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Longitudinal analysis of inflammation and microbiota dynamics in a model of mild chronic dextran sulphate sodium-induced colitis in mice

De Fazio L*et al*.A new murine model of colitis

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**Abstract**

**AIM:** To longitudinally characterize the inflammation and the gut microbiota trajectory in a mouse model of dextran sulphate sodium (DSS)-induced colitis.

**METHODS**: The method most commonly used to trigger colitis in animal models is based on oral administration of sulphated polysaccharides called DSS. The murine DSS colitis model has been widely adopted to cause severe acute, chronic or semi-chronic colitis, and has been validated as an important model for the translation of mice data to human IBD. Nevertheless, it is now clear that models characterized by mild intestinal damage are much more accurate for studying the effects of therapeutic agents. For this reason, we have developed a murine model of mild colitis to longitudinally study inflammation and microbiota dynamics during the intestinal repair processes, to obtain data suitable to support the recovery of gut microbiota-host homeostasis.

**RESULTS**: All plasma cytokines evaluated, except IL-17, started to increase (*P* < 0.05), after 7 d of DSS administration. IL-17 started to increase only 4 d after DSS withdrawal. IL-1β and IL-17 continue to increase during the recovery phase even when clinical signs of colitis had disappeared. IL-6 and IL-10 and IFN-γ reached a maximum 4 d after DSS withdrawal and decreased during the late recovery phase. TNFα reached a peak (3 fold increase, *P <* 0.05), after which it slightly decreased to increase again close to the end of the recovery phase. DSS administration prompted profound and rapid changes in the mice microbiota. After 3 d of DSS administration, we observed a major reduction of Bacteroidetes/Prevotella and a corresponding increase in Bacillaceae with respect to control mice. In particular, Bacteroidetes/Prevotella decreased from a relative abundance of 59.42 to 33.05%, while Bacillaceae showed a concomitant increase from 2.77% to 10.52%. Gut microbiota rapidly shifted toward a healthy profile during the recovery phase and returned normal 4 d after DSS withdrawal. Cyclooxygenase 2 (COX-2) expression started to increase 4 d after DSS withdrawal (*P <* 0.05), when dysbiosiswas recovered, and continued to increase during the recovery phase. All together, these data indicate that a chronic phase of intestinal inflammation, characterized by the absence of dysbiosis, can be obtained in mice by using a single DSS cycle.

**CONCLUSION**: Dysbiosis contributes to the local and systemic inflammation occurring in the DSS model of colitis, but chronic bowel inflammation is maintained even after recovery from dysbiosis.

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**Key words:** Colitis, Dysbiosis;Dextran sulphate sodium; Inflammation; Cyclooxygenase 2

**Core tip:** Experimental animal models of colitis are of primary importance for investigating the physiopathological mechanisms underlying inflammatory bowel disease (IBD) in humans. Murine dextran sulphate sodium colitis models have been widely adopted and have been validated as relevant models for the translation of mice data to human IBD. Nevertheless, it is now clear that models characterized by mild intestinal damages are much more accurate for studying the effects of therapeutic agents. In this study we have developed a reproducible mild chronic colitis model which allows the evaluation of intestinal repair processes, the modulation of systemic inflammation and the recovery of the gut microbiota homeostasis.

