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Partial normalization of pubertal timing in female mice with DSS colitis treated with anti-TNF- α antibody

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Abstract

Background—Inflammatory bowel disease (IBD) and resultant colitis occurring prior to puberty are frequently associated with delayed puberty and losses of growth and bone mineralization. Some of this delay may be due to colonic inflammation and associated systemic inflammation. To date no treatments for IBD have been shown to normalize the timing of puberty. Our objective in this study was to determine whether there is a normalization of the timing of puberty during treatment of colitis using mono-clonal antibodies (abs) to tumor necrosis factor (TNF)- α .

Methods—We induced colitis in 23-day-old C57Bl6 female mice using 3% dextran sodium sulfate (DSS) for 7 days, followed by removal of DSS for an additional 3 days, resulting in 10 days of worsening colitis. DSS-treated mice received either TNF- α ab or Control ab on days 4 and 8 of colitis, while non-colitic Control mice received injections of TNF- α ab (Control + TNF- α ab). All groups were followed for the timing of vaginal opening until day of life 33, when they were euthanized for serum and colon collection.

Results—The DSS + TNF- α ab group had lower levels of systemic interleukin (IL)-6 and a partial normalization of the timing of vaginal opening compared to the DSS + Control ab group. There were no differences in weight gain, growth, or colon histological inflammatory scores between the DSS + TNF α ab and DSS + Control ab groups over the course of the experiment.

Conclusions—We conclude that anti-TNF- α ab treatment causes a partial normalization of pubertal timing coincident with decreased systemic inflammation in DSS colitis. These data may have implications regarding growth and bone mineralization outcomes in pediatric IBD.

Keywords

Colitis; Puberty; Inflammation; TNF- α

Introduction

When inflammatory bowel disease (IBD) occurs prior to puberty, it frequently results in a delay in the timing of puberty [1], contributing to poor linear growth [2–5] and decreased bone mineral density [6–8]. The mechanism by which IBD contributes to delayed puberty has been postulated to result from IBD-mediated effects on the hypothalamic–pituitary–gonadal (HPG) axis [9]. In females this results in a decrease in luteinizing hormone (LH)- and follicle-stimulating hormone (FSH)-stimulated synthesis of estradiol by the ovaries.

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Ethical considerations These experiments were approved by the Animal Care and Use Committee at the University of Virginia.

Conflict of interest The authors declare that they have no conflict of interest.

These effects of IBD on HPG axis function have been postulated to be caused by two primary mechanisms. The first of these is poor weight gain, with subsequent decrease in fat mass and decrease in levels of leptin, an adipocyte-derived hormone that is essential for the normal timing of puberty [10, 11]. The second mechanism by which IBD may affect HPG function relates to increased systemic inflammation, given the known effects of inflammatory cytokines on levels of gonadotropins [9,12–14]. Thus far no treatments for IBD have been reported to normalize the timing of puberty.

We previously reported a model of colitis in pre-pubertal female mice, demonstrating that mice treated with 3% dextran sodium sulfate (DSS) exhibited a delay in vaginal opening (a validated marker of pubertal progression) beyond what was seen in a food-restricted group whose weight matched that of the DSS-treated mice [15, 16]. During the course of 10 days of worsening colitis, the DSS mice and the food-restricted group had similar food intake and—importantly—had similar levels of leptin. The major finding separating the DSS and food-restricted groups was worsening colonic and systemic inflammation, which was apparent in the DSS group by experimental day 4 and widened over the course of the experiment.

Anti-inflammatory treatment is a mainstay in IBD. One commonly used agent is infliximab, a monoclonal antibody to tumor necrosis factor (TNF)- α [17]. Infliximab treatment in humans attenuates disease severity and increases linear growth velocity [18, 19]. It is not known whether infliximab treatment affects the timing of puberty. Our goal was to use our mouse model of colitis to determine whether infliximab treatment normalizes the timing of vaginal opening and whether infliximab causes any change in the function of the HPG axis in the setting of colitis.

