Application Note

Immunotherapy

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Multiparametric Assessment of IL-2-Induced T Cell Activation

Combining imaging, luminescence, and ELISA-based assays to quantify T cell activation

Authors

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Abstract

This application note presents proof-of-concept data demonstrating the effectiveness of combining imaging, luminescence, and ELISA-based quantification methods of T cell activation. These complementary approaches provide distinct insights into T cell population dynamics and activation status, offering significant advantages over conventional methods such as the chromium release assay. Imaging enables direct visualization and enumeration of cells, whereas luminescence assays offer sensitive biochemical readouts of viability and cytokine secretion. ELISA provides high specificity and sensitivity for detecting key immune mediators like IFN-γ and TNF-α. Together, these methods provide a foundational approach to measuring T cell activation that complement functional readouts of immune cell killing.

Introduction

T cells play a central role in adaptive immunity, and their activation status is a critical indicator of immune function in both health and disease. As immunotherapies and cell-based treatments continue to evolve, there is a growing need for robust, scalable, and informative techniques to measure T cell activation and proliferation. Traditional assays, such as the chromium release assay, offer limited insight and often involve radioactive materials, making them less suitable for modern, high-throughput workflows.

Microplate imaging and detection-based assay formats provide convenient, well-established methods for measuring immune cell activation and proliferation. Automated imaging enables direct visualization and enumeration of cells, luminescence assays quantify metabolic activity and viability, and ELISA detects cytokine secretion with high specificity.

In this application note, we demonstrate the effectiveness of these techniques using well-established activation conditions and readouts for T cell proliferation and cytokine production. The results serve as a proof of concept for a broader, adaptable framework that can be applied across diverse immunological research and therapeutic development settings.

Interleukin-2 (IL-2) is a 15.5 kDa glycosylated protein that is a potent mitogen and growth regulator of T cells in vitro.[1] IL-2 initiates several responses in T cells upon binding with the trimeric IL-2 receptor (IL-2R) consisting of IL-2Rα, IL-2Rβ, and IL-2Rγ (Figure 1). Binding of IL-2 to its receptor initiates the Janus kinase-signal transducer and activator of transcription (JAK-STAT) signal transduction. Following their recruitment to the receptor, STAT5 proteins and SHC1 are phosphorylated by JAKs. Phosphorylation of STAT5 permits dimerization, nuclear translation, and transcription. Phosphorylation of SHC1 allows the recruitment of GRB2 and SOS to facilitate GTP loading of Ras and activation of the classical Raf-ERK mitogen-activated protein kinase (MAP kinase) cascade leading to cell proliferation.[2]

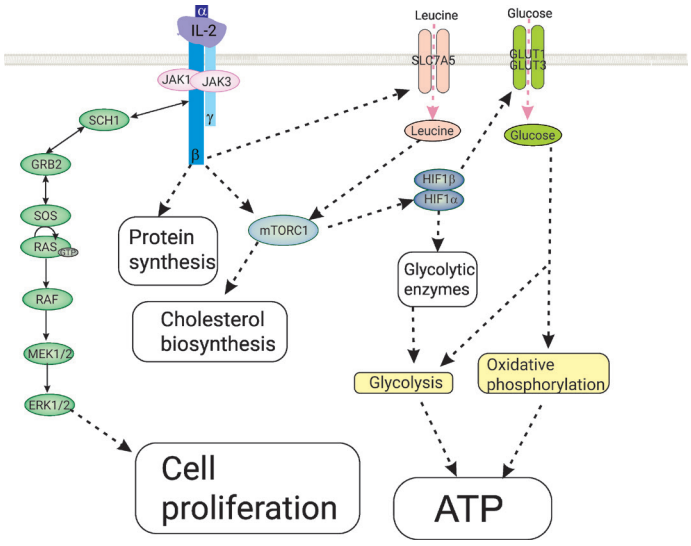

Figure 1. IL-2 binding-induced lymphocyte proliferation.