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**INTRODUCTION**

Inflammatory bowel disease (IBD), comprising Crohn's disease (CD) and ulcerative colitis (UC), is more common in the populations of developed countries. Assessment of the efficacy of novel and adjunct IBD therapies requires experimental animal models resembling human IBD. There is no ideal animal model for IBD, and myriad methods have been designed to induce colitis in mice, rats and other animals. Dextran sulphate sodium (DSS)-induced colitis is one of the most commonly used models. DSS-colitis reflects many of the clinical features of UC[1-3]. For example, acute, chronic or relapsing colitis can easily be induced by changing the DSS concentration or administration cycles. Moreover, the dysplasia frequently occurring after the chronic phase of DSS colitis resembles the clinical course of human UC[4]. Recent reports have focused on the multifunctional role of DSS for *in vivo* colitis modelling[5]. The most widely used DSS murine model treats animals with 3% DSS in their drinking water for seven days. This provides a model of acute intestinal injuries that allow clinical monitoring of colitis using parameters such as weight loss, stool consistency and blood in the stool. Together these parameters yield an average clinical score that is a powerful comparable number for identifying potential differences among groups for the total experimental time. However, the devastating intestinal injuries caused by 3% DSS remain for up to ten days after DSS administration. Therefore they do not provide a sensitive system to evaluate the role of therapeutic agents with different effectiveness[5], or the role of the various parameters involved in intestinal repair. By contrast, the milder seven-day 1% DSS treatment model seems to be a powerful means of evaluating the effect of most therapeutic agents and the repair phase of colitis. However, the 1% DSS model would not be characterized as a disease according to traditional disease activity indices and hence prevents clinical monitoring[5]. Histopathology, with quantification of morphological and immunological changes in the colon during and after 1% DSS treatment, is necessary to identify differences among groups.

The molecular events taking place after DSS ingestion and leading to established colitis are not completely understood, but are of primary importance to understand the strengths and weaknesses of this model. DSS is a sulphated polysaccharide with a variable molecular weight (MW) ranging from 5 to 1400 kDa. DSS is rapidly depolymerized in the stomach, to reach the cecum with a MW between 750 and 5000 Da, and it is reasonable to assume that these smaller sulphated polysaccharides are responsible for colon damage[6]. Hence, the MW of DSS is considered a major factor in the induction of colitis. The most severe colitis was obtained in BALB/c mice using DSS with a MW of 40 kDa, while higher or lower MW resulted in milder forms of colitis[7]. For this reason, some companies have developed DSS specifically designed for the induction of colitis and these specific products are strongly recommended to obtain much more repeatable results. DSS metabolism in the gut also involves the formation of complexes between DSS fragments and medium-chain fatty acids (MCFA), enriched in the large bowel[8]. The high toxicity of DSS-MCFA complexes explains why only the large bowel, and especially the terminal colon, is inflamed by the DSS moieties. Once entered into colonocytes, DSS impairs major cellular functions by inhibiting the activity of cellular enzymes such as ribonuclease[8] and iNOS[9] and ultimately causing cell cycle arrest and apoptosis in colonocytes[6] and probably also in other colonic wall cells. By interfering with intestinal barrier function, DSS is also able to stimulate local and systemic inflammation by locally increasing cyclooxygenase-2 (COX-2) expression and by secreting of a variety of cytokines and other inflammatory mediators that spread from the colon to the blood[10].

The importance of the microbiota and microbe-mucosa crosstalk in the pathogenesis of IBD is supported by several animal model studies. Colitis severity is dependent on the commensal bacterial strains maintained in gnotobioticanimals[11], and DSS treatment has been associated with a major shift in the composition of the intestinal microbiota, whose dynamics rapidly shifts toward an unhealthy state[12-14]. Moreover, antibiotic administration has been shown to improve both IBD and DSS-induced colitis[15], indicating that the microbiota plays a critical role in this disease as well as in the DSS model system. Evidence supporting this view is the finding that the simple ingestion of lysate of microbial cells belonging to Firmicutes, considered a healthy-type phylum, reduces DSS-induced experimental colitis in mice[14].

The present study is aimed to longitudinally characterize the inflammation and the gut microbiota trajectory in a highly sensitive DSS-induced murine model of colitis.

**MATERIAL AND METHODS**

***Animal treatment***

Of 24 male 8-week-old C57BL/6 mice were purchased from Charles River Laboratories (Lecco, Italy). Animals were housed in a controlled environment in collective cages at 22 ± 2 °C and 50% humidity, under a 12-h light/dark cycle. Mice were allowed to acclimate to these conditions for at least 14 days before inclusion in experiments and had free access to food and water throughout the study. Colitis was induced in 12 mice by oral administration of dextran sulphate sodium (DSS for colitis, TdB Consultancy, Sweden, MW 40000). DSS was added at a concentration of 1.5% in tap water. DSS–tap water was freshly prepared every day and administered to the mice for 9 d (day 0-9), followed by 21 d of tap water (day 10-29). The average amount of DSS taken was recorded daily. The control group (*n =* 12) received only tap water. The experimental design is schematized in Figure 1. The experiment, approved by the institutional review board of the University of Bologna and performed according to Italian and European guidelines, was repeated 3 times.

