Mixed Lymphocyte Reaction (MLR) in Immunotherapy

MLR measures the immune response of T cells when mixed with antigen presenting cells from two different blood donors. This is used to determine whether therapeutic antibody candidates can enhance immune response.

Methods

Peripheral blood mononuclear cells (PBMC’s) were isolated from two different human blood donors. PBMC’s from Donor 1 were stimulated with GMCSF and IL-4 to induce DC differentiation. On day seven, two populations of CD4+ cells (total and resting) are isolated from Donor 2 blood. DC’s from Donor 1 were then combined with T cells from Donor 2 along with antibodies capable of triggering the anti-cancer immune response. Markers of T cell activation such as cytokine production, viability, and proliferation are measured over eight days using the iQue3 flow cytometer.

Results

Cell Survival

Cell populations are distinguished based on light scatter of different colored lasers within the flow cytometer.

Sartorius iQue3 High Throughput Flow Cytometry

- Rapid analysis of cells surface antigens, cytokine secretion, activation and survival
- Requires smaller sample volumes
- Customizable and adaptable data analysis

Results

Cell Proliferation

Next Steps

- Perform assay with triplicate conditions rather than duplicate to decrease error
- Include intermediate timepoints between days 3 and 7 for more specific transient data
- Test therapeutic antibody candidates

Analysis

- Cell survival is maintained through test populations and slightly decreased in preactivated T cells due to over stimulation.
- INFg secretion in test samples increases as expected over 8 days.
- TNFa secretion remains low in all test populations except the positive control.
- Maximum CD69 expression is observed earlier in the co-culture, as expected of an early activation marker
- CD25 reaches maximum expression on day 6.
- Resting CD4+ T cell populations showed a more robust activation response overall than total CD4+ T cell populations.

Conclusions

- High throughput flow cytometry suggests a more effective method of conducting MLR readout.
  - More time efficient
  - Wider range of data
  - Simultaneous measurements of different parameters of T cell activation
  - Small sample volume required
- These improvements are extremely useful in the field of immunotherapeutic discovery as drug efficacy can be tested more efficiently and new treatments can reach patients in clinical trials faster.

References


Figure 1. Mixed lymphocyte reaction. Explicyte.