USE OF HIGH-PARAMETER HUMAN NK AND T CELL SPECTRAL FLOW CYTOMETRY FOR AN IN-DEPTH INVESTIGATION OF IMMUNE CELL VARIABILITY ACROSS HEALTHY DONORS

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Talk Summary

Inter-donor immune system variability is well-documented and poses challenges for the selection of normal controls or cell donors in biomarker or cell therapy studies. Spectral flow cytometry has facilitated efficient and extensive characterization of the immune system by enabling analysis of over 40 markers in a single tube. However, challenges in the development of such assays have so far limited their use to a broad and shallow analysis of several, distinct lineages. Furthermore, the lack of biological standards has hampered the assessment of the inevitable impact of spillover spread on biological resolution in very large panels. In this study, we developed a 38-color spectral flow cytometry panel for an in-depth analysis restricted to NK and T cells, including markers associated with maturation, senescence, activation, chemokine and inhibitory signaling. Smaller flow cytometry panels with very minimal spillover and spread impact were used as reference to monitor the maintenance of biological resolution, measured as frequency of populations of interest. The optimized panel was then used to characterize the NK and T cell compartments of N=10 healthy donors of different gender, age and ethnicity. Through conventional and unsupervised analysis, we show how inter-donor differences become more pronounced as the depth of cell analysis increases. Longitudinal analysis of N=4 donors showed remarkable immunophenotype stability. Ultimately, the panel was used for a comprehensive assessment of the impact of commonly used protocols for sample storage and handling of marker detection. This type of analysis can help to explain the plasticity of the immune responses in response to challenge.

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