Measuring Immunological Synapse Using the FlowSight Imaging Flow Cytometer

Abstract

Interaction between antigen-specific T cells and antigen presenting cells (APC) cognate ligand involve reorganization of the cytoskeleton and recruitment of adhesive and signaling molecules to the site of intercellular contact. Sustained adhesion of T cells to APCs and formation of the immunological synapse after T cell receptor stimulation are required for the antigen-specific response. One way to measure an immunological synapse is by fluorescently labeling the molecules that have been recruited to the synapse and imaging via confocal or conventional fluorescence microscopy. However, immunological synapses are often rare and therefore difficult to analyze objectively and statistically by traditional microscopy methods. In this study, the Amnis® FlowSight® imaging flow cytometry platform was employed to collect imagery of large numbers of cells to assess the percentage of T cells involved in an organized immunological synapse.

Quantifying the frequency of organized immunological synapse

In this experiment, Raji B cells were loaded with Staphylococcal enterotoxin B (SEB) to make APCs. The SEB-loaded APCs were incubated with human T cells purified from peripheral blood. After incubation the cells were fixed, permeabilized and labeled with CD3-PE-TexasRed (T cells, orange), CD19-AF488 (Raji B cells, green), phallloidin-AF647 (actin, red) and DAPI (nuclear stain, purple).

A FlowSight imaging flow cytometer equipped with the Quantitative Imaging (QI) option was used to assess the frequency of conjugates with an organized immunological synapse. Image analysis was completed using image-based algorithms available in the IDEAS 5.0 image analysis software package. Figure 1 demonstrates how to isolate cell conjugates using the IDEAS software.
Polymerization and concentration of actin at the immunological synapse results in a high local pixel intensity. A “Valley” mask operation on the DAPI image defined the region of contact between cell conjugates, as shown in Figure 2. The actin intensity was then quantified within the Valley mask.

In addition, the overlap between the cells was measured by using the Co-localization wizard in the IDEAS 5.0 software. The Co-localization wizard measures the Bright Detail Similarity between two images (the cross-correlation of the smaller fluorescence features), in this case CD3 and CD19. Higher Bright Detail Similarity scores are associated with greater overlap between the cells and an organized immunological synapse.

By plotting the Bright Detail Intensity of the actin in the Valley mask vs the Bright Detail Similarity of the CD3 and CD19, the number of cells with an organized immunological synapse were quantified, as shown in Figure 4. The percent of T cells in the Immune Synapse gate was 8.4% for the SEB-treated sample versus 1.6% for the control (no SEB) sample.

Conclusions

Imaging flow cytometry combines the quantitative power of large sample sizes common to flow cytometry with the information content of microscopy. This study utilized the FlowSight and its companion IDEAS data analysis software to demonstrate in an objective and statistically robust manner that there is a significant increase in number of cell conjugates with an organized immunological synapse when the APCs have been loaded with SEB.