical cell cycle compared to a G0-less inertial cell cycle. (A) In a typical cell cycle, SHM occurs at every round. Cells in a G0-less inertial cell cycle skip SHM until after the final division round. (B) The state of the cell cycle can be tracked with a DHB-tdTomato reporter. DHB moves from the nucleus to the cytoplasm after phosphorylation by CKD2. Using this reporter, the team could monitor cell cycle progression.

Image credit: Figure 3A-B. Pae et al. 2025. CC BY 4.0.



Separating cells based on protein localization. Cells were sorted with the BD FACSDiscover™ S8 Cell Sorter based on DHB-tdTomato intensity and diffusivity. Cells with concentrated, nuclear tdTomato are CDK2low and indicate G0-G1 cell cycle phases while cells with diffuse tdTomato are CDK2high and indicate G2-M phases. Thus, it is possible to use image-based cell sorting to separate cells by cell cycle phase.

Image credit: Figure 4A. Pae et al. 2025. CC BY 4.0.

CONCLUSION: Image-based single-cell analysis and sorting using BD CellView™ Image Technology allowed these researchers to identify and sort cell-cycle states and link them to functional outcomes — something that could only be done using image-based cell sorting. Findings from this work reshape our understanding of B cell biology, with implications for vaccine design and antibody discovery efforts that rely on B cell engineering, immunization or germinal center-like systems. This study also demonstrates how image-based single cell sorting and analysis can help biopharma teams uncover new insights in immune cell function even for rare or transient functional states such as B cell maturation in germinal centers.

Publications

1. Tas, J. M., Mesin, L., Pasqual, G., et al. Visualizing antibody affinity maturation in germinal centers. Science 351(6277), 1048–1054 (2016). https://doi.org/10.1126/science.aad3439
2. Pae, J., Schwan, N., Ottino-Loffler, B. et al. Transient silencing of hypermutation preserves B cell affinity during clonal bursting. Nature 641, 486–494 (2025). https://doi.org/10.1038/s41586-025-08687-8