IMMUNE CELL PROFILING USING FLOW CYTOMETRY

Immune cells can be broadly classified into innate and adaptive groups – the cells involved in innate immune response include macrophages, dendritic cells, natural killer cells etc. while the adaptive response cells include different types of T-cells and B-cells. The mix of immune cells changes in response to either pathogenic stimuli, vaccines or therapeutic modalities[1]. Immune cells can be profiled from blood samples by measuring changes in mRNA or protein levels of specific markers. The first step is isolating immune cells from peripheral blood, spleen and lymph nodes using several published protocols. Assays such as microarrays, RNA-Seq and NanoString's nCounter platform measure mRNA levels and high content fluorescence staining and flow cytometry measure protein markers[2].

Microarrays were one of the earliest methods to profile gene expression in various cell populations but scalability and limited data are major limitations. In contrast, RNA-Seq provides a global view of mRNA levels but requires very complex data analysis that can be expensive and typically requires high quality RNA preferably from fresh samples. The nCounter platform is becoming widely used in both preclinical and clinical trials, and will likely become the gold standard for cell profiling using RNA expression[2]. However, it is important to note that RNA levels do not always translate to protein expression and functional activity, so some researchers prefer to use protein-based methods to profile cell populations. Flow cytometry is a well-established method to measure cell surface and intracellular protein expression in a single cell suspension. While intracellular flow cytometry protocols are well established, they require fixation and permeabilization so cannot be used on live cells[1]. Additionally, the analysis of cell surface markers is comparatively simpler and immune cells express specific CD markers on the cell surface that are used to profile immune cell populations. One of the key advantages of flow cytometry is multiplexing using different fluorescent dyes that have different excitation and emission spectra[3]. A recent publication reported the development of a 17-plex flow cytometry panel to comprehensively profile multiple immune cell populations including T-cells, NK cells, monocytes, dendritic cells and macrophages[3]. Interestingly, the panel also includes markers to differentiate between specific immune cell subtypes – for example, macrophages can be pro-inflammatory (M1) or anti-inflammatory (M2) and each subtype can be characterized using specific CD markers. In the panel, CD80 is used to identify M1 macrophages while CD206 is used to identify M2 macrophages[3]. Specific markers to differentiate between cell states is also included in the panel – for example, NK cells can be in multiple states and the sub-populations can be identified using specific markers. Exhausted NK cells can be identified using TIGIT expression while activated NK cells can be identified using CD69 expression[3] and both markers are included in the panel.

The development of large multiplexed panels allows broad immune cell profiling in a single study that allows comparison of different cell populations very quickly. The panels have multiple applications across disease research and drug development. The mix of immune cell populations are altered in various diseases including cancer, autoimmune diseases, infectious diseases etc. and scientists are discovering alterations in immune cells during aging and other physiological states that could lead to disease development[4]. Another interesting area is evaluating changes in immune cell populations in response to therapies. A 2016 paper was one of the earliest reports on mapping the impact of drugs on the immune system[5] and since then, there has been significant development of immune-modulating therapies. The most well-known immune-modulating therapies in oncology are likely checkpoint inhibitors such as pembrolizumab that blocks PD-1 activity and increases T-cell killing of tumor cells. Another class of therapies are immunosuppressive therapies that reduce immune cell activity such as Rituximab and cyclosporin A. Given the increasing importance of immune cells in disease research and drug development, it is likely that flow cytometry panels will continue to be widely used to profile changes in immune cell populations.

References:

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