T-cell activation and suppression assays using flow cytometry

Immunological applications on the BD Accuri™ C6 Plus flow cytometer

Features

- Assess cell proliferation, activation, and suppression rapidly and reliably using flow cytometry
- Assess proliferation of different subsets of T cells in a single culture
- Visualize cells of different sizes simultaneously

Flow cytometry is a useful tool for assessing T-cell activation and validating immunosuppression, because it can measure multiple cellular activation parameters (such as proliferation, cytokine and surface marker upregulation) simultaneously and at the single-cell level. On the BD Accuri™ C6 Plus personal flow cytometer, one can perform these assays right on the benchtop, and study the influence of white blood cells and specific immunosuppressors on each other.

Suppression of immune response is an important element of normal cell function and immune homeostasis. While overt suppression leads to an immune-compromised host, as seen in various cancers where the tumor microenvironment actively silences the host T-cell response, excessive activation can result in disease states including autoimmunity and transplant rejection. Thus, the study of T-cell activation and suppression is an important part of basic research with translational implications. Frequently studied natural immune suppressors include regulatory T cells (Tregs), mesenchymal stromal cells (MSCs), and myeloid-derived suppressor cells (MDSCs).

Traditional approaches to assess cell proliferation have relied on colorimetric assays such as MTT, or assays that measure the uptake of radioactive thymidine by proliferating cells. Both of these approaches measure only average responses in a sample, and do not differentiate between different immune cell types such as $CD4^+$ vs $CD8^+$ T cells.

Flow cytometry is a powerful alternative technique for supplementing these conventional assays due to its ability to quantify responses at the single-cell level. Figure 1 shows an experiment on the BD Accuri C6 Plus in which MSCs were used to suppress proliferation of activated T cells in a sample of peripheral blood mononuclear cells (PBMCs). Proliferation was measured using CFSE, with a fluorescent signal that is reduced by half with each daughter generation. While unstimulated cells showed only one CFSE peak, indicating that they had not proliferated, stimulated cells showed multiple peaks corresponding to multiple generations of daughter cells. In contrast, stimulated cells co-cultured with MSCs showed only one peak, similar to unstimulated cells, indicating that proliferation was suppressed. Surface markers were used to distinguish $CD4^+$ from $CD8^+$ T cells, each exhibiting unique proliferation profiles. This ability of the BD Accuri C6 Plus to examine cell proliferation in different cell types is not shared with other conventional approaches.

