The impact of ruxolitinib and tocilizumab on IL-6 evoked phospho-STAT3 induction across human immune cell subsets

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ABSTRACT

Designing suitable biomarker assays to evaluate target engagement of therapeutic agents supports hypothesis testing in clinical trials. As an example, we have used phospho flow cytometry to investigate the phosphorylation of STAT3 in response to the inflammatory cytokine IL-6 across various human immune cell subsets including CD4 and CD8 T cells, NK and NK-T cells, Tregs, B cells and monocytes. IL-6 differentially induced phospho-STAT3 across immune subsets.

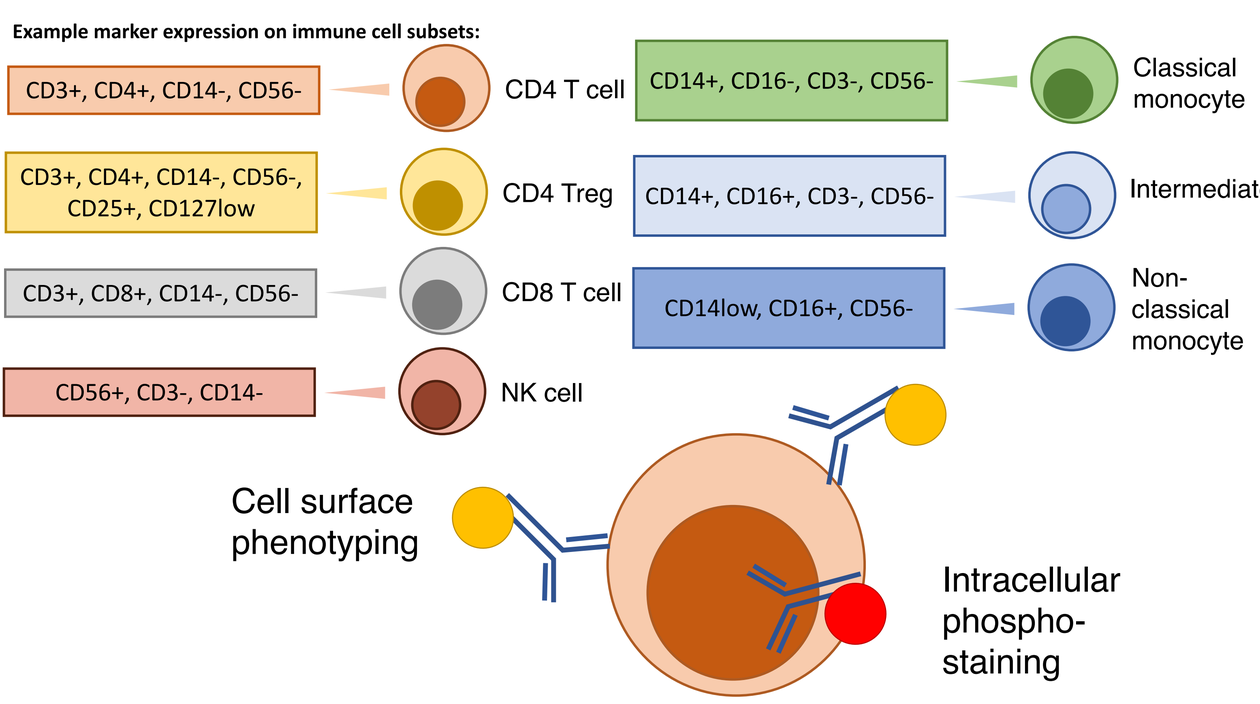
We have used this assay to compare two therapeutic agents targeting the IL-6 pathway, tocilizumab and ruxolitinib. Tocilizumab blocks IL-6 signaling at the IL-6 receptor whereas ruxolitinib inhibits IL-6-evoked STAT3 phosphorylation through inhibition of JAK1/2. We compare and contrast the sensitivity of human immune cell subsets to these two inhibitors by measuring the induction of STAT3 phosphorylation by a 'low' and 'high' concentration of IL-6.

On-going studies are utilising this approach to investigate the nature of IL-6 signaling in health and disease.

