Quantifying immune cell subsets in living cultures over time using IncuCyte® live-cell analysis

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Summary & Impact

- Heterogeneity exists in all populations, ranging from the cell type present to differences at the genetic level or stage of cell cycle. This heterogeneity plays an important role in how populations react in response to therapies and/or biological stimuli.
- To date, cellular analysis has been solely based on population-averaged measures whereas object (cell) data is considered essential to capture the cellular heterogeneity.
- However, effects on subpopulations can sometimes be masked by larger numbers of non-responsive/cell similar sized populations may produce opposite responses that result in net zero.

Monitoring PBMC activation: morphology & protein expression

- PBMCs (in 6-well) were activated with 10 ng/mL IL-2 and IFN-γ and incubated in medium with 10% FBS and 100 U/mL penicillin.
- Activated PBMCs exhibited clear heterogeneity in morphology with size and shape changing over 120 h.
- CD4+ T cells increased as a percentage of the total population over time when compared to other markers such as CD8+.
- Large cells were shown to preferentially express the CD71 marker as opposed to those cells that were smaller in area.

Linking PBMC subset proliferation to immune cell killing

- PBMCs (in 24-well) were labelled with either isotype or CD19 Abs to identify CD19+ cells.
- CD19+ cells were then isolated and incubated with IL-2 and irradiated with 2500 rad.
- The CD19+ subset was significantly enhanced to >60% of the total population in response to CD19 stimulation.
- The CD19+ subset was responsive to CD19 Ab treatment where potency was reduced to 60% when compared to the CD95 treatment.
- Using the same stimulation conditions in an immune cell killing assay shows that target cell death temporally aligns with CD19+ cell enrichment.
- Enrichment of the CD19+ population reveres to attack and kill target cells.

Monitoring heterogeneity in the tumour microenvironment

- A co-culture of A498 (clear red cancer cells) and PBMCs were plated and incubated with CD8-FasFluor-488 conjugate and IL-2.
- PBMCs were segmented and analysed, A498 cells were excluded from analysis.
- The total PBMC and CD8+ T cell subpopulation proliferation was able to be monitored.
- Numbers of CD8+ cells expressed as a percentage of the total PBMC population increased from 35% to 60% which exemplifies a significant enrichment of CD8+ cells.
- Classification mask gives information on CD8+ (green) and CD8− (blue) proximity to target cells.

Specific, non-perturbing fluorescent labelling

- Jurkat and Ramos cell mixtures (1:100%) were plated and monitored over 48 h.
- CD10 Ab was fluorescently labelled with IncuCyte FasFluor-488 reagent and used to identify the Ramos cells.
- Initial CD10+ subset percentages were comparable to the percentage of Ramos cells initially plated.
- CD10+ is present on both cell types and was represented by 100% of the total population.
- The CD10− subpopulation could be tracked over time.