Cells Coming to Life: Tools for Visualizing Immune Response

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ABSTRACT

Fluorescence visualization and technology is indispensable in biology and biochemistry, and in the field of immunity, this is no exception. Experiment complexity and sophistication is increasing due to the nature, number, and abundance of cellular targets, as well as from the variety of visualization methods available, including flow cytometry and fluorescence microscopy. Here, we describe an expanding set of reagents and instruments for measuring the immune response, including novel probes for cell cycle and DNA replication, cell proliferation, viability and phagocytosis. With the novel cell proliferation dye CellTrace™ Violet, we demonstrate the ability to track proliferating cells through several generations. This probe is then used to compare the efficiency of three different T cell-stimulation methods. This multi-parametric cell proliferation study is further refined with the addition of novel probes for DNA content and S phase detection through Click-iT™ Edu chemistry. Finally, a novel pH-sensitive probe is used to quantify the phagocytosis of bacteria and yeast by primary human macrophages. These probes provide mechanistic insight and increasingly granular information into the nature of immune response upon stimulus.

Comparing the Efficiency of T Cell Stimulation Methods

The mitogen Concanavalin A is perhaps the most widely used lectin and can be used as an alternative T cell stimulus. This lectin, an α-mannose / α-glucose-binding lectin, is frequently used as a surrogate for antigen-presenting cells in T cell stimulation experiments. Concanavalin A binds with high affinity to glycoproteins on the cell surface, specifically oligomannose- and hybrid-type N-glycans, and commits cells to proliferation.

The traditional combination of anti-CD3 and IL-2 is used to stimulate T cells to proliferation.

Here, we observe the efficiency of different stimulation techniques for T cell proliferation. We observe a high degree of efficiency for the CD3/CD28 Dynabead activation method, as a significant amount of the CellTrace™ Violet fluorescence has been imparted to T cells downstream. Concanavalin A and anti-CD3/IL-2 methods are also highly efficient, and show a distribution of fluorescent signal across generations.

Measuring Phagocytosis of Bioparticles in Real Time

The basic cellular internalization processes of endocytosis and phagocytosis are important to many areas of cell biology including receptor internalization, pathogen response, and apoptotic cell clearance. Here we present two fluorogenic, pH-sensitive probes, one green and one red. These dyes are non-fluorescent at neutral pH typically found in the cytosol and extracellular environment, but become brightly fluorescent in acidic cellular compartments, permitting direct monitoring of internalization and acidification processes.

The pH-sensitive, fluorogenic-based pHrodo™ Green and pHrodo™ Red dyes undergo a dramatic increase in fluorescence in response to an environmental shift from high to low pH. Both dyes have a pKa of approximately 6.8 – ideal for monitoring physiologically relevant pH changes.

Live-Cell Visualization of Phagocytosis

Live-Cell Visualization of Macrophage Phagocytosis of Zymosan

The green fluorescence of pHrodo™ Green conjugates enables multiplexed analysis of phagocytosis. Mouse monocytes/macrophages (MMM cells, ATCC™) were plated on 35mm MatTek dishes and labeled with 50 nM Lysotracker™ Deep Red in LCIS for 15 minutes. Cells were washed twice, labeled with 20 μg/ml pHrodo™ Green Zymosan BioParticles® conjugates, and incubated for 1h. The pHrodo™ BioParticles® conjugates appear blue outside the cell as they are labeled with NucleBlue™ Live Cell Stain, but no green signal is seen until the particles enter the acidic environment of phagolysosomes. Lysosomes are shown in red. Images were acquired with standard DAPI, FITC, and Cy5 filters.