INTRODUCTION

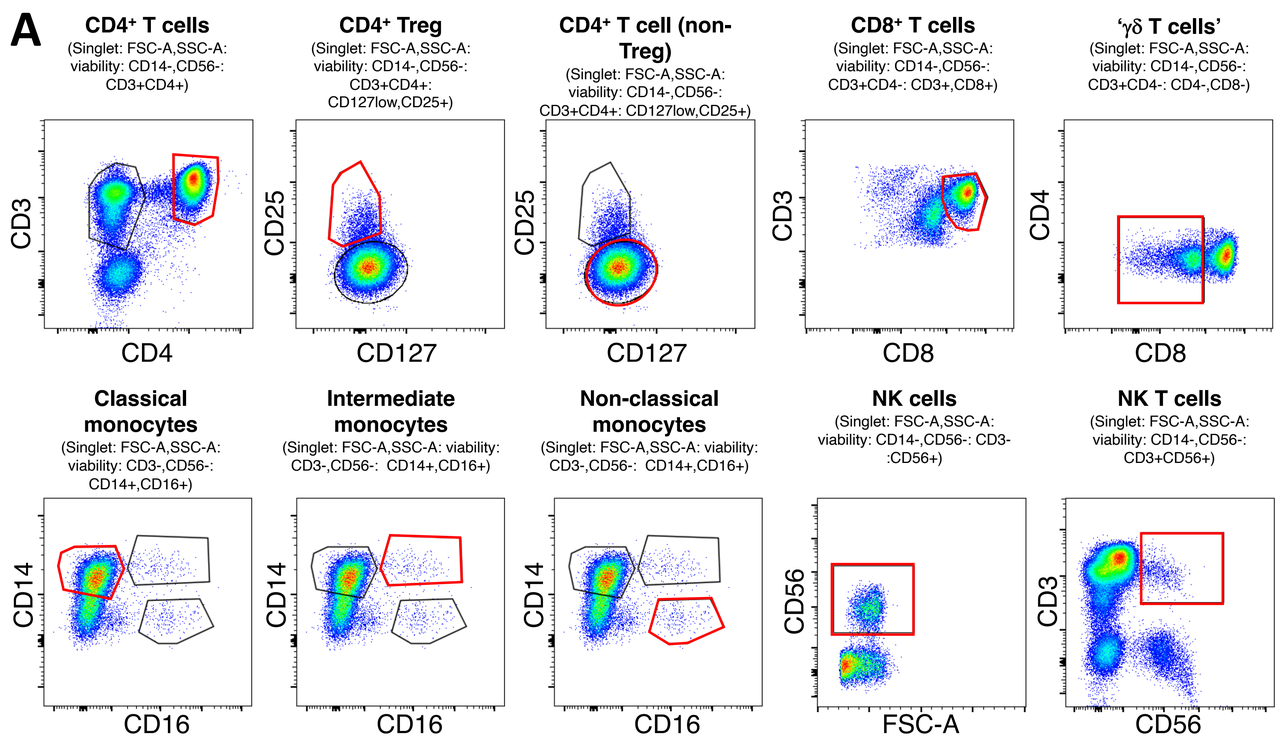
Activation of intracellular signaling pathways by several cytokines, including those elevated in autoimmune and inflammatory disease such as IL-6, IL-11 and IL-22, result in the phosphorylation of STAT3. The JAK family of non-receptor tyrosine kinases includes JAK1, JAK2 and Tyk2, which transduce signals following cytokine binding leading to phosphorylation of STAT3. Blockade of pro-inflammatory cytokines by agents such as the monoclonal anti-IL-6 receptor antibody, tocilizumab, or of pro-inflammatory JAK signalling by JAK inhibitors such as ruxolitinib and baricitinib is therefore a major therapeutic approach to treat autoimmune and inflammatory disease.