Materials and methods

C57BL6 mice were purchased from Jackson Laboratories (Bar Harbor, ME, USA) and were housed in standard wire-top cages and fed phytoestrogen-free chow. Two females and one male of childbearing age were housed together for breeding. Pups from litters of 6–10 mice were included in these experiments. Pups were weaned at 19 days of age by being removed from the mother and placed in a cage, without males, with moistened chow for three days before starting dry food exclusively.

Starting at day-of-life (DOL) 23, female mice were divided based on starting weight into three groups: Control + TNF- α ab, DSS + Control ab, and DSS + TNF- α ab. Mice in the DSS groups had 3% DSS (mol wt 36,100–45,500; TdB Consultancy, Uppsala, Sweden) placed in the water supply from DOL 23–30 before DSS treatment was discontinued and the animals were allowed to experience continued colitis from DOL 30 to 33. Mice in the Control groups received de-ionized water alone throughout the course of the experiment. All mice were given phytoestrogen-free food ad libitum throughout the experiment.

Antibody treatment was performed on DOL 27 and 31 (day 4 and day 8 of colitis treatment) via IP injection. Antibodies were generously provided by Centocor Ortho Biotech (Horsham, PA, USA). Animals were given 0.1 mg of either murine monoclonal anti-murine TNF- α or non-specific antibody suspended in phosphate-buffered saline. Additional confirmatory experiments included administration of 0.2 mg of anti-TNF- α ab on days 4 and 8 or 0.1 mg on days 3 and 7.

Vaginal opening was determined by daily inspection using surgical loupes and recorded as the day on which the vaginal orifice transitioned from tightly closed to patent [9, 20].

Animals were euthanized at DOL 33 via injection of standard mouse cocktail followed by cardiac puncture. In a subset of animals, gonadotropin-releasing hormone (GnRH)

stimulation testing was performed, as described previously [15]. GnRH stimulation was performed using 100 mg/kg of GnRH (Bachem Americas, Torrance, CA, USA) injected s.c., with blood drawn 15 min after injection. Serum was stored at -80° until the time of testing.

At the time of euthanizing, colons were removed and fixed in Bouin's reagent prior to embedding in paraffin, sectioning, and staining. Slides from individual animals were read by a blinded examiner and scored for chronic inflammatory index [21, 22].

Serum measurements

Serum cytokines were tested in the University of Virginia Digestive Health Research Center using a Becton Dickinson multi-plex system (Becton Dickinson, San Jose, CA, USA). Serum LH and FSH measurements were tested using a Milliplex assay (Luminex, Austin, TX, USA) by the Ligand Core Laboratory of the University of Virginia Center for Research in Reproduction. Additional animals were placed through the same protocol for measurement of cytokine and gonadotropin levels.

Statistics

Statistics were calculated using GraphPad Prism (La Jolla, CA, USA) graphing and statistics software and SAS (Research Park Triangle, NC, USA). Comparisons of mean values between groups were performed using *t*-tests or analysis of variance (ANOVA) with Bonferroni post-test comparison of all groups, as appropriate. Kaplan–Meier curves of timing of vaginal opening were analyzed via log-rank test [23].

Results

Growth and food intake

Daily weights for each group are shown in Fig. 1a. Mice in the DSS + Control ab and DSS + TNF- α ab groups had similar weights throughout the experiment. Mice in the DSS + TNF- α ab and the DSS + Control ab groups were significantly different from the Control + TNF- α ab group starting at DOL 28 and 29, respectively. Daily and cumulative food intake for each group is presented in Fig. 1b, c. Again, mice in the DSS + Control ab and DSS + TNF- α ab groups had similar food intake to each other but ate less than the Control + TNF- α ab group. Change in length was not different between the three groups (Fig. 1d). An additional comparator group of Control mice receiving Control ab (Control + Contr ab) was not different from the Control + TNF- α ab group in terms of daily weight, food intake, and linear growth (data not shown).

Colonic and systemic inflammation

Representative histological sections of colons from each group are shown in Fig. 2a–c. Blinded histological evaluation revealed that the DSS + Control ab and DSS + TNF- α ab groups had similar amounts of colonic inflammation, as assessed by the chronic inflammatory index [21, 22] (Fig. 2d). Conversely, the Control + TNF- α ab group had no apparent inflammation.