IL-2 binding also activates of the mammalian target of rapamycin complex 1 (mTORC1)[3] and the hypoxia inducible factor 1 transcriptional complex (HIF1α/HIF1β) that maintain the uptake of nutrients (for example glucose). IL-2 binding also sustains the expression of the amino acid transporter SLC7A5, which transports many of the essential amino acids, such as leucine, into cells. Amino acids are required to sustain mTORC1 activity and fuel translation. Glucose metabolism, via glycolysis and oxidative phosphorylation drives ATP generation. Protein synthesis is essential for maintaining the expression of proteins, such as MYC, with high turnover rates. Through the mTORC1 pathway, IL-2 can also sustain glycolytic metabolism and other biosynthesis in cytotoxic T lymphocytes to support cell proliferation, growth, and the expression of effector molecules.

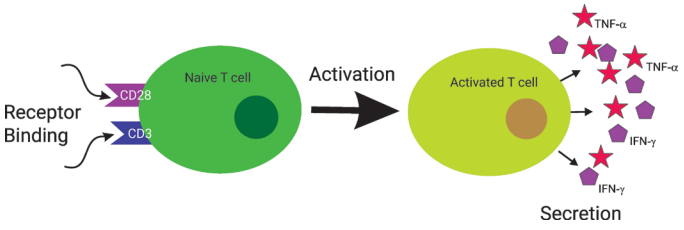

Figure 2. T cell activation-induced cytokine secretion.

T cell activation induces the upregulation of several cell surface markers that occur in a temporal progression. CD69 and the serum iron-transport protein CD71 are expressed quite rapidly after activation.[4] The CD25 antigen, which upregulates later in the activation process and is considered the hallmark of T cell activation, is present constitutively on a subset of peripheral blood lymphocytes with antigen density increasing in vitro, upon activation by stimulation of the TCR/CD3 complex.[2,5] In addition to the induction of cell surface molecules, activated lymphocytes also produce several cytokine proteins involved in regulating lymphocyte function (Figure 2). Two such cytokines are tumor necrosis factor alpha (TNF-a) and interferon-gamma (IFN-γ). TNF-α represents both cellular immunity and humoral immunity type cytokines.[6] IFN-γ, the prototypic cellular immunity cytokine, is one of the most tightly regulated of all cytokines consistent with its critical role in an inflammatory response.[7]

Experimental

For lymphocyte proliferation experiments, EpCAM-specific CAR T cells were thawed from liquid nitrogen frozen stocks and seeded into Agilent 96-well microplates, black-sided, clear bottom plates (part number 204626-100) at 20,000 cells per well in 100 µL of ImmunoCult XF cell expansion media. Various concentrations of IL-2 were then added as 2x solutions in 100 µL of ImmunoCult XF cell expansion media. After five days, the cells were imaged, and the number of lymphocytes determined using image analysis. After imaging, the cells were vigorously agitated to disperse any clumps and 100 µL was removed and placed into a solid white microplate (Greiner, part number 655075). Luminescence was then determined using CellTiter-Glo (Promega, part number G7570) according to the assay kit instructions. The luminescence was plotted as a function of IL-2 concentration.

For T cell activation cytokine secretion experiments, normal human lymphocytes were thawed from liquid nitrogen frozen stocks and seeded into two separate wells of a six-well microplate (Corning, part number 3506) in 8 mL of ImmunoCult XF cell expansion media supplemented with 10 ng/mL IL-2. One well received CD3/CD28 T cell activator (StemCell Technologies, part number 10971) while the other did not. Each day for five days, 1 mL was removed from each well, the cells counted, and the supernatant collected after centrifugation and stored frozen at –20 °C until assayed. On the day of the assay, supernatants were thawed and a portion was diluted 1:5 and 1:25 with culture media. Undiluted and diluted supernatant samples for each day were assayed in duplicate using ELISA kits for IFN-γ (R&D Systems, part number FIF50C) and TNF-α (part number DTA00D) according to the assay kit instructions.

Results and discussion

T cell proliferation

IL-2 can stimulate T lymphocyte proliferation. As seen in Figure 3, CAR T cells increase in number when incubated with 100 ng/mL of IL-2 for five days. However, the observed increase is not statistically different than wells with untreated CAR T cells. When a portion of the cells are removed and assayed for cell viability using luminescence, the cells receiving IL-2 were found to be significantly more viable than the cells in media only.

