Oral administration of dextran sodium sulphate induces a caecum-localized colitis in rabbits

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Abstract: Trichuris suis ova (TSO) have shown promising results in the treatment of inflammatory bowel disease (IBD) but the mechanisms which underlies this therapeutic effect cannot be studied in mice and rats as T. suis fails to colonize the rodent intestine, whilst hatching in humans and rabbits. As a suitable rabbit IBD model is currently not available, we developed a rabbit colitis model by administration of dextran sodium sulphate (DSS). White Himalayan rabbits (n = 12) received 0.1% DSS in the daily water supply for five days. Clinical symptoms were monitored daily, and rabbits were sacrificed at different time points. A genomewide expression analysis was performed with RNA isolated from caecal lamina propria mononuclear cells (LPMC) and intestinal epithelial cells (IEC). The disease activity index of DSS rabbits increased up to 2.1 ± 0.4 (n = 6) at day 10 (controls <0.5). DSS induced a caecum-localized pathology with crypt architectural distortion, stunted villous surface and inflammatory infiltrate in the lamina propria. The histopathology score reached a peak of 14.2 ± 4.9 (n = 4) at day 10 (controls 7.7 ± 0.9, n = 5). Expression profiling revealed an enrichment of IBD-related genes in both LPMC and IEC. Innate inflammatory response, Th17 signalling and chemotaxis were among the pathways affected significantly. We describe a reproducible and reliable rabbit model of DSS colitis. Localization of the inflammation in the caecum and its similarities to IBD make this model particularly suitable to study TSO therapy in vivo.

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Oral administration of dextran sodium sulphate induces a cecum localized colitis in rabbits

Short title: DSS model of colitis in rabbit

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Summary

Background: The therapeutic effect and the underlying mechanisms of the helminth species *T. suis*, that has shown initially promising results for treatment of inflammatory bowel disease (IBD) but more recently no benefit in Crohn's disease in human studies, cannot be studied in mice and rats as *T. suis* fails to colonize the rodent intestine whilst hatching in humans and rabbits. Since a suitable rabbit IBD model is currently not available, we developed a rabbit colitis model by administration of dextran sodium sulphate (DSS).

Methods: White Himalayan rabbits (n = 12) received 0.1 % DSS in the daily beverage for 5 days. Clinical symptoms were monitored daily and rabbits were sacrificed at different time points. A genome wide expression analysis was performed with RNA isolated from cecal lamina propria mononuclear cells and epithelial cells.

Results: The disease activity index of DSS rabbits increased up to 2.1 ± 0.4 (n = 6) at day 10 (controls < 0.5). DSS induced a cecum localized pathology with crypt architectural distortion, stunted villous surface and inflammatory infiltrate in the lamina propria. The histopathology score reached a peak of 14.2 ± 4.9 (n = 4) at day 10 (control animals 7.7 ± 0.9, n = 5). Expression profiling revealed an enrichment of IBD related genes in both lamina propria mononuclear cells and intestinal epithelial cells. Innate inflammatory response, Th17 signalling and chemotaxis were among the significantly affected biologically relevant pathways.

Conclusions: We describe a reproducible and reliable rabbit model of colitis by administration of DSS. Localization of the inflammation in the cecum and its similarities with IBD make this model suitable to study *T. suis* therapy in vivo.

Keywords: rabbit model of inflammatory bowel disease; Crohn's disease; Ulcerative colitis; DSS colitis; *Trichuris suis*; RNA sequencing.

Introduction

Inflammatory bowel diseases (IBD) can be defined as a "post-industrial revolution epidemics" since their frequency has increased dramatically in the last 60 years (Molodecky*, et al.*, 2012). Initially, this increase was explained on the basis of the hygiene hypothesis that linked the improved hygienic conditions and the consequent reduction of childhood-infections with an increase in the prevalence and incidence of immune-related diseases (Strachan, 1989). Today, it is assumed that the increase in hygiene standards reduces the interactions with microorganisms that coevolved with the immune system and influences the balance between immune-regulatory and effector mechanisms (Rook, 2011).