Levels of systemic cytokines are shown in Fig. 3. There were no differences between groups in levels of IL-1 β , IL-12, and IL-10 ($p > 0.05$). Mice in the DSS + Control ab group had greater levels of IL-6 than either the Control + TNF- α ab or DSS + TNF- α ab groups. Mice in the DSS + Control ab group also had higher levels of keratinocyte-derived chemokine (KC) than the Control + TNF- α ab group, but not the DSS + TNF- α ab group.

Timing of vaginal opening and levels of gonadotropins

The timing of vaginal opening over the course of the experiment is shown in Fig. 4. As assessed by both log-rank test of the Kaplan–Meier curves (Fig. 4a) and average age at vaginal opening (Fig. 4b), the DSS + Control ab group had later timing of vaginal opening than either the Control + TNF- α ab or DSS + TNF- α ab groups; there was no significant difference in the timing of vaginal opening between the DSS + TNF- α ab and Control + TNF- α ab groups. Use of a higher dose of anti-TNF- α antibody (0.2 mg) and an alternative dosing regimen (day 3 and day 7 of colitis) resulted in a similar difference in the timing of vaginal opening and failed to improve weight gain associated with the underlying colitis (data not shown). Additionally, assessment for correlations between levels of serum cytokines and age at vaginal opening failed to reveal significant associations (data not shown).

Levels of gonadotropins are shown in Fig. 5. Compared to the DSS + Control ab group, the DSS + TNF- α ab group had higher LH levels after stimulation with GnRH but lower pre-stimulation FSH levels. Compared to the Control + TNF- α ab group, the DSS + TNF- α group was not different with respect to levels of LH or FSH (data not shown).

Discussion

We have demonstrated a partial normalization in the timing of puberty during treatment with a monoclonal antibody to TNF- α . To our knowledge, this is the first time that a treatment for IBD has resulted in a difference in pubertal timing. We had previously shown that female (and male) mice with DSS colitis had a later timing of vaginal opening (or prepuce-glans separation for males) than food-restricted mice of the same body weight [15, 16]. In those experiments, the DSS-treated mice and the food-restricted mice had similar weight gain, food intake, and levels of leptin. The major differences in mice with colitis compared to those with food restriction were in a gradual increase in levels of systemic cytokines—beginning by day 4 of DSS treatment—and an increase in colonic inflammation as judged by histological scoring. Following those experiments we concluded that an element of colitis contributed to a delayed puberty beyond what was seen following food restriction alone. The present experiment extends these findings to suggest that this element of colitis delaying puberty is lessened during treatment with anti-TNF- α antibody—a common treatment used to treat IBD in children and adults.

Pubertal timing is determined by initiation of activity of the HPG axis, starting with the GnRH-secreting neurons in the hypothalamus. These neurons in turn lead to the release of LH and FSH from the pituitary, resulting in the production of estradiol and secondary sexual characteristics. While estradiol measurements in mice at pubertal age are at or below the limit of detection even in untreated controls [16], a validated measure of puberty in female mice is the physical examination feature of vaginal opening [9, 20], which occurred earlier in the DSS + TNF- α ab group compared to the DSS + Control ab mice. We were also able to test levels of gonadotropins at baseline and after stimulation with GnRH, demonstrating higher levels of LH in the DSS + TNF- α ab group, suggesting that these mice were more primed from regular exposure to GnRH, as is seen in the progression of puberty [24].

Clearly there are multiple potential mechanisms by which anti-TNF- α treatment may serve to normalize the timing of puberty. We noted similar food intake between the DSS + TNF- α ab group and the DSS + Control ab group. Although differences in absorption of food may have been different between groups, it is notable that the two colitis groups had similar weight curves as well. In some ways this is surprising, given the role of inflammation in

mediating anorexia associated with chronic disease [25]. The DSS + TNF- α ab group continued their poor weight gain despite anti-inflammatory treatment.

