

Effects of a Janus kinase (JAK)1/3-inhibition with tofacitinib on T cells, activated under differing inflammatory conditions

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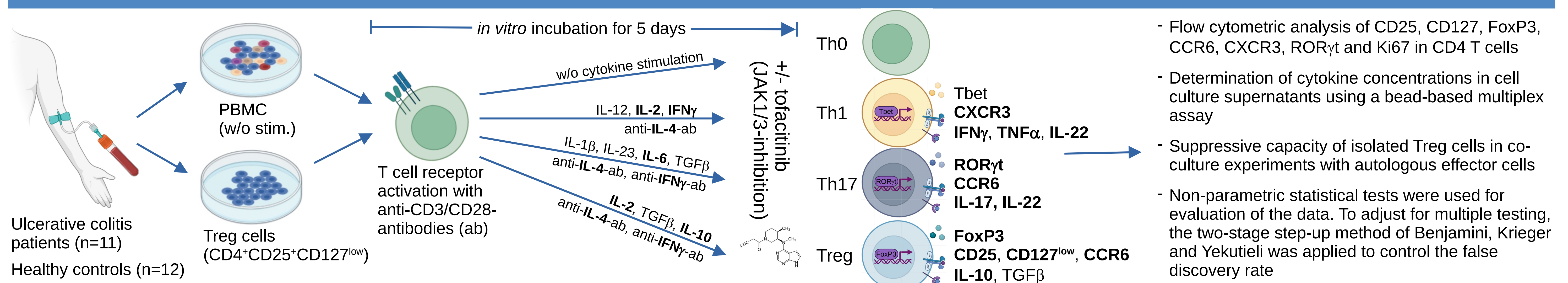
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Background

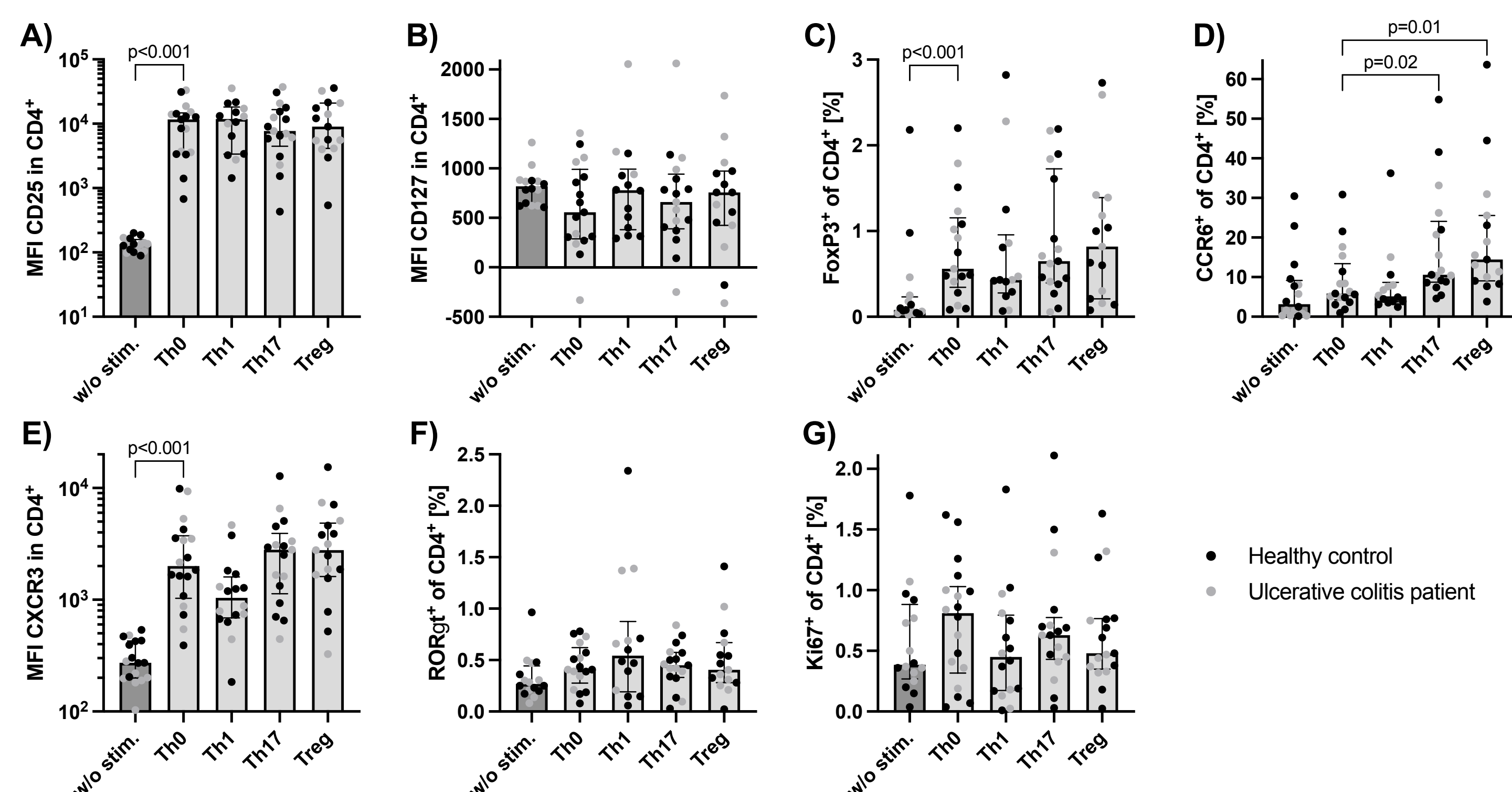
The JAK1/3-inhibitor tofacitinib is licensed for a variety of inflammatory diseases, including rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, juvenile idiopathic arthritis and ulcerative colitis. The anti-inflammatory effects of tofacitinib are mediated by inhibition of a broad spectrum of cytokine receptors. Secondary effects of the cytokine receptor inhibition on T helper (Th) cell activation and proliferation, however, are less well investigated.