In 2000, Elliot and colleagues focused their attention on the complementarity between the distribution of IBD and of helminth infections (Elliott*, et al.*, 2000)·(Weinstock*, et al.*, 2002). In their work, they set the basis for the clinical application of a helminth therapy and proposed the whipworm parasite *Trichuris suis* as a therapeutic agent (Summers*, et al.*, 2003). Overall, the treatment with *T. suis* ova (TSO) proved to be safe with only mild and transient gastrointestinal effects reported (Scholmerich, 2013). Those early results for efficacy in IBD were promising, however, two recent large multicenter trials in mild to moderate Crohn's disease with our without immunosuppression could not demonstrate a significant benefit of TSO treatment over placebo. Further clinical trials in ulcerative colitis are still under discussion. a,. So far, most studies have focused on the clinical efficacy and safety and the mechanisms underlying the TSO treatment effects remain unsolved. The discussion on whether further clinical studies should be undertaken (i.e. in ulcerative colitis) has given rise to a request for a better understanding of potential mechanisms and thus establishment of suitable animal models.

Animal models of colitis are essential for the understanding of the aetiology and pathophysiology of IBD and constitute an essential tool in the development of new therapies. Currently, more than 66 different IBD models have been developed in several species

including mouse, rat, rabbit and tamarin (Wirtz and Neurath, 2000). Generally, IBD models can be subdivided in four categories of experimental colitis depending on the method of induction: congenital, genetically engineered, chemically induced, and cell transfer induced (Mizoguchi, 2012).

Most methods are used successfully in both mouse and rat. Unfortunately, research on the therapeutic application of *T. suis* in model organisms is complicated by the unsuccessful hatching of the ova in the mouse and rat intestine (un-published data). In contrast, the life cycle of these parasites in the human and rabbit intestine is similar. In both hosts *T. suis* hatch and establish in the distal intestine region where the larvae seem to die prematurely without reaching sexual maturity (un-published data). Therefore, a colitis model in the rabbit would be a valuable tool to investigate the mechanisms underlying *T. suis* therapy.

Thus far, the established rabbit IBD models present some drawbacks that limit their use for translational research. Rectal application of acetic acid causes severe acute inflammation, ischemia and erosion within one day post application, but fails to induce chronic inflammation (Hathaway*, et al.*, 1999)·(Murthy, 2006). Similarly, trinitrobenzene sulfonic acid (TNBS) dissolved in ethanol is also applied in the rectum. Within a week after application, TNBS induces a fully developed inflammation that presents ulcerative lesions and trans mural inflammation (Anthony*, et al.*, 1995, Anthony*, et al.*, 2007). However, the development of chronic inflammatory lesions in the TNBS model is highly variable and does not warrant good reproducibility (Knollmann*, et al.*, 2002). Furthermore, the short-term and self-limiting nature of these colitis models is poorly adequate to study the relapsing and remitting course of IBD. Both acetic acid and TNBS are introduced as an enema in the rabbit rectum and induce an inflammation that is usually confined to the distal colon, whereas *T. suis* ova hatch and develop in the ileum and cecum. Further colitis models in rabbits are of limited use either because of the complicated induction procedure (Hodgson*, et al.*, 1978)·(Hotta*, et*

al., 1986) or because of the high variability of the induced pathology(Watt and Marcus, 1970).

Thus a novel colitis model in the rabbit that allows to study the mechanisms underlying the therapeutic effects of *T. suis* treatment is needed.

In mice and rats experimental colitis is commonly induced by the heparin-like polysaccharide dextran sodium sulphate (DSS). DSS increases the trans-epithelial permeability by decreasing the expression, and by inducing the redistribution of tight junction proteins (occludin, zonula occludens-1, claudins) and by enhancing epithelial cell apoptosis (Poritz*, et al.*, 2007),(Yan*, et al.*, 2009),(Mennigen*, et al.*, 2009). Furthermore, DSS causes a hyperosmotic stimulus that leads to the activation of NF-κB in the epithelium (Schwartz*, et al.*, 2008). This is consistent with the accepted role of epithelial barrier dysfunction in the pathogenesis of IBD (Clayburgh*, et al.*, 2004). In both IBD and DSS colitis the damaged epithelium allows the entry of luminal content into the mucosa thereby facilitating the onset of inflammatory processes (Nell*, et al.*, 2010). In mice, short term administration of DSS (1 - 10 % w/v) in drinking water is used to induce “acute” colitis whereas long-term or cyclic administration produces chronic colitis (Wirtz*, et al.*, 2007). The development of pathology can be easily monitored based on body weight, stool appearance, rectal bleeding and behavioural changes. Such clinical changes are usually preceded by alterations in histopathological parameters including colon shortening, mucosal injury, immune infiltration and epithelial damage. These changes initially appear in focal regions of the distal colonic mucosa and then expand progressively although the inflammation remains confined to the colon (Melgar*, et al.*, 2005).

