Use of flow cytometry for investigating immunomodulatory effects of medicines

Richard Stebbings, David Eastwood, Chris Bird, Meenu Wadhwa, Lucy Findlay, Stephen Poole, Robin Thorpe

In 2006 a "cytokine storm", not predicted by pre-clinical safety testing, occurred during the phase-I clinical trial of the immunotherapeutic mAb TGN1412. We subsequently developed in-vitro procedures that would have predicted cytokine release syndrome in man, but did not compare other therapeutic antibodies that can cause clinical infusion reactions. In contrast to other immunotherapeutics tested we found that IL-2 was a key biomarker of TGN1412-mediated cytokine release. Using flow cytometry, we identified that TGN1412-mediated IL-2 and IFN-γ release came from mainly CD4+ effector memory T-cells and TNF-α release from both central and effector memory CD4+ T-cells, a mechanism of cytokine release different from that of other immunotherapeutics tested. In contrast, other immunotherapeutics tested stimulated IFN-γ and TNF-α release not from CD4+ T-cells, but from CD8+ T cells and NK cell subsets. No IL-2 release was detected with other immunotherapeutics tested. Differences in the pattern of CD8+ T cell and NK cell subsets activated by different immunotherapeutics were also evident. These differences in the mechanism of IFN-γ and TNF-α release are not seen by ELISA of culture supernatants from immunotherapeutic stimulated un-fractionated cells. Polychromatic flow cytometry offers investigators a powerful tool for revealing differences in the unwanted immunomodulatory effects of medicines.