An explorative analysis was set up in ulcerative colitis patients and healthy controls to investigate the effects of JAK1/3-inhibition on the activation of T cells in a Th1-, Th17-, or regulatory T cell (Treg)-promoting cytokine milieu *in vitro*.

Methods

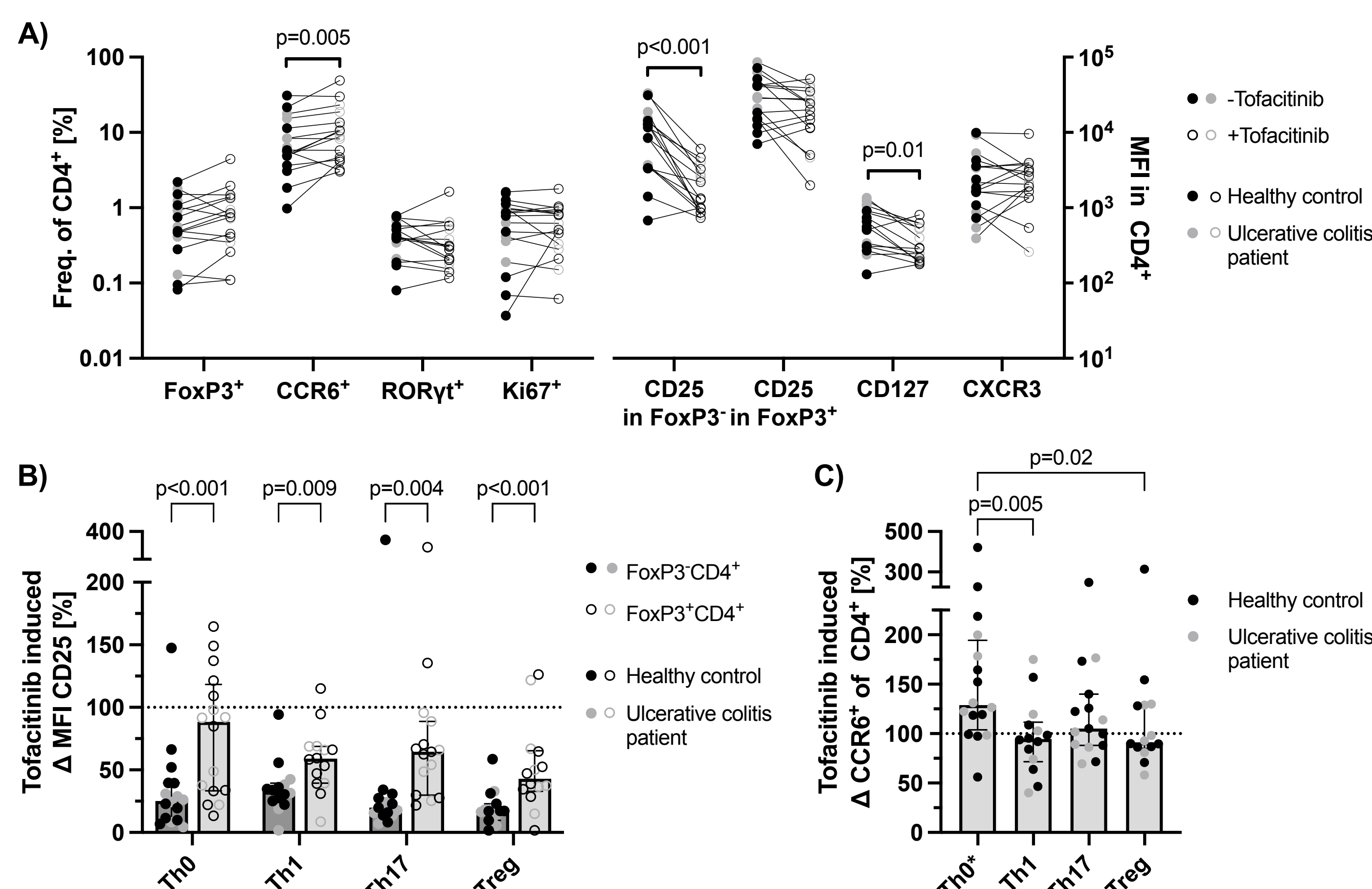


Results

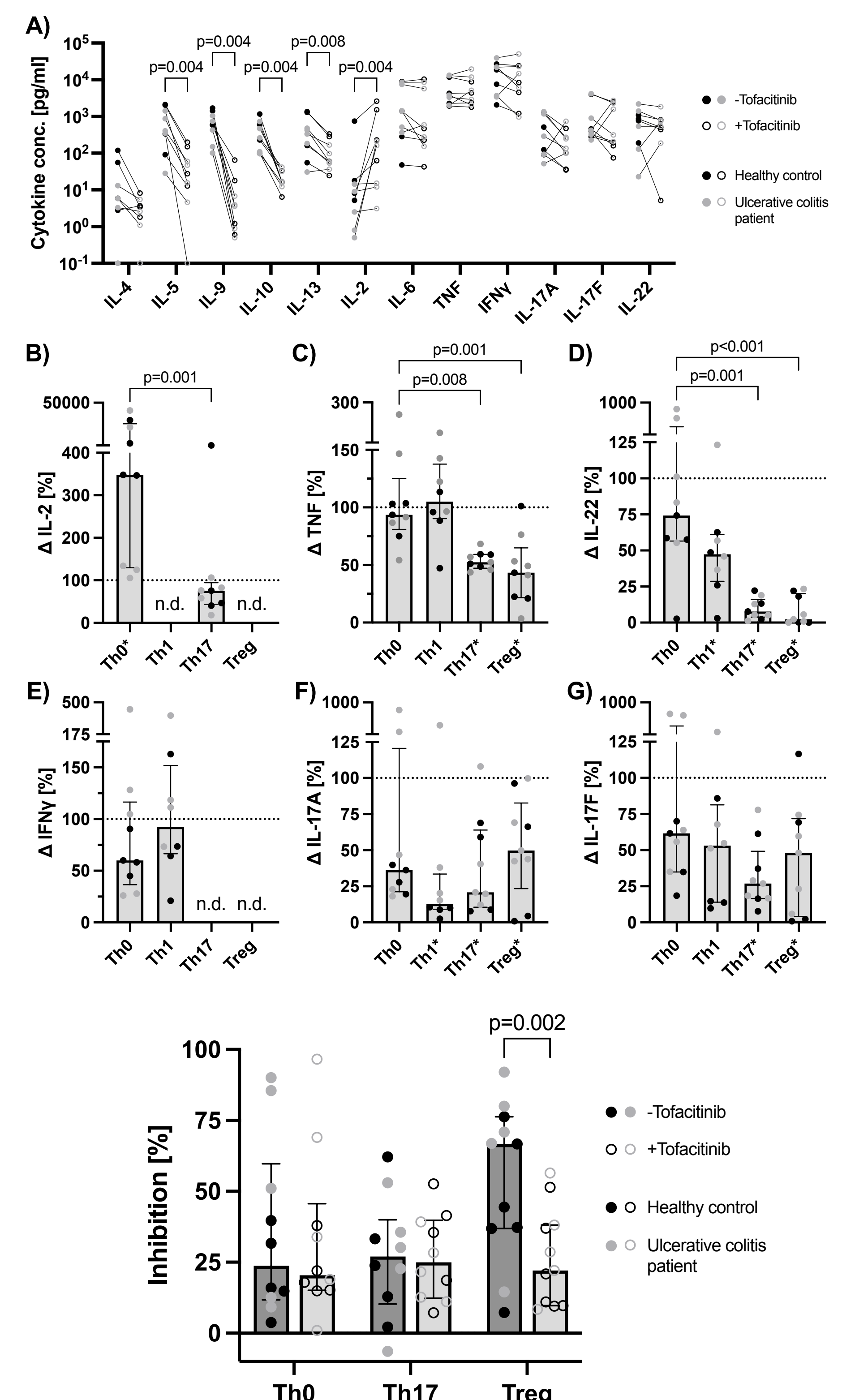


(1) T cell receptor activation with anti-CD3/CD28-ab induced a marked up-regulation of CD25 (A) and CXCR3 (E), and increased the proportion of FoxP3⁺ Th cells (C). Furthermore, T cell activation in a Th17- or Treg-promoting cytokine milieu induced an up-regulation of the C-C motif chemokine receptor (CCR) 6 expression, an established marker of Th17 and Treg cells (D).

(2) Tofacitinib induced the down-regulation of the IL-2-receptor (IL-2R, CD25) and the IL-7-receptor α -chain (CD127) (A), independent of the surrounding cytokine milieu (data not shown), whereas the amount of proliferating, Ki67-expressing CD4 T cells was not altered. The tofacitinib induced suppression of the IL-2R was less pronounced in FoxP3⁺ CD4 T cells than in FoxP3⁻ CD4 T cells (B). In a neutral cytokine milieu (Th0), tofacitinib induced the upregulation of CCR6, whereas