The lack of improvement in appetite among the DSS + TNF- α ab group may have occurred because of a lack of apparent attenuation of disease activity with treatment, as evidenced by similar histological scores between colitis groups. Humans treated with infliximab exhibit impressive attenuation of disease activity and histological findings of disease alongside decreases in systemic inflammation [17,18]. In this way, DSS colitis likely represents an imperfect model of human disease. Although DSS administration produces histological and systemic inflammatory findings reminiscent of human disease, DSS produces these effects via chemical irritation and does not require Th2 lymphocyte activity. Past studies have suggested that blocking local or systemic inflammation does not decrease the mucosal irritation caused by the DSS polymers [26]. It is conceivable that this ulceration continued to affect appetite and food intake in the DSS + TNF- α ab group.

It appeared that the major difference between the DSS-treated groups was in systemic inflammation. As may have been anticipated, treatment with anti-TNF- α ab resulted in a decrease in systemic inflammation, regardless of the lack of effect on histological features of colitis. This was seen in differences in lower levels of IL-6 in the DSS + TNF- α ab group relative to the DSS + Control ab group, though both groups had higher levels of IL-6 than the Control + TNF- α ab group. Similarly, the DSS + Control ab group had higher levels of KC, an important cytokine in the pathogenesis of colitis [27]. A weakness of our study is that while it is presumed that there were also differences in TNF- α between groups, we were unable to test this hypothesis because the TNF- α antibody interferes with the TNF- α assay.

Peripheral inflammation is associated with a decrease in LH release [14], potentially via an increase in the central expression of inflammatory cytokines [28]. Additionally, intracerebroventricular injection of inflammatory cytokines—including IL-6, TNF- α , and IL-1 β —decreases the release of gonadotropin and sexual function [12, 13]. Although measurements of baseline and stimulated levels of gonadotropins were similar between mice in the DSS + TNF- α ab and DSS + control ab groups (with an increase in stimulated levels of LH), it should be noted that these levels were tested at the end of the experiment on DOL 33, when the symptoms of colitis were worst. Continued progression through puberty in the DSS + TNF- α ab group occurred at earlier time points (DOL 28–31) when symptoms of disease were more moderate. We also noted a decrease in the levels of FSH prior to GnRH stimulation, and this is of unclear significance. Relative differences in LH and FSH are determined by the frequency of pulses of GnRH from the hypothalamus, with more rapid pulses resulting in higher LH levels and lower FSH levels [29], though it is uncertain why such a pattern would be more common among anti-TNF- α -treated animals. Our overall interpretation of the increased responsiveness of anti-TNF- α -treated mice to GnRH stimulation is that these mice were more attuned to GnRH signaling. Clearly, further experiments related to gonadotropin regulation in this setting are necessary.

Clinical parallels to this study remain to be seen. While treatment with infliximab has been shown to increase growth velocity [19, 30] and bone mineral density [31] in human subjects, no data exist regarding effects on the timing of puberty. There is reason to expect that improvements are likely to be present in clinical settings as well. In our rodent model of colitis, mice that received anti-TNF- α antibody treatment exhibited decreased systemic inflammation (and improvements in the timing of puberty) but continued to exhibit severe mucosal ulceration and body weight loss. By contrast, in human disease, infliximab treatment is associated with improved weight gain, a decrease in mucosal injury, and a decrease in systemic inflammation [18, 32, 33]. Many of these differences between our model and clinical disease are due to the source of ulceration, which is from immune-

mediated action in human disease and which is thus suppressed following TNF- α inhibition [17]. Given the improvements in the intestinal mucosa and body weight, and the decrease of systemic inflammation, it is our hypothesis that the timing of puberty would be normalized in the setting of human disease as well.

In conclusion, we noted an earlier timing of puberty among mice with DSS colitis treated with a monoclonal antibody to TNF- α . Although treatment did not improve weight gain or the histological score, the decrease in systemic inflammation was associated with an increase in HPG activity. Given the importance of puberty for bone mineralization and the importance of the pubertal growth spurt, these data may bear some implications for other important outcome measures for pediatric IBD, though further research is necessary.