***Disease activity index***

The disease activity index (DAI) was calculated by the combined score of weight loss, stool consistency and bleeding, as detailed in Table 1. All parameters were scored from day 1 to day 29.

***Histological evaluation of colitis***

Mice (*n =* 2) were anesthetized and sacrificed by cervical dislocation on day 3 (after 3 d of DSS intake), 7 (after 7 d of DSS intake), 13 and 19. The colon was excised, rinsed with saline solution, fixed in 4% formalin and embedded in paraffin. Of 4 μm sections were stained with haematoxylin–eosin and observed for histological assessment of epithelial damage by a pathologist in a blinded manner.

***Determination of plasma cytokine levels***

Blood samples (200 uL) were taken from the tail vein on day 3, 7, 13, 19, and 29. Blood, collected in eppendorf tubes containing sodium citrate, was centrifuged at 1000 RPM for 10 minutes, and plasma was collected and stored at -80°C until BioPlex analysis. Cytokine levels were determined using a multiplexed mouse bead immunoassay kit (Bio-Rad, CA, United States). The 6-plex assays (IL-1α, IL-6, IL-10, IL-17A, IFN-γ, TNFα) were performed in 96-well filter plates as previously described[16], following the manufacturer’s instructions. Microsphere magnetic beads coated with monoclonal antibodies against the different target analytes were added to the wells. After 30 min incubation, the wells were washed and biotinylated secondary antibodies were added. After incubation for 30 min, beads were washed and then incubated for 10 min with streptavidin-PE conjugated to the fluorescent protein, phycoerythrin (streptavidin/phycoerythrin). After washing, the beads (a minimum of 100/analyte) were analyzed in the BioPlex 200 instrument (BioRad). The concentrations of the samples were estimated from the standard curve using a fifth-order polynomial equation and expressed as pg/ml after adjusting for the dilution factor (Bio-Plex Manager software 5.0). Samples below the detection limit of the assay were recorded as zero. The intra-assay CV averaged 15%.

***RNA extraction and real-time PCR***

Total RNA from colon samples was extracted using Trizol® reagent (Life Technologies, CA, United States) according to the manufacturer’s instructions. Extracted RNA samples were treated with DNase I to remove any genomic DNA contamination using DNA-free kit (Ambion, United States) and reverse-transcripted using RevertAid™ First Strand cDNA Synthesis Kit (Fermentas, Canada). COX-2 and β-actin mRNAs were reverse-transcribed using random hexamer primers (Fermentas, Canada). COX-2 and β-actin mRNA levels were analysed by real-time PCR using SYBR® Select Master Mix (Life Technologies, CA, United States) and StepOnePlusTM system (Applied Biosystems, CA, United States) according to the manufacturer’s instructions. The melting curve data were collected to check PCR specificity. Each cDNA sample was analysed as triplicate. COX-2 mRNA levels were normalized against β-actin mRNA and relative expressions were calculated using the 2-2ΔCt formula. COX-2 primer pair: 5’- TTC TCT ACA ACA ACT CCA TCC TC -3’ and 5’- GCA GCC ATT TCC TTC TCT CC -3’ (247 bp product); β-actin primer pair: 5’- ACC AAC TGG GAC GAC ATG GAG -3’ and 5’- GTG GTGGTG AAG CTG TAG CC -3’ (380 bp product).