there was no additive effect of tofacitinib on the CCR6 expression in a Th17- or Treg-promoting cytokine milieu (C). B, C: depicted is the ratio of the expression of CD25 (B) / the proportion of CCR6⁺ CD4 T cells (C) following the addition of tofacitinib to the respective values without addition of tofacitinib. * Statistically significant ($p < 0.05$) tofacitinib induced increase of the proportion of CCR6⁺ CD4 T cells.



(3) The secretion of IL-5, IL-9, IL-10 and IL-13 by anti-CD3/CD28-antibody stimulated PBMC was significantly inhibited by tofacitinib (A), independent of the surrounding cytokine milieu (data not shown). The production of IL-2 was enhanced by tofacitinib upon T cell activation without further cytokine stimulation. This effect, however, was abrogated by a Th17-promoting cytokine milieu (B). Furthermore, tofacitinib inhibited the secretion of TNF (C) and IL-22 (D) in a Th17- or Treg-promoting cytokine milieu, but not under neutral or Th1-stimulating conditions. B-G: depicted is the ratio of the cytokine concentrations in cell culture supernatants following the addition of tofacitinib to the cytokine concentration without tofacitinib. * Statistically significant ($p < 0.05$) tofacitinib induced alteration of the cell culture supernatant cytokine concentration.



(4) Whereas tofacitinib had no effect on the suppressive function of Treg cells in a neutral (Th0) or Th17-promoting cytokine milieu, the effects of a Treg-promoting cytokine milieu were abrogated by JAK1/3-inhibition.

Isolated Treg cells were incubated for 5 days in a neutral (Th0), Th17- or Treg-promoting cytokine milieu in the presence or absence of tofacitinib, followed by co-culture with activated autologous effector cells for 4 days. Proliferation of effector cells in the presence or absence of Treg cells was determined using CFSE-dilution. The suppressive capacity of the Treg cells incubated in the presence or absence of tofacitinib was compared for each cytokine milieu using Wilcoxon matched-pairs signed rank test.

Conclusion

Whereas JAK1/3-inhibition effectively suppresses inflammation in a variety of diseases, it also inhibits the secretion of the cytokines IL-10 and IL-22, playing roles in the regulation of inflammation and tissue regeneration. Furthermore, JAK1/3-inhibition with tofacitinib abrogates the effects of a Treg-promoting cytokine milieu on the suppressive function of Treg cells.