A comparison of systemic versus targeted anti-TNFα antibody in treatment of colitis induced by adoptive transfer of CD44-/CD62L+ T-cells into RAG2-/- mice recipients

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Introduction

Inflammatory bowel disease (IBD) is characterized by a disproportionate inflammatory response in gastrointestinal tissues leading to damage and clinical symptoms. TNFα is a potent proinflammatory cytokine exerting pleiotropic effects and is generated in a precursor form called transmembrane TNFα expressed on activated macrophages and lymphocytes. A soluble form of TNFα can be released at the cell surface by TNFα converting enzyme (TACE). Soluble TNFα mediates biologic activities through TNFα receptors type I and type II (TNFR1 and TNFR2). The development of therapies targeting TNFα such as infliximab, adalimumab and others has revolutionized the treatment of IBD. These antibodies bind soluble TNFα, thereby blocking receptor binding and subsequent cytokine-driven inflammatory processes. In addition, when these anti-TNFα antibodies bind transmembrane TNFα many are capable of targeted cell death through antibody-dependent cell-mediated cytotoxicity (ADCC). Recent publications have established that there may be inadequate anti-TNFα antibody drug reaching diseased tissue in patients with active IBD and that the high TNFα burden is not adequately suppressed.[1] In the present study we evaluated the efficacy of targeted intracecal (IC) anti-mouse-TNFα antibody (a surrogate for human anti-TNFα antibodies) when compared with systemic intraperitoneal (IP) injection in an adaptive T-cell transfer induced chronic colitis mouse model.

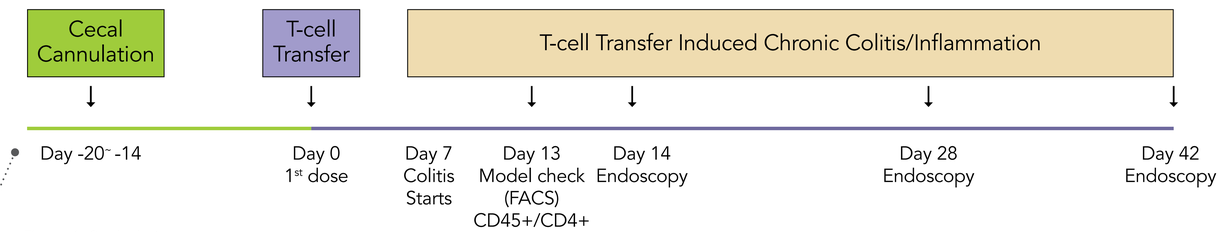

Figure 1. Study design

Methods

All animals underwent surgical implantation of a cecal cannula 2 weeks prior to the experiment for the ease of bolus topical delivery to the cecum. Colitis was induced by intraperitoneal (IP) injection of $0.5x10^6$ CD44-/CD62L+ T-cells isolated and purified from C57Bl/6 donor to the male RAG2-/- recipient mice on Day 0. To minimize variation due to different routes of administration, animals were treated with both IP every third day (Q3D) and intracecal (IC) once daily (QD) of either the anti-TNFα or the control (Vehicle solution or IgG1 controls) from Day 0 to 42 (Figure 1).

Groups were treated from Day 0 to 42 as follows: Group 1: Naïve no surgery not treated; Group 2: Naïve not treated; Group 3: Vehicle (IP) 3x/wk + Vehicle (IC) QD; Group 4: IgG (IP) 3x/wk + Vehicle (IC) QD; Group 5: Vehicle (IP) 3x/wk + IgG (IC) QD; Group 6: Anti-TNFα (IP) 3x/wk + Vehicle (IC) QD; Group 7: Vehicle (IP) 3x/wk + anti-TNFα (IC) QD. Mice underwent endoscopy on Days 14, 28, 42 to assess colitis severity and stool consistency. The Disease Activity Index (DAI) was calculated as a combination of body weight loss score plus colitis score and stool consistency score at endoscopy. At the Day 42 termination, blood and tissues were collected for bioanalysis of inflammatory cytokines and tissues were fixed for histopathologic analysis.

Results

- Significant body weight loss was observed in groups treated with Vehicle or IgG control (IP/IC) starting at Day 7 to 14 after T-cell transfer with a 98% T-cell engraftment rate found on Day 13 indicating successful development of colitis (Figure 1).
- Treatment with either IP or IC anti-TNFα antibody led to a significant reduction of body weight loss (%) AUC from Day 0 to Day 42 (Figure 2) and DAI at Day 28 and Day 42 (Figure 3).

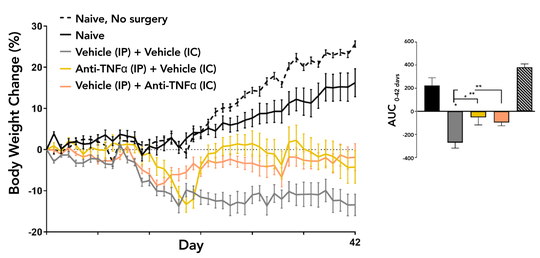

Figure 2. Body weight (BW) changes (mean % ± SEM) (left) and BW AUC (Day 0 to 42) (mean % ± SD) (right) for Naïve group and groups post CD44-/CD62L+ T-Cell transfer and administration of anti-mouse TNFα antibody (IP and IC) or Vehicle control. Pair-wise comparisons by two-tailed Mann-Whitney U-Test for the comparison of treatment effects; $p<0.05$* and $p<0.01$**.