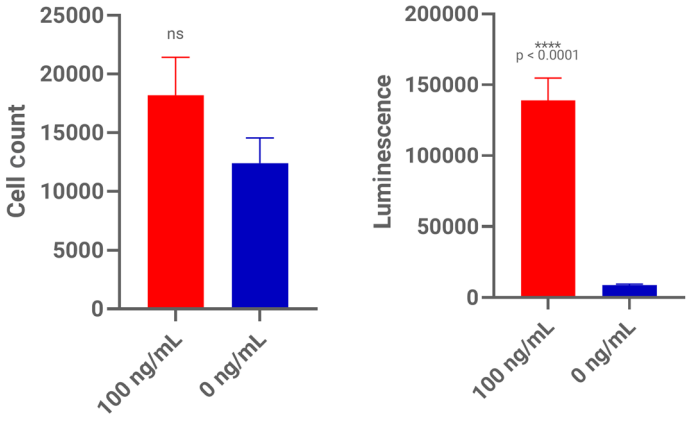

Figure 3. Cell count and luminescence of CAR T cells incubated with IL-2. Freshly thawed CAR T cells were incubated with media containing either 100 or 0 ng/mL of IL-2 for five days. Wells were then imaged and counted using image analysis. After imaging, 100 µL of the cell suspension was transferred to a fresh plate and the luminescence was determined. Data represents the means and standard deviation of 20 replicates.

The effect of IL-2 concentration on T cell proliferation can be monitored using luminescence. Figure 4 demonstrates that CAR T lymphocytes respond to IL-2 in a concentration independent manner. Cells not exposed to any IL-2 demonstrate significantly less luminescence than cells exposed to the lowest concentration tested.

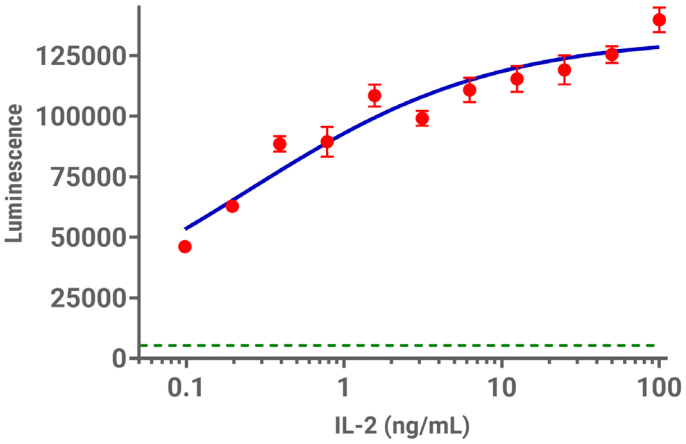

Figure 4. Effect of IL-2 on CAR T cell growth. Freshly thawed CAR T cells were incubated with various concentrations of IL-2 for five days, after which a portion was transferred to a fresh plate and the luminescence determined. The line indicates the luminescence signal of CAR T cells treated with media only. Data represents the mean and standard deviation of eight replicates.

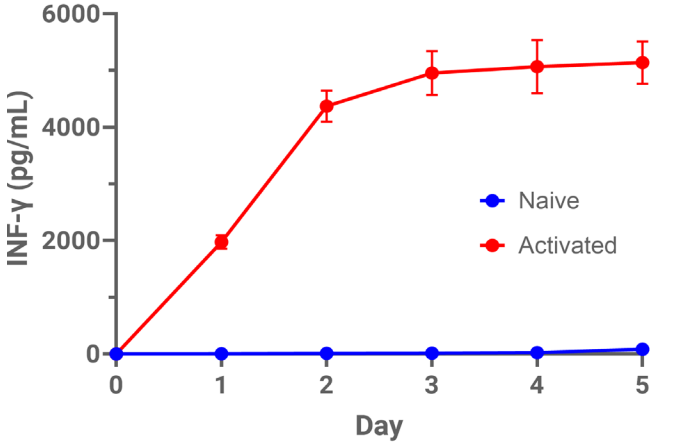

Figure 5. INF-γ secretion in activated and naïve T cells. Naïve and activated T cell culture supernatant was collected daily for five days and assayed for IFN-γ using an ELISA assay kit specific for the cytokine. After analysis, samples were corrected for dilution and plotted as a function of time. Data represents the mean and standard deviation of all replicates within the range of the calibration curve.