***Immunohistochemistry***

Tissue sections (4 um) were mounted on slides, sections were deparafﬁnised with xylene and rehydrated through a series of graded alcohols, then were and incubated overnight at 4 C with anti-COX-2 antibody (Cayman Chemicals, Ann Arbor, MC, United States) at a 1:200 dilution in PBS/BSA-1.5%. In control slides, the primary antibody was omitted. Sections were then incubated with secondary anti-rabbit antibody for 15 min at room temperature and then in 3,3-diaminobenzidine tetrahydrochloride (DAKO) for 1 min. Sections were counterstained with haematoxylin.

***Characterization of the intestinal microbiota by HTF-Microbi.Array***

The intestinal mice microbiota was characterized using the fully validated diphylogenetic DNA microarray platform HTF-Microbi.Array[17].Targeting 33 phylogenetically related groups, this LDR-based Universal Array covers up to 95% of the mammalian gut microbiota[18]. Gut microbiota analysis was performed at day 3, 7, 9, 13 and 19. Total DNA from faecal material was extracted using the QIAamp DNA Stool Mini Kit (Qiagen) according to the modified protocol previously reported[17]. Final DNA concentration was determined using NanoDrop ND-1000 (NanoDrop Technologies). A nearly full-length portion of 16S rDNA gene was amplified using universal forward primer 27F and reverse primer 1492R, according to the protocol previously described[17]. PCR amplifications were performed in a Biometra Thermal Cycler T Gradient (Biometra, Göttingen, Germany). PCR products were purified using the High Pure PCR CleanupMicrokit (Roche, Mannheim, Germany), eluted in 30 µl of sterile water and quantified with NanoDrop ND-1000. Slide chemical treatment, array production, LDR protocol and hybridization conditions were performed as previously reported[19, 20]. Briefly, LDR reactions were carried out in a final volume of 20 µl containing 500 fmol of each LDR-UA HTF-Microbi.Array probe[18], 50 fmol of PCR product and 25 fmol of the synthetic template (5’-AGCCGCGAACACCACGATCGACCGGCGCGCGCAGCTGCAGCTTGCTCATG-3’). LDR products were hybridized on Universal Arrays, setting the probe annealing temperature at 60°C. All arrays were scanned and processed according to the protocol and parameters already described[17]. Fluorescence intensities were normalized on the basis of the synthetic ligation control signal[18]. The relative abundance of each bacterial group was obtained by calculating the relative fluorescence contribution of the corresponding HTF-Microbi.Array probe as a percentage of the total fluorescence.

***Statistical analysis***

Al data are expressed as mean ± SEM of at least 3 independent determinations. Statistical differences between groups were determined by one-way ANOVA by using GraphPad Prism 6 (GraphPad Software Inc., San Diego, CA, USA). Differences were considered statistically significant at *P <*0.05.

**RESULTS**

***Colitis activity indexes***

Mice started to show mild clinical signs of disease after the end of the 1.5% DSS treatment (day 9) due to the simultaneous increase in stool consistency and bleeding index (maximum DAI score = 2). The most evident clinical signs were recorded between day 11 and 15 (Figure 2) with a significant weight loss that peaked between day 9 and 12 (see Figure 3) with a maximum DAI score = 7.

***Histological evaluation of colitis***

Histological evaluation of the colon was made from the colocaecal junction to the anus. Overall, the tissue damage tended to be limited to the terminal colon and rectum regions, and can be classified as mild to moderate colitis (Figure 4). After 3 d of DDS treatment (Figure 4B), the colon mucosa appeared normal, except for focal inflamed areas in which we observed leukocyte infiltration with a prevalence of granulocytes, indicating active inflammatory colitis, and solid lymphatic follicles. After 7 d of DDS treatment (Figure 4C), the terminal colon mucosa showed the same features of spotted focal leukocyte infiltrations, with a prevalence of lymphocytes, showing the colitis had shifted toward a chronic status, with mucus discharge and architectural abnormalities and depletion of goblet cells. At day 13, (Figure 4D), the lymphocyte infiltration was widespread and epithelial damage was evident, with complete crypt disappearance and mucosal erosion in some areas and a mild thickening of the muscularis mucosa. During weight recovery, at day 19 (Figure 4E), leukocyte infiltration tended to return to be focalized. Mucosal erosions were still evident, with flattened villi. At day 29 (Figure 4F), much focal infiltration, also involving glands, was still present with villi that still appeared flattened.