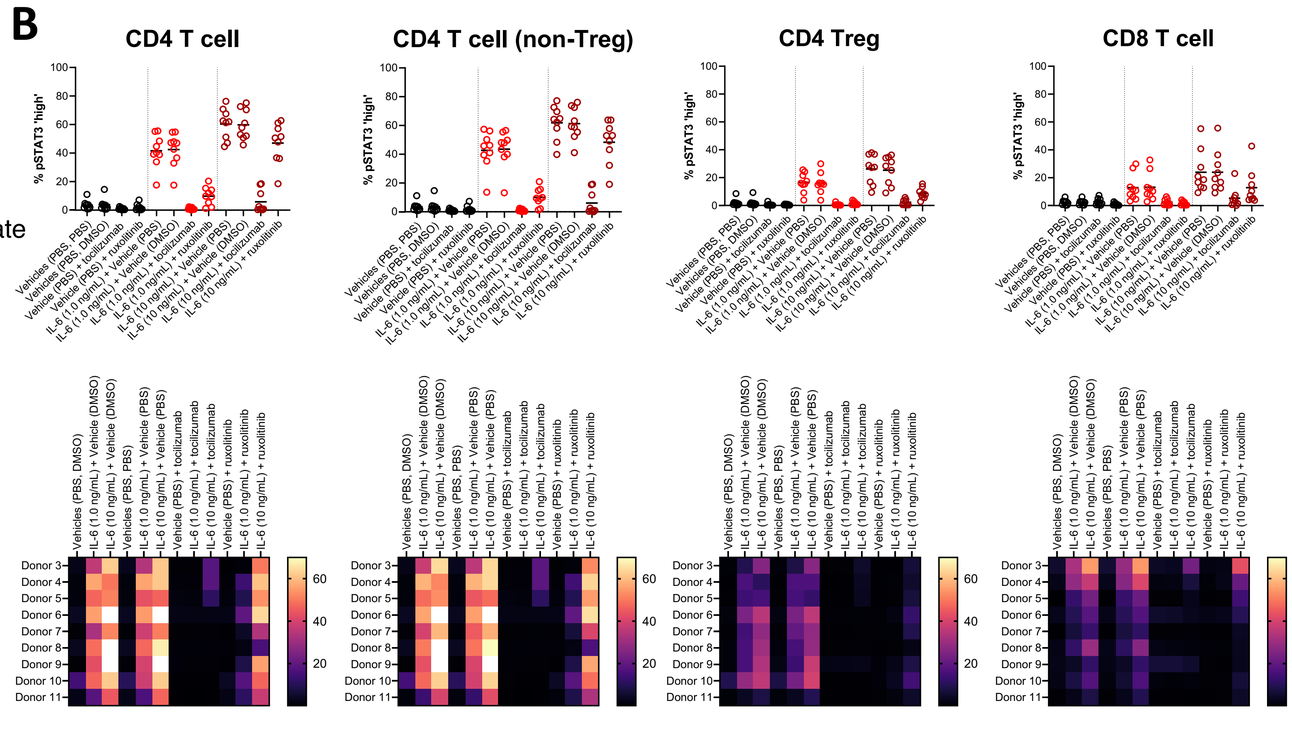
Multiparametric flow cytometry provides a method to quantify 'target proximal' signals such as the phosphorylation of STAT3 across multiple human primary immune cell subsets at several stages of the drug discovery process for use ranging from early screening to target engagement biomarkers. Here we describe the application of a flow cytometric method to explore the sensitivity of immune cell subsets to IL-6-mediated STAT3 phosphorylation to the therapeutic agents ruxolitinib, baricitinib and tocilizumab.