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References

1. Ballinger AB, Savage MO, Sanderson IR. Delayed puberty associated with inflammatory bowel disease. *Pediatr Res*. 2003; 53:205–10. [PubMed: 12538776]
2. Pfefferkorn M, Burke G, Griffiths A, Markowitz J, Rosh J, Mack D, Otley A, Kugathasan S, Evans J, Bousvaros A, Moyer MS, Wyllie R, Oliva-Hemker M, Carvalho R, Crandall W, Keljo D, Walters TD, LeLeiko N, Hyams J. Growth abnormalities persist in newly diagnosed children with Crohn disease despite current treatment paradigms. *J Pediatr Gastroenterol Nutr*. 2009; 48:168–74. [PubMed: 19179878]
3. Sawczenko A, Ballinger AB, Savage MO, Sanderson IR. Clinical features affecting final adult height in patients with pediatric-onset Crohn's disease. *Pediatrics*. 2006; 118:124–9. [PubMed: 16818557]
4. Wong SC, Macrae VE, McGrogan P, Ahmed SF. The role of pro-inflammatory cytokines in inflammatory bowel disease growth retardation. *J Pediatr Gastroenterol Nutr*. 2006; 43:144–55. [PubMed: 16877976]
5. DeBoer MD, Barnes BH. The importance of treatment regimens and pubertal status for growth in IBD. *J Pediatr*. 2009; 154:936–7. [PubMed: 19446107]
6. Dubner SE, Shults J, Baldassano RN, Zemel BS, Thayu M, Burnham JM, Herskovitz RM, Howard KM, Leonard MB. Longitudinal assessment of bone density and structure in an incident cohort of children with Crohn's disease. *Gastroenterology*. 2009; 136:123–30. [PubMed: 19026647]
7. Paganelli M, Albanese C, Borrelli O, Civitelli F, Canitano N, Viola F, Passariello R, Cucchiara S. Inflammation is the main determinant of low bone mineral density in pediatric inflammatory bowel disease. *Inflamm Bowel Dis*. 2007; 13:416–23. [PubMed: 17206686]
8. Thayu M, Leonard MB, Hyams JS, Crandall WV, Kugathasan S, Otley AR, Olson A, Johanns J, Marano CW, Heuschkel RB, Veereman-Wauters G, Griffiths AM, Baldassano RN. Improvement in biomarkers of bone formation during infliximab therapy in pediatric Crohn's disease: results of the REACH study. *Clin Gastroenterol Hepatol*. 2008; 6:1378–84. [PubMed: 19081527]
9. Azooz OG, Farthing MJ, Savage MO, Ballinger AB. Delayed puberty and response to testosterone in a rat model of colitis. *Am J Physiol Regul Integr Comp Physiol*. 2001; 281:R1483–91. [PubMed: 11641119]
10. Farooqi IS. Leptin and the onset of puberty: insights from rodent and human genetics. *Semin Reprod Med*. 2002; 20:139–44. [PubMed: 12087499]
11. Kaminski BA, Palmert MR. Genetic control of pubertal timing. *Curr Opin Pediatr*. 2008; 20:458–64. [PubMed: 18622205]
12. Kalra PS, Sahu A, Kalra SP. Interleukin-1 inhibits the ovarian steroid-induced luteinizing hormone surge and release of hypothalamic luteinizing hormone-releasing hormone in rats. *Endocrinology*. 1990; 126:2145–52.