T cell activation

T cells that have been activated by exposure to CD3/CD28 rapidly secrete substantial amounts of TNF-α and INF-γ. As demonstrated in Figure 5, conditioned media from activated T cells has over 2,000 pg/mL of INF-γ within 24 hours of activation. These levels continue to increase, leveling off after three days at nearly 5,000 pg/mL. In the same cultures, TNF-α follows a parallel path, rapidly increasing in concentration to over 8,000 pg/mL in conditioned media (Figure 6). Conditioned media from T cells without CD3/CD28 activation has virtually no measurable amounts of these cytokines after five days (Figures 5 and 6). These differences are not the result of cell number differences. Both stimulated and unstimulated cells were cultured in media supplemented with 10 ng/mL IL-2. Manual cell counts from the daily samples indicated that both stimulated and unstimulated T cells proliferated at approximately the same rate (Figure 7).

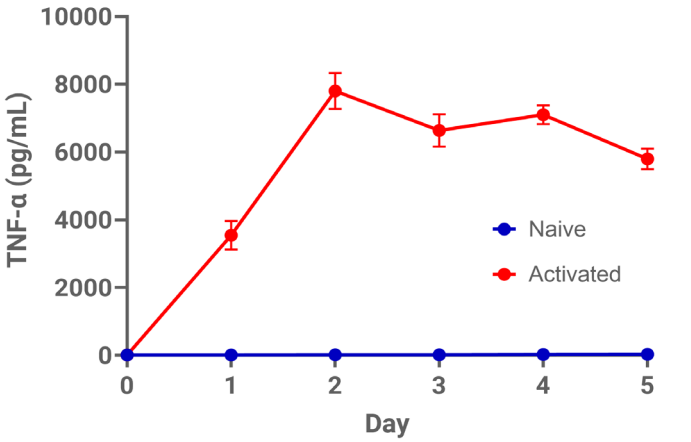

Figure 6. TNF-α secretion in activated and naïve T cells. Naïve and activated T cell culture supernatant was collected daily for five days and assayed for TNF-α using an ELISA assay kit specific for the cytokine. After analysis, samples were corrected for dilution and plotted as a function of time. Data represents the mean and standard deviation of all replicates within the range of the calibration curve.

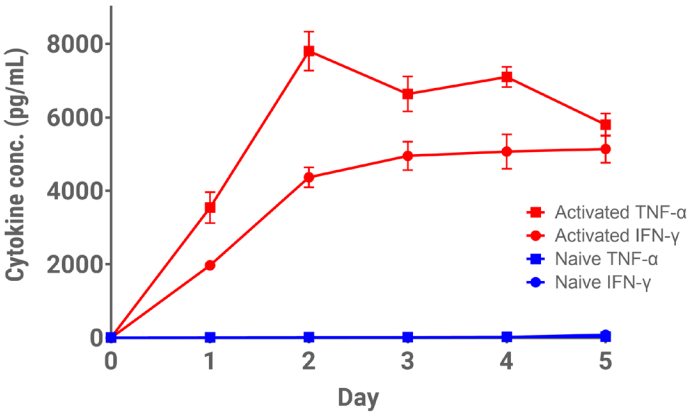

Figure 7. Cell concentration in activated and naïve T cells. Naïve and activated T cell cells were collected daily for five days and counted. Data represents the mean of four determinations for each day.

While the data presented here serves as proof-of-concept, the methodologies themselves are broadly applicable across diverse experimental systems and research objectives. These approaches are not limited to a specific cytokine, cell type, or stimulation protocol; rather, they can be adapted to a wide range of immunological assays, including studies of T cell differentiation, exhaustion, cytotoxicity, and response to therapeutic agents.

Each technique contributes unique and complementary insights: imaging enables direct visualization and enumeration of cell populations, luminescence assays provide sensitive and rapid assessments of metabolic activity and viability, and ELISA offers high specificity for quantifying secreted cytokines. Together, these methods support a comprehensive understanding of T cell behavior in both basic and translational research settings. Their compatibility with high-throughput formats and nonradioactive workflows makes them especially valuable for applications in drug screening, immunotherapy development, and immune monitoring.

Conclusion

Combining imaging, luminescence, and ELISA-based assays provides researchers with a powerful, adaptable toolkit for quantifying T cell activation. These orthogonal approaches for characterizing immune cell activation complement functional readouts of immune cell killing and support innovation across immunology, therapeutic development, and translational research. Agilent BioTek multimode readers and imaging platforms are well-suited to support these advanced workflows, facilitating robust and reproducible immune cell analysis.

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Products used in this application

Agilent products

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