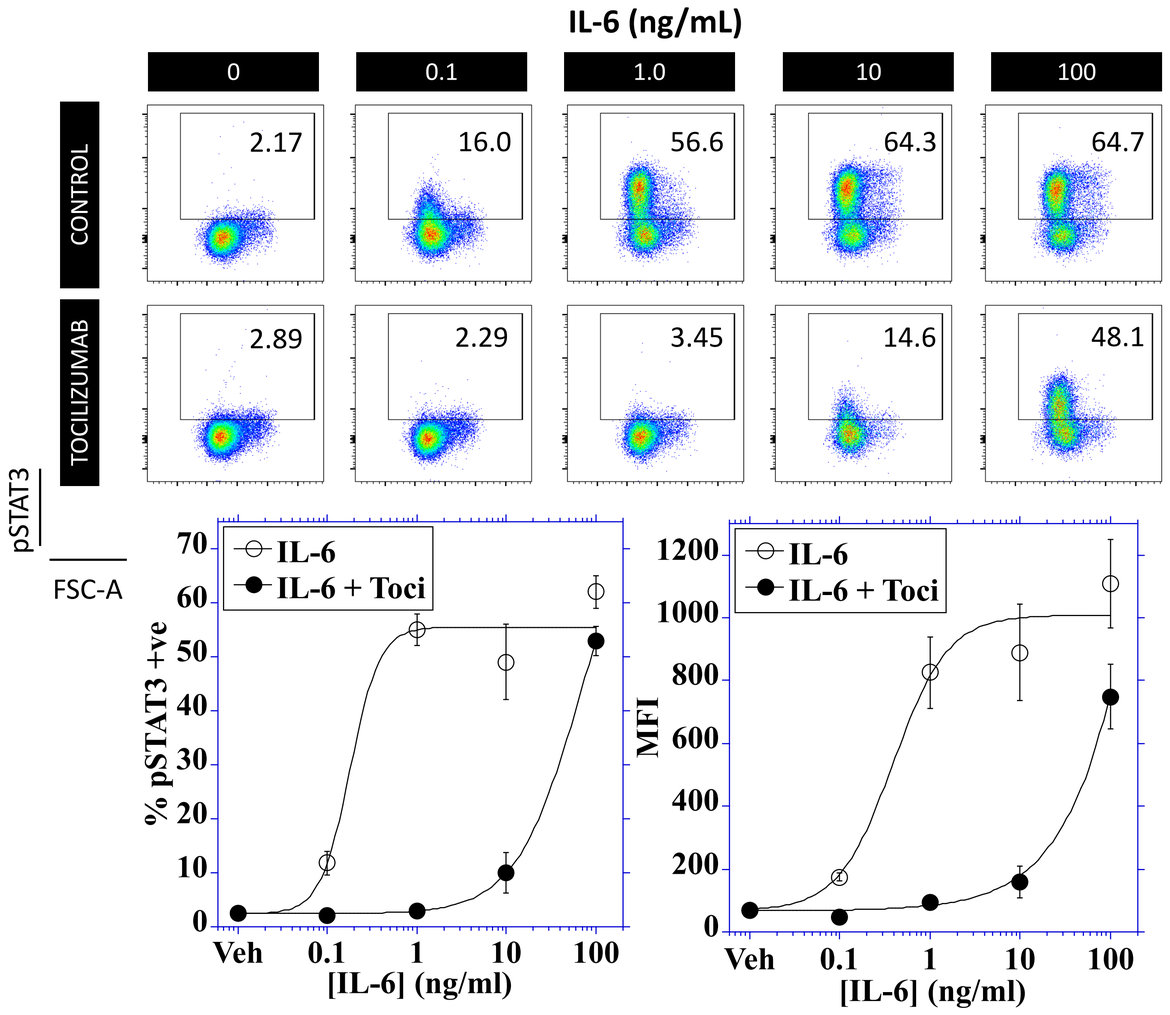
ANALYSIS OF IL-6-EVOKED STAT3 PHOSPHORYLATION IN IMMUNE CELL SUBSETS

A Representative pseudo-colour scatter plots showing positive gating identification of cellular populations (following exclusion of other populations by negative gating, and following gating for singlets, scatter and viability).
B Analysis of pSTAT3 responses to 'low concentration' and 'high concentration' IL-6 in selected T cell populations. Quantification of responses from nine healthy donors revealed a greater induction of phospho-STAT3 by IL-6 in conventional CD4 T cells compared to regulatory CD4 T cells. At 'high concentration' IL-6 (10 ng/mL), inhibition of pSTAT3 by ruxolitinib was reduced compared to tocilizumab.



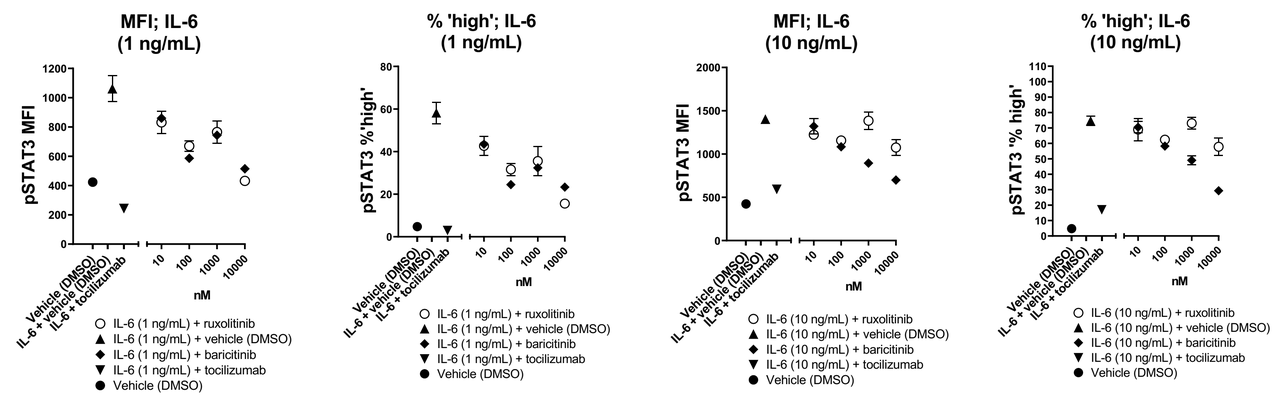


IL-6-EVOKED STAT3 PHOSPHORYLATION AND BLOCKADE BY TOCILIZUMAB IN PBMC



COMPARISON OF RUXOLITINIB AND BARICITINIB

The incomplete blockade of IL-6-evoked pSTAT3 by ruxolitinib may result from activation of Tyk2 at which this inhibitor has limited activity. We therefore tested the concentration-dependent impact of ruxolitinib, and another inhibitor, baricitinib (which has activity at Tyk2) on IL-6 evoked STAT3 phosphorylation. At the lower concentration of IL-6 (1.0 ng/mL), the two inhibitors displayed comparable activity, whereas at the higher concentration of IL-6 (10 ng/mL), baricitinib displayed greater activity. These results would be consistent with activation of Tyk2 at higher concentrations of IL-6. While this concentration of IL-6 is unlikely to be physiological, synergy between cytokines is common, and therefore activity at additional JAK family members such as Tyk2 may contribute to therapeutic efficacy.



FUTURE WORK

Studies investigating phospho-STAT3 induction by other cytokines, and inhibition by other therapeutic agents are on-going. In addition, this flow cytometric approach is being used in a major interventional study exploring the role of inflammation in psychosis.

ACKNOWLEDGEMENTS

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