13. Rivier C, Vale W. Cytokines act within the brain to inhibit luteinizing hormone secretion and ovulation in the rat. *Endocrinology*. 1990; 127:849–56.
14. Nappi RE, Rivest S. Effect of immune and metabolic challenges on the luteinizing hormone-releasing hormone neuronal system in cycling female rats: an evaluation at the transcriptional level. *Endocrinology*. 1997; 138:1374–84. [PubMed: 9075691]
15. DeBoer MD, Li Y. Puberty is delayed in male mice with dextran sodium sulfate colitis out of proportion to changes in food intake, body weight, and serum levels of leptin. *Pediatr Res*. 2011; 69:34–9. [PubMed: 20940665]
16. DeBoer MD, Li Y, Cohn S. Colitis causes delay in puberty in female mice out of proportion to changes in leptin and corticosterone. *J Gastroenterol*. 2010; 45:277–84. [PubMed: 20072791]
17. Hyams J, Crandall W, Kugathasan S, Griffiths A, Olson A, Johanns J, Liu G, Travers S, Heuschkel R, Markowitz J, Cohen S, Winter H, Veereman-Wauters G, Ferry G, Baldassano R. Induction and maintenance infliximab therapy for the treatment of moderate-to-severe Crohn's disease in children. *Gastroenterology*. 2007; 132:863–73. (quiz 1165–1166).
18. Sinitsky DM, Lemberg DA, Leach ST, Bohane TD, Jackson R, Day AS. Infliximab improves inflammation and anthropometric measures in pediatric Crohn's disease. *J Gastroenterol Hepatol*. 2010; 25:810–6. [PubMed: 20492339]
19. Walters TD, Gilman AR, Griffiths AM. Linear growth improves during infliximab therapy in children with chronically active severe Crohn's disease. *Inflamm Bowel Dis*. 2007; 13:424–30. [PubMed: 17206672]
20. Nelson JF, Karelus K, Felicio LS, Johnson TE. Genetic influences on the timing of puberty in mice. *Biol Reprod*. 1990; 42:649–55. [PubMed: 2346773]
21. Burns RC, Rivera-Nieves J, Moskaluk CA, Matsumoto S, Cominelli F, Ley K. Antibody blockade of ICAM-1 and VCAM-1 ameliorates inflammation in the SAMP-1/Yit adoptive transfer model of Crohn's disease in mice. *Gastroenterology*. 2001; 121:1428–36. [PubMed: 11729122]
22. Marini M, Bamias G, Rivera-Nieves J, Moskaluk CA, Hoang SB, Ross WG, Pizarro TT, Cominelli F. TNF-alpha neutralization ameliorates the severity of murine Crohn's-like ileitis by abrogation of intestinal epithelial cell apoptosis. *Proc Natl Acad Sci USA*. 2003; 100:8366–71. [PubMed: 12832622]
23. Bland JM, Altman DG. The logrank test. *BMJ*. 2004; 328:1073. [PubMed: 15117797]
24. Eckert KL, Wilson DM, Bachrach LK, Anhalt H, Habiby RL, Olney RC, Hintz RL, Neely EK. A single-sample, subcutaneous gonadotropin-releasing hormone test for central precocious puberty. *Pediatrics*. 1996; 97:517–9. [PubMed: 8632938]
25. DeBoer MD, Marks DL. Therapy insight: use of melanocortin antagonists in the treatment of cachexia in chronic disease. *Nat Clin Pract Endocrinol Metab*. 2006; 2:459–66. [PubMed: 16932335]
26. Kojouharoff G, Hans W, Obermeier F, Mannel DN, Andus T, Scholmerich J, Gross V, Falk W. Neutralization of tumour necrosis factor (TNF) but not of IL-1 reduces inflammation in chronic dextran sulphate sodium-induced colitis in mice. *Clin Exp Immunol*. 1997; 107:353–8. [PubMed: 9030875]
27. Bento AF, Leite DF, Claudino RF, Hara DB, Leal PC, Calixto JB. The selective nonpeptide CXCR2 antagonist SB225002 ameliorates acute experimental colitis in mice. *J Leukoc Biol*. 2008; 84:1213–21. [PubMed: 18653784]
28. Wong ML, Bongiorno PB, Rettori V, McCann SM, Licinio J. Interleukin (IL) 1beta, IL-1 receptor antagonist, IL-10, and IL-13 gene expression in the central nervous system and anterior pituitary during systemic inflammation: pathophysiological implications. *Proc Natl Acad Sci USA*. 1997; 94:227–32. [PubMed: 8990190]
29. Marshall JC, Kelch RP. Gonadotropin-releasing hormone: role of pulsatile secretion in the regulation of reproduction. *N Engl J Med*. 1986; 315:1459–68. [PubMed: 3097540]
30. Malik S, Wong SC, Bishop J, Hassan K, McGrogan P, Ahmed SF, Russell RK. Improvement in growth of children with Crohn disease following anti-TNF-alpha therapy can be independent of pubertal progress and glucocorticoid reduction. *J Pediatr Gastroenterol Nutr*. 2011; 52:31–7.

