Tofacitinib and a selective Janus Kinase 1 inhibitor are equally potent in suppressing human macrophage function and T cell proliferation

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Background

Non-selective Janus Kinase (JAK) inhibitors such as tofacitinib have shown efficacy in treatment of ulcerative colitis. Tofacitinib inhibits signaling of multiple cytokines involved in the pathogenesis of IBD.

Side effects are attributed to JAK2 signaling leading to the development of more selective JAK inhibitors.

Aim: To investigate the potency of a selective JAK1 inhibitor (JAK1i, GSK2276186), a JAK3 inhibitor (JAK3i, GSK2864192A) and tofacitinib (CP-690,550, Pfizer) to supress innate and adaptive immune responses in vitro.

Methods

CD14+ monocytes (n=6) were stimulated with LPS (100 ng/ml) and IFNγ (10 ng/ml) for 6 hours in presence or absence of JAK1i, JAK3i or tofacitinib (10-1000 nM). Chemokine production was measured by ELISA.

JAK1i, JAK3i and tofacitinib (10-10.000 nM) were added to PBMC's in a mixed lymphocyte reaction (MLR).

Lymphocytes (n=3) were stimulated with anti-CD3/CD28 beads in the presence of JAK1i, JAK3i or tofacitinib. T cell proliferation was measured with a Tritium proliferation assay. Cell viability was assessed using an MTS colorimetric assay.

Conclusion

- In vitro, JAK1i and tofacitinib, but not JAK3i inhibit CXCL10 secretion produced by human monocyte-derived macrophages
- JAK1i, tofacitinib and JAK3i at a higher concentration inhibit proliferation of human T cells
- T cell viability was not affected at effective concentrations of JAK1i, JAK3i and tofacitinib

Results

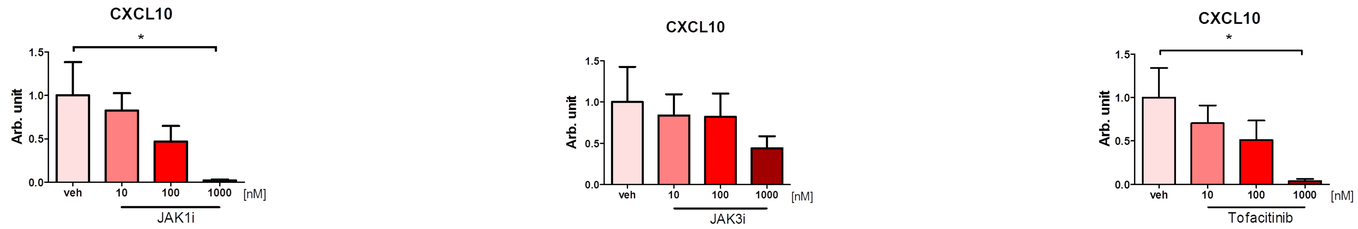

Fig.1| ELISA: In human CD14+ monocyte-derived macrophages, JAK1i and tofacitinib, but not JAK3i decreased CXCL10 secretion at 1000 nM, while TNFα, IL6, IL12 and IL10 secretion was unaffected by all inhibitors. Statistics: 1-Way ANOVA, posthoc test Bonferroni. Error bars represent SEM; *** p<0.001; ** 0.001≤p≤0.01; * 0.01<p≤0.05. Data of six separate experiments