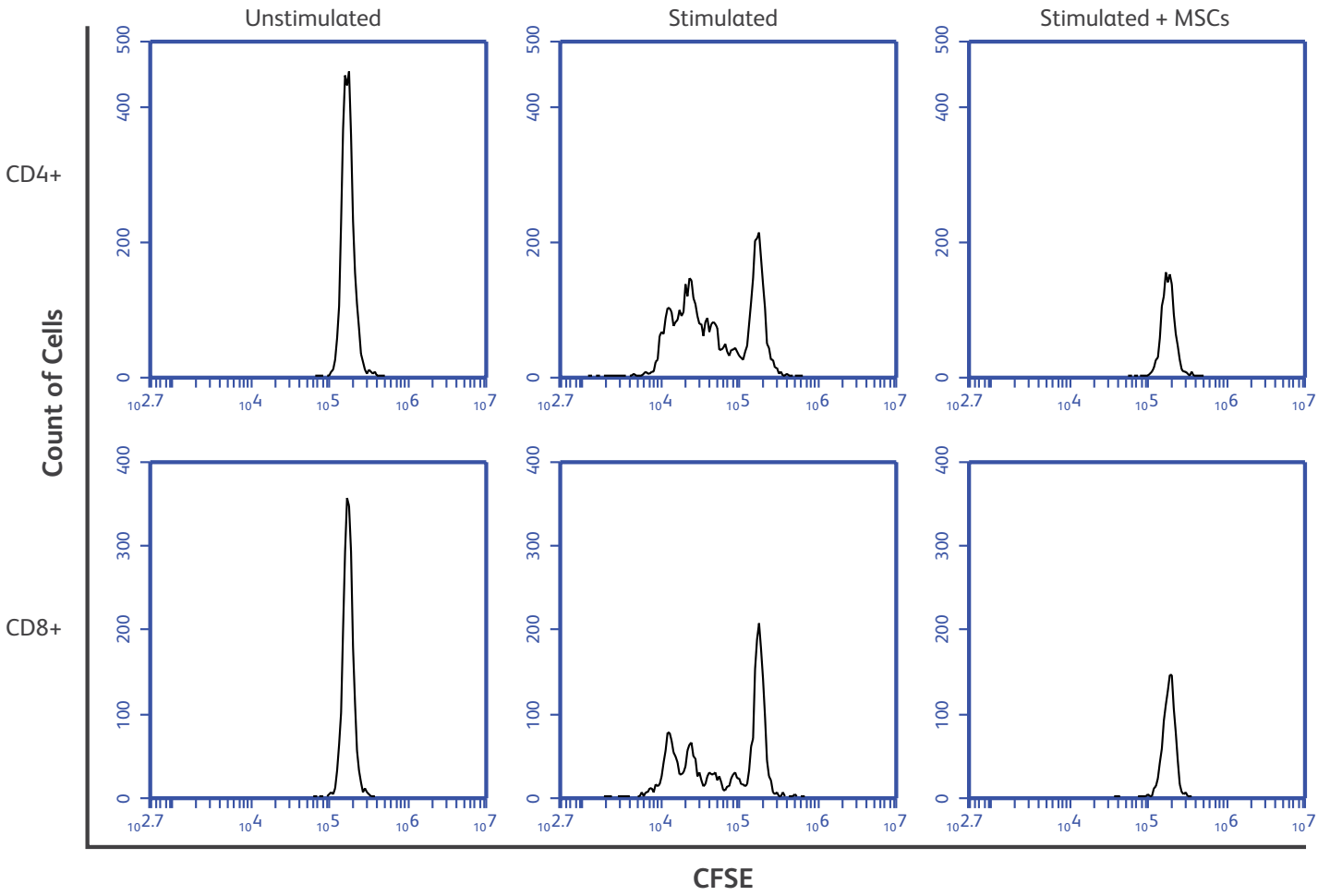

Figure 1. Suppression of immune cell proliferation by MSCs

Human PBMCs were stained with BD Horizon™ CFSE and stimulated with Dynabeads™ Human T-Activator CD3/CD28 beads (Thermo Fisher Scientific) for three days in the presence or absence of human MSCs. Unstimulated PBMCs (no Dynabeads and no MSCs) were used as controls. On day 3, cells were harvested and stained with BD Pharmingen™ Alexa Fluor™ 647 Mouse Anti-Human CD4 (Cat. No. 557707) and BD Pharmingen™ PerCP-Cy™5.5 Mouse Anti-Human CD8 (Cat. No. 560662), and then analyzed on a BD Accuri C6 Plus. **Results:** Compared to unstimulated PBMCs (left plots), stimulated PBMCs (middle plots) showed multiple CFSE peaks, indicating that they proliferated through multiple generations. $CD4^+$ PBMCs (top) proliferated more than $CD8^+$ PBMCs (bottom) in response to stimulation, as shown by a higher CFSE dilution (more peaks to the left of the parental peak). Stimulated PBMCs cultured in the presence of MSCs (right plots) showed a single CFSE peak (similar to unstimulated cells), indicating that proliferation was suppressed in both $CD4^+$ and $CD8^+$ PBMCs.

Figure 2 shows how the BD Accuri C6 Plus, with two light scatter detectors and four fluorescence channels, can investigate interactions between co-cultured cells. In Figure 2A, the cells were initially distinguished using light scatter, based on the larger size and granularity of MSCs vs PBMCs. With its 7-decade dynamic range, the BD Accuri C6 Plus can visualize these large and small cells on the same scale, without the need for voltage adjustments. Characteristic surface markers, detected in two fluorescence channels, were then used to distinguish MSCs (CD105⁺) from PBMCs (CD45⁺) and confirm the initial separation of the cell populations by scatter properties.

The other two fluorescence channels were used to assess activation of both MSCs and PBMCs. In Figure 2B, MSCs co-cultured with PBMCs up-regulated the MSC activation marker CD54 compared to MSCs cultured alone. However, co-culturing had the opposite effect on PBMCs. In Figure 2C, stimulated PBMCs co-cultured with MSCs expressed intermediate levels of the PBMC activation marker CD25—more than unstimulated PBMCs, but less than stimulated PBMCs cultured alone—again showing the inhibitory effect of MSCs on PBMC activation.

Easy to use, simple to maintain and versatile, the BD Accuri C6 Plus personal flow cytometer is equipped with a blue laser, a red laser, two light scatter detectors and four fluorescence detectors. A compact design, fixed laser alignment, pre-optimized detector settings and automated instrument QC result in a system that is simple to use. For walkaway convenience, the optional BD CSampler™ Plus accessory offers automated sampling from 24-tube racks or multiwell plates.