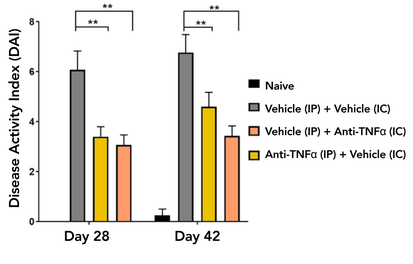

Figure 3. Disease Activity Index (DAI) (mean % ± SEM) in Naïve group and groups receiving CD44-/CD62L+ T-Cell transfer and administration of anti-mouse TNFα antibody (IP and IC) or Vehicle control. Pair-wise comparisons by two-tailed Mann-Whitney U-Test for treatment effects; $p<0.05$* and $p<0.01$**.

- Significant reduction in mean concentration of inflammatory cytokines was found in groups treated with anti-TNFα by IC or IP route when compared with Vehicle (IP/IC) control and respective IgG controls (IC or IP) in colon tissue (Figure 4).
- Targeted IC anti-TNFα treatment showed a significant improvement in mean histopathologic score when compared with the Vehicle controls (IP and IC) groups in proximal and distal colon tissues indicating that anti-TNFα treatment was generally more effective in this group (Figure 5).

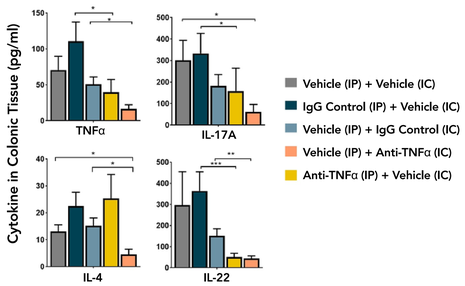

Figure 4. Inflammatory cytokines (pg/mL) (mean % ± SEM) in the Naïve group and groups receiving CD44-/CD62L+ T-Cell transfer and administration of anti-mouse TNFα antibody (IP and IC) or Vehicle control. Pair-wise comparisons by two-tailed Mann-Whitney U-Test for treatment effects; $p<0.05$*, $p<0.01$**, and $p<0.001$***.

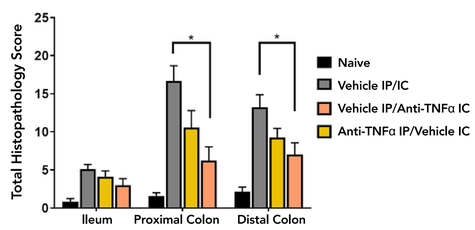

Figure 5. Comparison of total histopathology score (mean % ± SEM) in ileum, proximal colon and distal colon tissues. Pair-wise comparisons by two-tailed Mann-Whitney U-Test for treatment effects; $p<0.05$*.

- Targeted IC anti-TNFα treatment showed the greatest magnitude of lymphocyte reductions in all counted field from inner lumen to submucosa when compared with vehicle control groups (Figure 6). Similar trend of lymphocyte count reduction was found in distal colon but with less lymphocyte count in general.

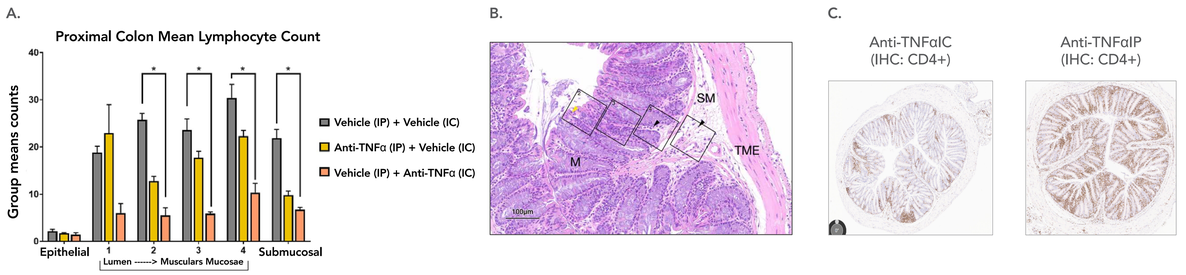

Figure 6. Mean lymphocyte counts from luminal to external submucosal and represented images of H&E stains and IHC stains of the proximal colon. A. Mean lymphocyte count from most inner lumen to submucosal of the proximal colon in groups treated with Vehicle controls, anti-TNFα (IP) and anti-TNFα (IC), Group mean +/- SEM. Kruskal-Wallis Test with Dunn's multiple comparison for treatment effects; $p<0.05$*. B. Represented image of H&E stain of proximal colon in proximal colon of anti-TNFα (IC) group. An intraepithelial lymphocyte (yellow arrowhead), example lamina proprial lymphocytes (black arrowheads), and the tunica muscularis externa (TME) are indicated; C. Represented images of IHC stain of CD4 marker for lymphocytes in proximal colon of anti-TNFα (IC) or anti-TNFα (IP) group.

Conclusion

- Human studies have shown there is inadequate anti-TNFα antibody reaching the diseased tissue resulting in persistent inflammation. Thus, we hypothesized that targeted and topical administration of anti-TNFα antibody would result in improved efficacy when compared to systemic (IP) distribution in an adoptive T-cell transfer mouse model of colitis.
- Significantly reduced weight loss (%), decreased Disease Activity Index, improved histological score and reduced tissue inflammatory cytokines was found in animals receiving targeted (IC) anti-TNFα antibody when compared with vehicle controls.
- Targeted IC delivery was significantly more efficacious when compared to systemic (IP) anti-TNFα antibody in a most important end point total histologic score and lymphocyte count from inner lumen to submucosa of proximal colon.
- Orally administered, minimally absorbed drugs designed to act locally in the gastrointestinal tract are becoming of increasing interest and we believe that the present findings provide a proof of efficacy for targeted IC anti-TNFαantibody to treat IBD.

References

1. Yarur et al. The association of tissue anti-TNF drug levels with serological and endoscopic disease activity in inflammatory bowel disease: the ATLAS study. Gut. 2016 Feb;65(2):249-55.

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