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Discovery and validation of immunological biomarkers using an advanced flow cytometry platform

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Introduction

Immune mediated inflammatory diseases (IMIDs), such as rheumatoid arthritis, psoriasis and Crohn's disease are a group of chronic conditions sharing common inflammatory pathways. Inflammatory cytokine imbalance is central in IMID pathophysiology suggesting a key role for CD4+ T cells in shaping the immune response via cytokine production. Given the phenotypic and functional complexity of the T-cell compartment, multiparameter flow cytometry represents one of the best experimental tools to study T cell biology.

Aim

The aim of our immuno-monitoring project is to develop a flow cytometry-platform to define and validate immune cell signatures in inflammatory diseases. In particular we want to simultaneously analyze the frequency and functional state (cytokine production, STAT protein phosphorylation) of T helper cell subsets relevant for IMIDs.

Patients and methods

Multicolour flow cytometry panels were developed for the simultaneous detection of up to 10 parameters both at surface and intracellular levels. PBMC are stimulated with PMA/Ionomycin and then stained in 96 well plates containing lyophilized reagents (lyoplates) to ensure consistency and reproducibility between different primary samples.

Cellular activation and signalling was assessed by phosphoflow cytometry after *ex vivo* stimulation with relevant proinflammatory cytokines.

Results

Three polychromatic (10 colours) flow cytometry panels using the lyoplate platform have been developed to analyse the number, cytokine production (IFNγ, IL17A, IL22 and IL10) and transcription factor expression (T-Bet, RORC and FoxP3) of Th1, Th17 and Treg cells within PBMC. A fourth panel has been designed to study T cell activation by detecting constitutive and cytokine induced phosphorylation of Stat1 and Stat3.

Conclusion

The multicolour flow cytometry platform we have optimized is an excellent tool to characterize CD4+ T cell subsets in both healthy controls and patients with immune mediated inflammatory diseases.

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