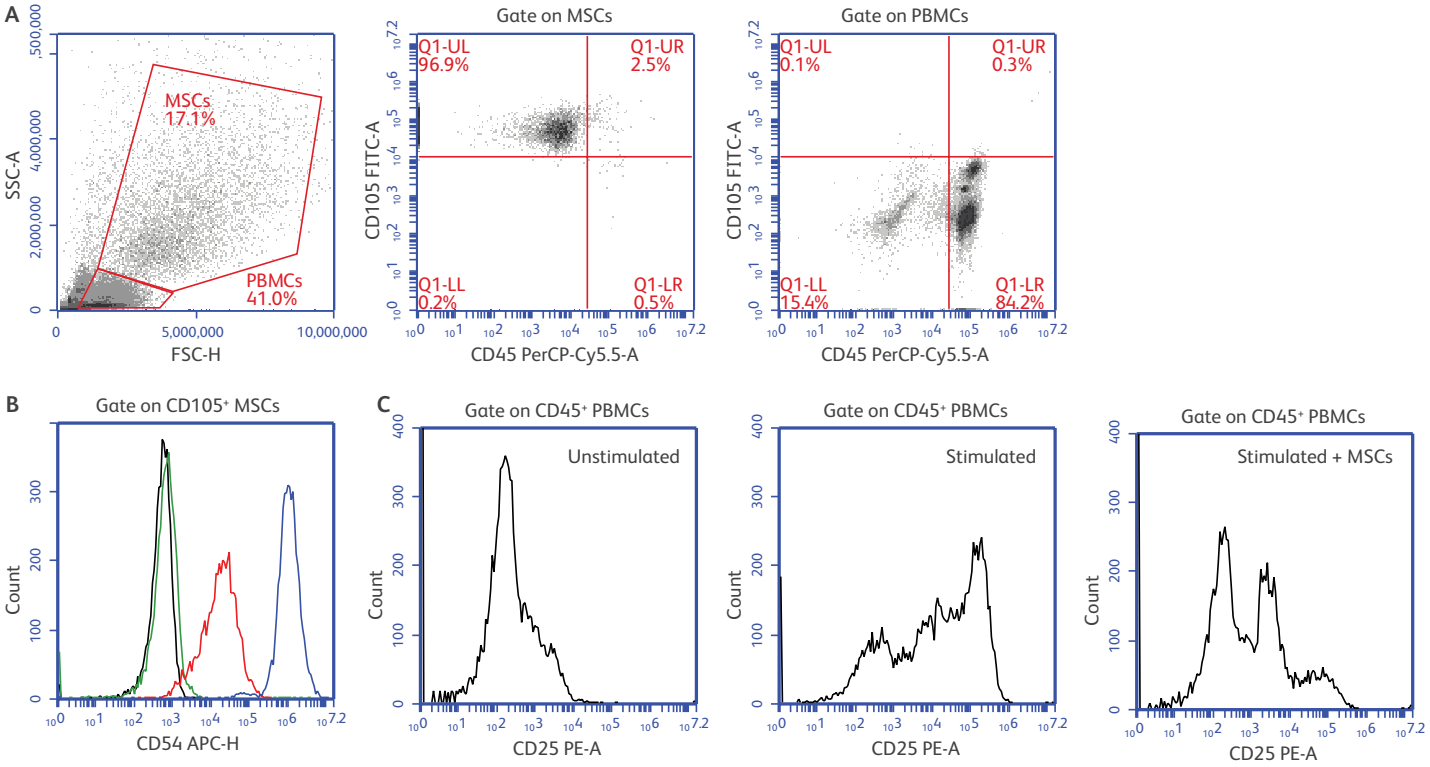

Figure 2. Surface activation marker expression in a PBMC/MSC co-culture

Human PBMCs were stained with BD Horizon CFSE and stimulated with Dynabeads Human T-Activator CD3/CD28 beads (Thermo Fisher Scientific) for three days in the presence or absence of human MSCs. Unstimulated PBMCs (no Dynabeads and no MSCs) were used as controls. On day 3, cells were harvested and stained with BD Pharmingen™ FITC Mouse Anti-Human CD105 (Cat. No. 561443), BD Pharmingen™ PE Mouse Anti-Human CD25 (Cat. No. 555432), BD Pharmingen™ PerCP-Cy5.5 Mouse Anti-Human CD45 (Cat. No. 564105), and BD Pharmingen™ APC Mouse Anti-Human CD54 (Cat. No. 559771). Cells were then analyzed on a BD Accuri C6 Plus. **Results: A.** MSCs and PBMCs can be separately identified based on scatter properties (left plot) and lineage marker expression (middle and right plots). MSCs were characterized and gated as $FSC^{high}SSC^{high}CD105^{+}$, while PBMCs were characterized and gated as $FSC^{low}SSC^{low}CD45^{+}$ **B.** $CD105^{+}$ MSCs cultured in the presence of PBMCs (blue, stained; green, negative control) showed increased expression of the activation marker CD54 compared to MSCs cultured alone (red, stained; black, negative control). **C.** Compared to unstimulated $CD45^{+}$ PBMCs (left histogram), stimulated $CD45^{+}$ PBMCs (middle) showed increased expression of the activation marker CD25. However, stimulated $CD45^{+}$ PBMCs cultured in the presence of MSCs (right) showed decreased expression of CD25 compared to stimulated $CD45^{+}$ PBMCs.

Ordering information	
Description	Cat. No.
BD Accuri™ C6 Plus Flow Cytometer System	660517
BD Accuri™ C6 Plus Workstation and Software	661391
BD CSampler™ Plus Automated Sampling System (optional)	660519

Class 1 Laser Product.
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