***Inflammatory cytokine profile of colitis***

All cytokines evaluated, except IL-17, started to increase after 7 days of DSS administration (Figure 5). The IL-17 plasma level started to increase at day 13. IL-1β (Figure 5A) had a sudden 4-fold increase at day 13, when colitis reached the maximum DAI score, and slightly increased until day 29, when clinical signs of colitis had disappeared but IL-1β reached its peak plasma level. IL-6 (Figure 5B) started to increase at day 7 and reached a peak at day 13 (4.5 fold increase), after which it decreased to reach a value twice the normal level at the end of the experiment (day 29). IL-10 (Figure 5C) reached a peak at day 13 (4.2 fold increase), after which it decreased to resume a physiological level at the end of the experiment (day 29). IL-17 (Figure 5D) started to increase at day 13 and continuously increased to peak at day 29 (3-fold increase) at the end of the experiment. IFN-γ (Figure 5E) increased at day 7, reaching a peak at day 13 (6 fold increase), after which it rapidly decreased to resume a physiological level at the end of the experiment (day 29). Finally, TNFα (Figure 5F) increased at day 7, reaching a peak at day 13 (3 fold increase), after which it slightly decreased at day 19 to increase again to the peak level at the end of the experiment (day 29). At the end of the recovery (day 29), IL-1b, IL-17 and TNF are at their maximum levels, indicating that colitis has become chronic.

***Colitis induces COX-2 overexpression in colonocytes and colon wall***

Since COX-2 plays a crucial role in the production of many lipid mediators involved in intestinal inflammation and since it is one of the targets of IBD pharmacological therapy, we analysed COX-2 mRNA expression in colon tissue during DSS-induced colitis. Interestingly, COX-2 mRNA start to increase (2 fold) only 5 d after DSS removal and continue to increase (up to 7 fold) until the end of the recovery phase, a trend in agreement with the chronicization of the inﬂammatory process (Figure 6).

***Intestinal microbiota modifications induced by colitis***

To characterize the trajectory of the gut microbiota in our DSS murine model of mild colitis, the temporal dynamics of the faecal microbiota of DSS-treated mice was compared with that of healthy controls. In particular, for DSS-treated mice the gut microbiota was characterized in control mice and at day 3, 7, 9, 13, 19 (Figure 7). The faecal microbiota of healthy control mice was sampled at the same time points. According to our data, DSS administration prompted profound and rapid changes in the mice microbiota (Figure 7). After 3 days of DSS treatment we observed a major reduction of Bacteroidetes/Prevotella and a corresponding increase in Bacillaceae with respect to control mice. In particular, Bacteroidetes/Prevotella decreased from a relative abundance (rel.ab.) of 59.42% to 33.05%, while Bacillaceae showed a concomitant increase from 2.77% to 10.52%. During the course of colitis, from day 7 to day 9, there was a progressive increase in the rel.ab. ofBacillaceae (from 10.52% to 17.90%), Lactobacillaceae (from 2.07% to 6.55%), Verrucomicrobiae (from 0.82% to 1.07%), Enterococcales (from 1.52% to 2.07%) and Enterobacteriaceae (from 0.66% to 1.18%) and a parallel progressive reduction of members of the Clostridium cluster XIVa (from 23.79% to 7.19%). On the other hand, the progression of colitis did not affect Bacteroidetes/Prevotella, which remained constant at the low rel.ab value detected 3 dafter DSS administration. Unlike DSS-treated mice, healthy control mice showed a constant gut microbiota profile throughout the study. Interestingly, during the recovery from DSS-induced colitis, at day 13 and 19, we observed a rapid shift of the gut microbiota toward a healthy profile, comparable to that shown by healthy control mice. In particular, 5 d after the interruption of DSS administration, the mice microbiota recovered a rel.ab. value of Bacteroidetes/PrevotellaandBacillaceae analogous to that observed in healthy controls (71.08 and 1.79%, respectively).