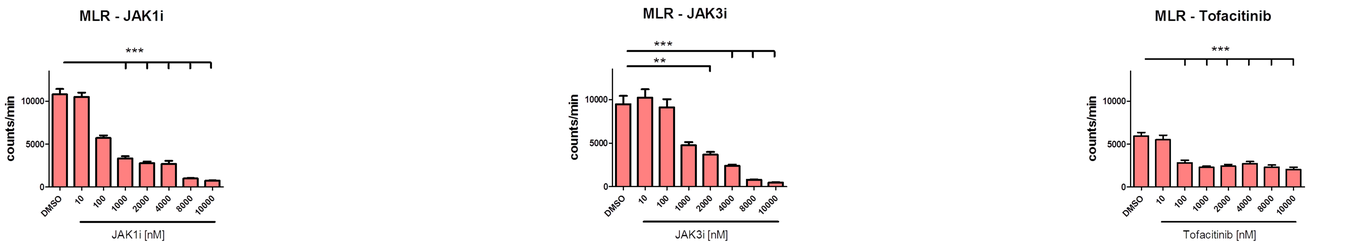

Fig.2| Mixed Lymphocyte reaction: JAK1i and tofacitinib inhibited T cell proliferation at respectively 1000 nM and 100 nM and up. JAK3i inhibited T cell proliferation at 2000 nM and up. Statistics: Kruskall-Wallis, Dunn's multiple comparison test. Error bars represent SEM; *** p<0.001; ** 0.001≤p≤0.01; * 0.01<p≤0.05. Data of three separate experiments

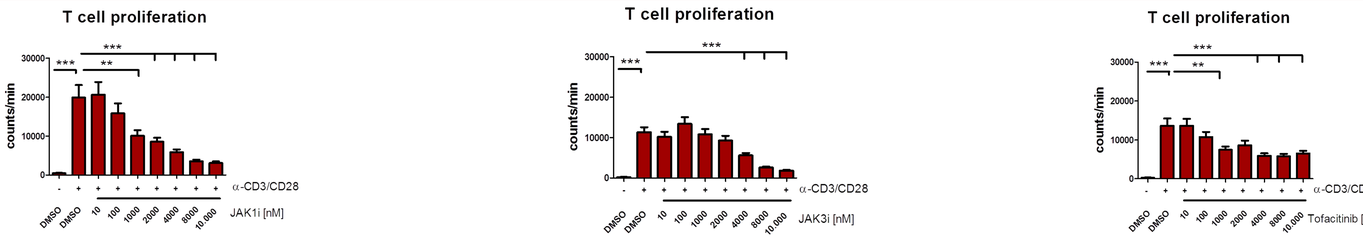

Fig.3| T cell proliferation: JAK1i and tofacitinib both inhibited T cell proliferation at 1000 nM. JAK3i inhibited T cell proliferation at 4000 nM (p=0.004).Statistics: 1-Way ANOVA, posthoc test Bonferroni. Error bars represent SEM; *** p<0.001; ** 0.001≤p≤0.01; * 0.01<p≤0.05. Data of three separate experiments

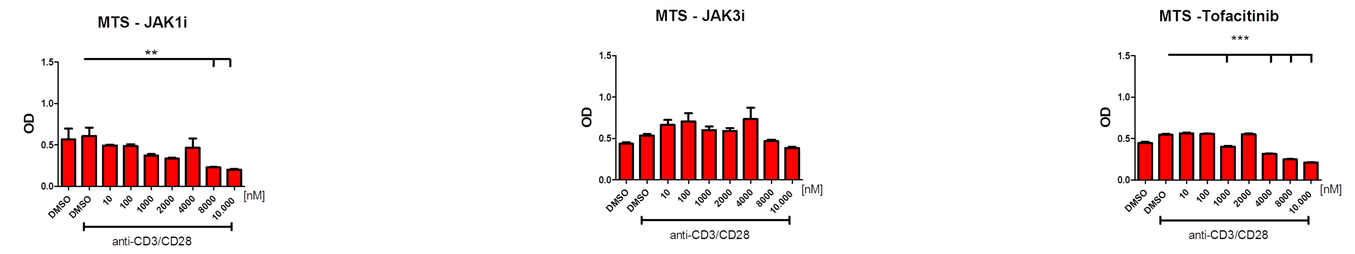

Fig.4| MTS viability assay lymphocytes: T cell viability was only affected by JAK1i, JAK3i and tofacitinib at higher concentrations. Statistics: 1-Way ANOVA, posthoc test Bonferroni. Error bars represent SEM; *** p<0.001; ** 0.001≤p≤0.01; * 0.01<p≤0.05.