31. Bernstein M, Irwin S, Greenberg GR. Maintenance infliximab treatment is associated with improved bone mineral density in Crohn's disease. *Am J Gastroenterol.* 2005; 100:2031–5. [PubMed: 16128948]
32. DeBoer MD, Barnes BH, Stygles NA, Sutphen JL, Borowitz SM. Changes in inflammation and quality of life after a single dose of infliximab during on-going treatment: Differences between patients with and without IBD symptoms at time of administration. *J Pediatr Gastroenterol Nutr.* 2011 doi:10.1097/MPG.0b013e3182382ee3.
33. Rutgeerts P, Sandborn WJ, Feagan BG, Reinisch W, Olson A, Johanns J, Travers S, Rachmilewitz D, Hanauer SB, Lichtenstein GR, de Villiers WJ, Present D, Sands BE, Colombel JF. Infliximab for induction and maintenance therapy for ulcerative colitis. *N Engl J Med.* 2005; 353:2462–76. [PubMed: 16339095]

Fig. 1.

Weight, length, and food intake following colitis induction and food restriction. **a** Weight curve over the course of the experiment, day of life (DOL) 23–33. **b** Daily food intake. **c** 10-Day cumulative food intake during the experiment. **d** Linear growth determined as follows: (length at DOL 33)/(length at DOL 23). Animal numbers: Control + tumor necrosis factor (*TNF*)- α antibody (*ab*) group 10, dextran sodium sulfate (*DSS*) + Control *ab* group 15; *DSS* + *TNF*- α *ab* group 11. Error bars represent standard error of the mean. ** $p < 0.01$, *** $p < 0.001$, NS not significant ($p > 0.05$)

Fig. 2. Colonic cytology, acute inflammatory index, and chronic inflammatory index. Representative $\times 10$ magnification histological sections of colons from mice from the Control + TNF- α ab group (a), DSS + Control ab group (b), and DSS + TNF- α ab group (c); *bar* equals 0.25 mm. **d** Chronic inflammatory index scores. Animal numbers: Control + TNF- α ab group 10, DSS + Control ab group 15; DSS + TNF- α ab group 11. *** $p < 0.001$; *NS* not significant ($p > 0.05$). *Arrows* in **b** and **c** show areas of ulceration of colonic mucosa

Fig. 3.

Systemic inflammation. Serum measurement of cytokines at end of experiment (day of life 33). **a** Interleukin (*IL*)-6, **b** IL-1 β , **c** keratinocyte-derived chemokine (*KC*), **d** IL-12, **e** IL-10. Animal numbers: Control + TNF- α ab group 16, DSS + Control ab group 14; DSS + TNF- α ab group 21. Error bars represent standard error of the mean. * p < 0.05, *** p < 0.001, *NS* not significant (p > 0.05)

Fig. 4.

Timing of vaginal opening (VO). **a** Kaplan–Meier curve showing the proportion of mice without vaginal opening over time. DSS + TNF- α ab group versus DSS + Control ab group $p < 0.001$ as determined by log-rank test. **b** Mean age at vaginal opening as assessed by daily examination. Animals that had not experienced vaginal opening by the end of the experiment (day of life 33) were assigned a value of 34 days old. Animal numbers: Control + TNF- α ab group 10, DSS + Control ab group 15; DSS + TNF- α ab group 11. Error bars represent standard error of the mean. * $p < 0.05$, *** $p < 0.001$

Fig. 5.

Gonadotropin-releasing hormone (*GnRH*) stimulation test. Serum levels of luteinizing hormone (*LH*) (**a**) and follicle-stimulating hormone (*FSH*) (**b**) for DSS + Control ab and DSS + TNF- α ab groups both before and after stimulation with GnRH. Test performed at the end of the experiment (day of life 33). Animal numbers: DSS + Control ab group pre-GnRH 8 and post-GnRH 8; DSS + TNF- α ab group pre-GnRH 6, post-GnRH 10. * $p < 0.05$