**DISCUSSION**

The DSS murine model described here induces a milder colitis than the classical 3% DSS model, with a mortality rate very close to zero. Moreover, the signs and symptoms of colitis induced by this model are much more homogeneous in all the treated mice. We are aware that the responses to DSS observed in laboratory animals do not only depend on DSS type and treatment protocol[21] but this model, in our hands, has proven to be highly reproducible.

Histological examination of the colon of 1.5% DSS-treated mice showed that the mucosal damage starts to be evident 6 days before the DAI starts to increase. This early damage was limited to the terminal colon mucosa and ascended toward the proximal colon when colitis severity increased. Colitis showed peak histological damage at day 13, in association with the maximum DAI. Histological damage remained evident even when the DAI score had returned to zero and the weight loss had been completely recovered. The persistence of histological damage, lymphocyte infiltration and COX-2 overexpression after complete clinical recovery (day 29) mimics the features of chronic UC in humans. The histological changes in this model are much easier to follow with respect to those observed in the 3% DSS model in which epithelial architecture is constantly lost for many days[5].

Circulating cytokine levels are indicative of the whole inflammatory profile. While studies in IBD patients have focused on serum cytokines, most investigations in mice models tended to evaluate tissutal cytokines, losing information on systemic inflammation. Circulating IL-1, IL-6, IL-17 and TNF play a key role in the pathogenesis of IBD[22]. IL-1 and IL-6 levels are in line with IBD activity. IL-17 is a delayed-type immune reaction cytokine produced by Th17 and by CD8+ T cells during chronic inflammation. Even if its role in IBD remains controversial[23], it seems to have a prominent pro-inflammatory role in the DSS model[24, 25]. IL-10 is the most important antinflammatory cytokine in humans. Its role has been extensively studied in IL-10 knockout mice and IL-10 mRNA expression in the inflamed mucosa is increased in UC patients but decreased in CD patients[26, 27]. IFN secretion has been linked to IL-17 secretion[28] in experimental colitis and its relative mRNA expression transiently increases during DSS-induced acute colitis, with a peak close to the maximum DAI score[5]. TNF is a master cytokine in IBD pathogenesis and its orchestrating role in colonic inflammation is verified by the efficacy of anti-TNF therapy in IBD[29]. The serum TNF level correlates with clinical activity both in UC and CD[29]. Alex and collaborators[30] reported that acute DSS colitis in mice significantly increases circulating IL-1, TNF, IL-6 and IL-17, while chronic colitis increases IL-4, IL-10, IL-6 and IFN, but they only analyzed a single time point for each condition. In our model, while IL-6, IFN and IL-10 peaked at day 13 (maximum DAI score) and decreased during the recovery of colitis, IL-1, IL-17 and TNF levels remained high even when the symptoms and signs of colitis had disappeared. So, while circulating IL-6, IFN and IL-10 levels seem to correlate with the major clinical signs, IL-1, IL-17 and TNF mainly correlate with the histological damage that tends to become chronic.

DSS (2%–5%) administration for 5 up to 7 days has been used to develop an acute form of colitis while an inflammatory condition reminiscent of human chronic IBD can be induced by repeated DSS cycles[31]. Our model allows obtaining a chronic colitis by using a single DSS cycle.

