Element Biosciences

Data Spotlight

Profiling T Cell Activation with Teton™ Immuno and Focus Panels on AVITI24™

Introduction

T cell activation is a critical step in the immune response, driving the detection and elimination of pathogens and cancer cells, while also shaping how the body reacts to transplanted organs and autoimmune triggers. Activation of T cells generally includes antigen recognition, TCR binding, co-stimulation, and cytokine signaling. A variety of markers can be used to confirm that activation has occurred[1,2], and the Jurkat T cell line is a standard in vitro cellular model commonly used for studying T cell activation[3].

In this data spotlight, we use AVITI24 and Teton CytoProfiling to profile T cell activation produced by treatment with anti-CD3 and anti-CD28 in Jurkat cells over time. By applying 5D multiomics, we can capture dynamic cellular changes across layers of biology simultaneously, revealing a more comprehensive and nuanced view than would be possible with single-modality approaches.

Detecting T Cell Activation with Teton

To capture the dynamic response of T cells after activation, Jurkat cells were activated with anti-CD3 and anti-CD28 antibodies and profiled at three different time points post-activation: 30 minutes, 6 hours, and 24 hours. Anti-CD3 and anti-CD28 stimulation activates T cells by triggering the signaling pathway of the T cell receptor (TCR)[1]. Positive and negative controls were also included (Table 1).

For all treatment conditions, cells were co-stimulated with anti-CD3 and anti-CD28 in a plate-bound format. Cells in the 24-hour condition were transferred to media with FBS post-activation for incubation. The two other activation conditions (30 minute and 6 hour activation time points) did not use FBS because not all cells in these time points were expected to be fully activated. Ionomycin and PMA treatment was included as a non-specific positive control, since it bypasses the TCR and directly activates the signaling molecules required for T cell responses[1,4,5]. Negative controls with media and media with DMSO were also included.

Treatment	Conditions
30 minutes	10 µg/mL anti-CD3, 10 µg/mL anti-CD28
6 hours	
24 hours	
Positive Control	1 µM Ionomycin, 50 ng/mL PMA
Negative Control 1	Media only
Negative Control 2	Media, DMSO

Table 1. Description of treatment conditions and controls.

Prior to seeding, all cells were washed and resuspended in 1X PBS. Cells in all treatment conditions and the negative controls were seeded at a density of 60,000 cells per well, while the positive control (Ionomycin + PMA) was seeded at a density of 30,000 cells per well due to cell number limitations. Each treatment and control included two replicates (2 wells) on a 12-well Teton slide. All conditions and controls were prepared for cytoprofiling on the AVITI24 according to the protocol for suspension cells in the Teton CytoProfiling User Guide.

We used the Teton Immuno Panel and three Focus Panels to simultaneously capture RNA, morphology, and protein with spatial resolution. The Teton Human Immuno Panel Kit (#830-00039) detects 343 RNA, 6 cell paint, and 50 protein and phospho-protein targets, including immune cell markers, cytokines, and signaling molecules. The Teton Focus Panels provide an additional 72 total protein and phospho-protein targets covering pathways involved in T Cell Activation (#830-0047), Cytokine Signaling (#830-0046), and Innate Immunity (#830-0048).

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Cells were filtered using standard Teton criteria, which includes removing cells with assignment rates less than 50% and removing outliers in total counts and cell size. Each well was randomly downselected to a maximum of 3,000 cells for analysis, and images were analyzed with CellProfiler, which created a comprehensive cell-by-feature matrix. This matrix was imported into Seurat[6], and RNA and protein counts were normalized before UMAP generation.

Dynamic T Cell Activation Response

Treatment conditions are clearly differentiated in the UMAP (Figure 1), which shows distinct clustering of cells in groups that align with each condition or time point. As expected, the negative controls cluster away from the positive control and treatment conditions. Cells in the earlier time point conditions (30 minutes and 6 hours) show a progression towards the 24 hour treatment condition (Figure 1). This progression is also supported by the overlap of the activated treatment conditions with the positive control, though the combination of PMA and ionomycin in the control is known to affect a broader range of cytokines and is thus not expected to exactly match the activated treatment condtions[4].

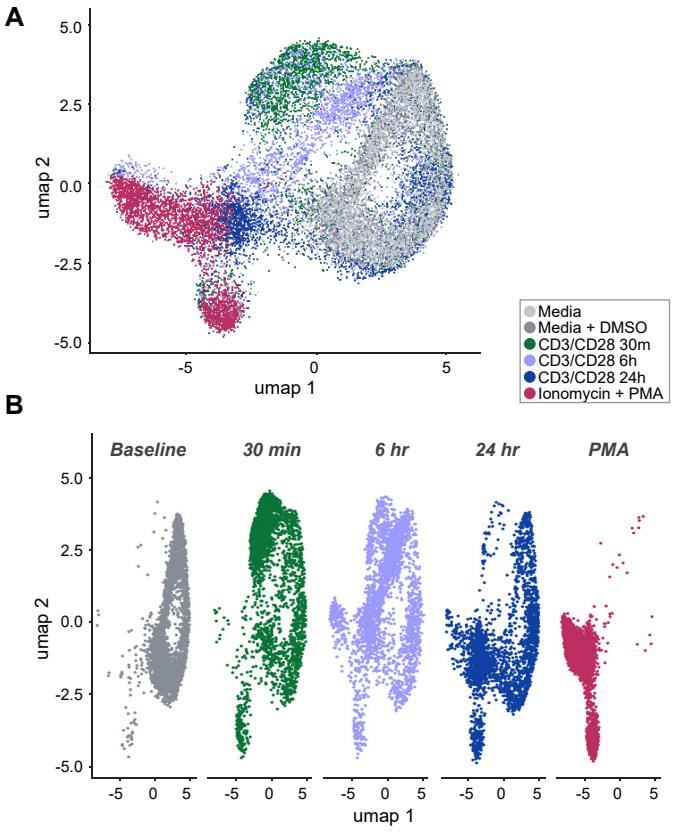

Figure 1. Uniform manifold approximation and projection (UMAP) plots of Jurkat cells colored by condition: (A) All conditions overlaid on single plot and (B) conditions plotted individually.