To our knowledge, this is the first study to characterize the gut microbiota trajectory in a mouse model of DSS-induced colitis. In particular, the longitudinal approach allowed the assessment of microbiota changes immediately after the induction of colitis, during the course of disease progression and during the recovery phase. According to our data, the induction of colitis rapidly compromises the homeostasis of the gut microbial ecosystem, leading to a dramatic reduction of Bacteroidetes/Prevotella, a major mutualistic group of the mice gut microbiota, and a corresponding increase in Bacillaceae. Confirming these findings, a rapid decrease in Bacteroidetes has already been observed in mice models of DSS-induced colitis[32]. Moreover, disease progression in our model was flanked by a gradual, but weak, increase in the pro-inflammatory gut microbiota components Enterococcales and Enterobacteriaceae, the minor symbiotic member Lactobacillaceae, and the mucus-degrading Verrucomicrobiae Akkermansia muciniphila. On the other hand, during the progression of colitis we also noted a gradual decrease in members of the Clostridium cluster XIVa. Asa major component of a healthy gut microbiota, this cluster is involved in the production of short-chain fatty acids, microbial metabolites essential for several aspects of the host physiology: nutrition, immune modulation and protection from pathogen colonization[33]. Taken together, these data demonstrate a progressive impairment of the gut microbiota with advancing colitis, resulting in a dysbiotic profile which can violate mutualism and support the disease. Interestingly, at the end of DSS administration, during weight recovery, we observed a rapid shift of the gut microbial community toward a healthy profile. Within two days after the end of DSS administration, the mice gut microbiota showed rel.ab. values of Bacteroidetes/Prevotella, Bacillaceae, Enterococcales, Enterobacteriaceae, Lactobacillaceae, Verrucomicrobiae and Clostridium cluster XIVa analogous to those observed in healthy controls. These data demonstrate the high degree of resilience of the gut microbiota that shows the potential for rapid recovery of its healthy mutualistic profile after DSS-induced dysbiosis.

One of the unanswered questions regarding IBD and DSS-induced colitis is to establish to what extent the dysbiosis is a contributory cause of the local and systemic inflammation especially during the recovery phase. Dysbiosis can cause increased mucus secretion[7] and exacerbate intestinal inflammation that further contribute to the microbiota shifts. In DSS-colitis, microbiota homeostasis is rapidly compromised. After 3 d of DSS treatment the microbiota is profoundly changed, and this alteration is maintained during DSS administration. When the maximum DAI is reached, the microbiota is returning to a healthy composition. Thus, dysbiosis precedes the systemic inflammation that starts to increase after 7 d of DSS treatment, reaching its maximum when the maximum DAI is reached and remaining high until the late recovery phase.

COX-2 is a very good marker of colonocytes and colon mucosa inflammation. Its expression in the colon of DSS treated mice starts to increase only when the maximum DAI is reached and remains very high until the late recovery phase. The 10 days delay between the dysbiosis and the increased COX-2 expression in colonocytes suggests that dysbiosis alone is not capable to trigger COX-2 expression. On the other hand, the recovery from the dysbiosis is not sufficient to ameliorate the inflammatory profile of DSS colitic mice, nor their colonocytes inflammation.

These results emphasize that the microbiota certainly contributes to intestinal inflammation, but also that the pro-inflammatory response elicited by DSS in the colon wall continues even when dysbiosis is recovered. It is therefore reasonable to sustain that the observed deviations in the gut microbiota structure can foster changes in cytokine expression[34]. However, the interactions between microbiota and the immune system are very complicated and they remain to a great extent to be elucidated[35]. More studies are needed to be able to draw conclusions regarding this point.

The overexpression of COX-2 in colonocytes associated with leukocyte mucosal infiltration creates a pro-inflammatory loop from which it is difficult to get out. Moreover, the circulating IL-10, one of the major anti-inflammatory cytokines, decreases during the recovery phase until it returns to basal levels at the end of the recovery, when both COX-2 expression and circulating IL-1, IL-17 and TNFα are at their maximum levels. It is very likely that this kind of pro-inflammatory loop, which is responsible for the chronicity of the DSS-induced colitis, is also activated in UC patients.

***Concluding remarks***

Decreasing the DSS concentration to 1.5% and increasing the treatment's duration to 9 days induces chronic colitis with a short milder acute phase, followed by a mild chronic active disease. This mild disease is a much more accurate condition for studying the dynamics of colitis during clinical remission. This model also represents a step forward in reducing the suffering of animals and, given the very low mortality rate, it allows to reduce the number of animals necessary to obtain statistically significant results.

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**COMMENTS**

***Background***

Progress in understanding the molecular basis of inflammatory bowel disease (IBD) in humans has accelerated, thanks to the generation of animal models of colitis. Experimental colitis allows to study complex physiophatological mechanisms which cannot be simulated *in vitro* or *in silico*. The most commonly-used method to trigger colitis in animal models is based on oral administration of a sulphated polysaccharide called dextran sulphate sodium (DSS). This model has been validated as a relevant model for the translation of mice data to human inflammatory bowel diseases.

***Research frontiers***

The aetiology of IBDs still remains largely unknown, and their prevalence is increasing in developed countries, with a total number of IBD patients nowadays estimated to be between 1 and 1.5 million in the United States. A genetic basis for IBD has long been recognized, due to the increased familial risk. However, significant discordance for Crohn’s disease (CD) in twins, and a much less robust phenotypic concordance for ulcerative colitis (UC), suggest that environmental factors play a major role in IBDs pathogenesis. Among these, the gut microbiota seems to have a crucial role in CD and UC, since an altered immune response to the normal microbiota has been discovered to be a common feature in IBD patients.

***Applications***

This study represents a step forward in view of using the DSS model in preclinical studies. It describes new experimental procedures useful to dissect the role of microbiome-immune system interactions in colitis pathogenesis and to evaluate new possible IBDs treatments.

***Terminology***

IBDs, including CD and UC, are chronic inflammatory disorders of the intestine. DSS is a synthetic sulphated poly- saccharide composed of dextran with sulphated glucose. It is capable of triggering the development of colitis in mice by binding to medium-chain-length fatty acids present in the colon and inducing in this way inflammation.

***Peer review***

The study is well designed and very interesting for evaluating treatments for ulcerative colitis with mild to moderate activity. It describes a new murine model of colitis, based on the administration of 1.5% DSS. The results are highly interesting having a strong potential for being used as a benchmark for further studies that evaluate possible treatments of colitis.

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**Figure 1 Experimental design of the study**. Faeces, blood and tissue collection are indicated (dark blue) in the grid.

**Figure 2 Disease activity index score of colitis in 1.5% dextran sulphate sodium-treated mice.** At day 28, an average disease activity index (DAI)score of 0.5 is still present.

**Figure 3 Weight loss in dextran sulphate sodium-treated mice**. The maximum weight loss (11%) was recorded between days 9 and 12. Weight recovery ends at days 19-20.

**Figure 4 Differences in histological parameters during experimental colitis**. Colons were collected from mice on day3 (B), 7 (C) 13 (D), 19 (E) and29 (F). Histopathological changes in individual crypts are shown in representative hematoxylin and eosin-stained sections. Loss of crypt architecture associated with epithelial damage and flattened villi (red arrows) and leukocyte infiltration (black arrows) are evident following DSS treatment (bar = 200 m).

**Figure 5 Plasma cytokine variations during experimental colitis.** Data are expressed as mean ±SEM of at least three animals. a*P*≤0.05 with respect to controls.

**Figure 6 Evaluation of COX-2 mRNA during dextran sulphate sodium-induced colitis.** On day 3, 7, 13, 19 and 29 colon tissue was collected and processed for real-time PCR. The COX-2 mRNA significantly increases during the recovery phase (A).a*P*≤0.05. Immunohistochemistry analysis confirms that COX-2 expression is limited to the apical mucosa in healthy mice (B) while it is increased and spread over the entire thickness of the mucosa at day 29 (C).

**Figure 7 Temporal dynamics of the faecal microbial community of dextran sulphate sodium-treated mice and healthy controls.** Cl: *Clostridium* cluster.

**Table 1 Disease activity index score parameters**

|  |  |  |
| --- | --- | --- |
| **Stool consistency** | **Bleeding** | **Weight loss** |
| 0 = Formed | 0 = Normal color stool | 0 = No weight loss |
|  |  |  |
| 1 = Mild-soft | 1 = Brown color | 1 = 5%-10% weight loss |
|  |  |  |
| 2 = Very soft | 2 = Reddish color | 2 = 11%-15% weight loss |
|  |  |  |
| 3 = Watery stool | 3 = Bloody stool | 3 = 16%-20% weight loss |
|  |  |  |
|  |  | 4 ≥ 20% weight loss |