Analysis of individual targets (Figure 2) also provides evidence for T cell activation in the treatment conditions and positive control. The activated time points (30 minutes, 6 hours, and 24 hours) capture a progression from immediate signaling and early gene expression to surface activation and metabolic ramping, and eventually cytokine secretion and transcriptional reprogramming.

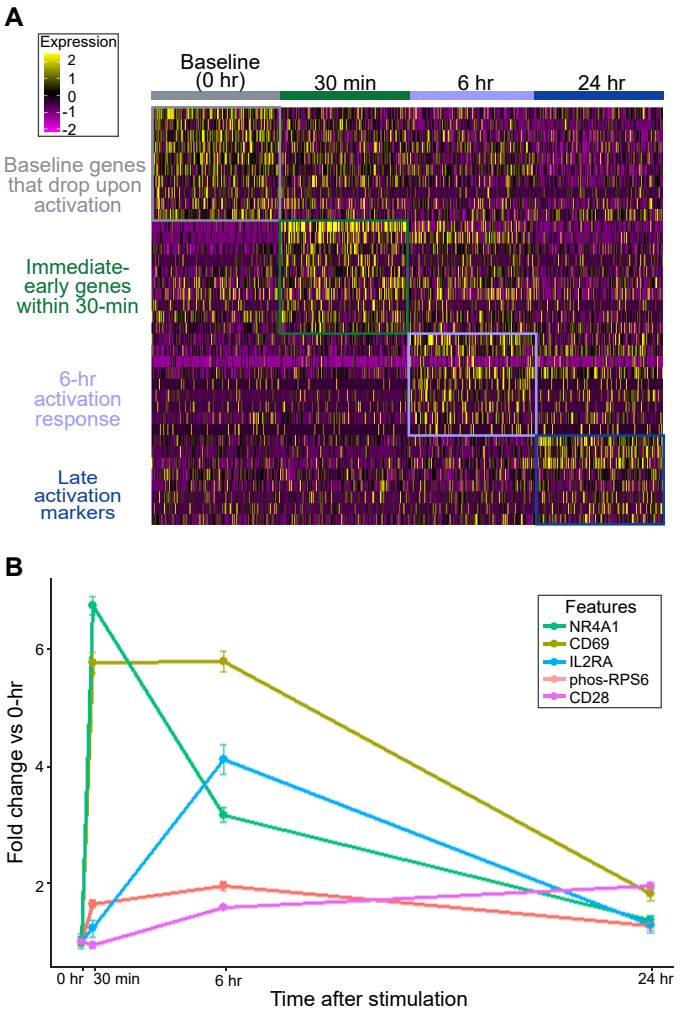

Figure 2. (A) Heatmap showing expression of RNA targets following stimulation at 0 hours (negative control), 30 minutes, 6 hours, and 24 hours. RNA targets are represented on the y-axis and single cells are represented on the x-axis. (B) Fold change in expression of select RNA or protein targets vs baseline (0 hr) after stimulation.

The transcription factor NR4A1, an immediate-early response gene[7], shows peak expression at the earliest activation time point (30 minutes), remains elevated at 6 hours, and returns to baseline by 24 hours (Figure 2). The surface marker CD69, another early response gene[8,9], is expressed in the earlier treatment time points and the positive control (not shown)

before declining at the 24 hour time point (Figure 2). This is consistent with findings that CD69 is detected early after activation, then declines rapidly after approximately 6 hours[10]. The negative controls (0 hrs) show lower expression of CD69 and NR4A1 than the activated time points, as expected.

There is also evidence of T cell activation and progression in later time points. The soluble form of IL-2R is also known to be a characteristic marker of T cell activation[11], and expression of IL2RA is clearly rising in the 6 hour time point, then falls by the 24 hour time point (Figure 2). It is not expressed in resting T cells[12] and in our dataset shows almost no expression at baseline. The costimulatory receptor CD28 shows increasing expression by the 6 hour time point and remains expressed at 24 hours, helping to sustain signaling over the longer activation period (Figure 2). At 24 hours, Jurkat cells show an upregulation of CD84, which plays a role in facilitating cell-cell interactions[14,15]. Sustained, high expression of TOX at 24 hours suggests the cells may be headed toward exhaustion[16]. Together, these markers show that the cells have moved past the initial signal into a state of full, persistent activation.

The inclusion of phospho-proteins in this assay provides an additional modality for profiling T cell activation. The protein phospho-RPS6 is elevated in the early time points of 30 minutes and 6 hours (Figure 2). Phospho-RPS6 is also known for its involvement in T cell infiltration, regulation, and activation[13]. Both the RNA and protein data are concordant and show a clear progression of targets important for T cell activation over time in the 30 minute, 6 hour, and 24 hour time points.

Conclusion

The Teton Immuno and Focus panels provide robust profiling of T cell activation over time, which represents a crucial part of the immune response, all on a single flow cell within a single cytoprofiling run. Cytoprofiling with Teton on AVITI24 provides 5D multiomics data by simultaneously capturing RNA, morphology, protein, spatial resolution, and dynamic response, all at single cell resolution and with minimal hands-on time.

Data and an analysis notebook from this experiment are available for download at elementbiosciences.com

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