The DSS model guarantees low risk of mortality, high reproducibility and good uniformity of the induced mucosal inflammation (Melgar*, et al.*, 2008). We therefore chose to develop a DSS colitis model in the rabbit. We found that in rabbits, administration of 0.1 % DSS for five days induces a clear cecum localized inflammation that mimics histological features of ulcerative colitis and is characterized by a similar gene expression profile as observed in

125 biopsies from IBD patients. Furthermore, we describe a scoring system to correlate clinical
126 parameters with histopathological findings that should facilitate the evaluation of the tested
127 therapeutic approach.

Methods

Rabbits

All animal experiments were carried out according to Swiss animal welfare laws and approved by the veterinary office of Zurich. Female white Himalayan rabbits and New Zealand white rabbits (Charles River, Kisslegg, Germany) weighing 1.9-2.1 kg were used for the experiments. Rabbits were maintained single-housed with water and food (standard rabbit maintenance diet – Provimi Kliba AG, CH-4303 Kaiseraugst-, hay and straw) *ad libitum* on a 12:12 hour light/dark cycle. Upon arrival, animals were kept for at least 4 days under routine husbandry. One week prior to DSS exposure, drinking water was substituted by organic fennel tea (Hipp, Pfaffenhofen, Germany) *ad libitum*.

Colitis induction and clinical evaluation

Colitis was induced by DSS (MP Biomedicals, Illkirch, France) dissolved in cold fennel tea at 0.1 % w/v (if not specified otherwise). Control rabbits received fennel tea as vehicle. The beverage was prepared freshly and changed at least every second day. Of every animal daily weight, daily food and beverage intake, daily stool appearance and behaviour were monitored. A disease activity index was calculated according to table 1. The disease activity (range: 0-4) index represents the sum of individual scores for weight loss, presence of uneaten cecotrophs, food intake and beverage intake divided by 4. Euthanasia was performed following sedation with barbiturates with an overdose of ketamine hydrochloride (Vétoquinol, Bern, Switzerland) and xylazine (Bayer, Lyssach, Switzerland).

The abdominal cavity was exposed by a midline laparotomy, and samples were collected from the ileum, jejunum, duodenum, cecum and colon. For RNA extraction and Myeloperoxidase activity analysis, the excised samples (0.5 cm in length) from the duodenum, jejunum, ileum and colon were opened by a longitudinal incision and rinsed with cold PBS. 1 cm^2 sections of the cecum were extensively washed with cold PBS until complete removal of the luminal content. The samples were immediately snap-frozen in liquid nitrogen, and stored at -80° C

until analysis. For histological analysis, samples (0.5 cm^2 sections of the cecum samples or 0.5 cm length sections of the other tissues) were either cut longitudinally or cut into smaller (0.2 cm) sections for fixation. The samples were carefully washed and fixed with phosphate buffered 10 % formalin solution. For genome wide mRNA expression studies cecal samples (2 cm^2) were extensively washed with cold PBS and stored on ice in 5 % BSA in PBS until further processing.

Whole cecal tissue RNA extraction and quantitative real-time PCR

Total RNA was isolated using the RNeasy Mini Kit (Qiagen, Hilden, Germany) and the automated sample preparation system Qiacube (Qiagen, Hilden, Germany) following the manufacturer's recommendations. cDNA was synthesized with the High-Capacity cDNA Reverse Transcription Kit (Life Technologies, Carlsbad, California, U.S.A)

To study the transcription of immune response markers in rabbit intestine tissues, sequence-specific primers were applied (table 2) and q-rtPCR was performed according to Schnupf et al. with some modifications (Schnupf and Sansonetti, 2012). Amplifications were performed in a total volume of 15 µl including 50 ng of cDNA, primers (0.2 µM each) and 7.5 ml of Power SYBR Green mix (Life Technologies, Carlsbad, California, U.S.A). Reactions were run in triplicate on an ABI 7900HT (Life Technologies, Carlsbad, California, U.S.A) using the universal thermal cycling parameters (2 min 54° C, 94.5° C 10 min, 40 cycles of 15 sec at 97° C and 60 sec at 59.7° C; dissociation curve: 15 sec at 95° C, 15 sec at 60° C and 15 sec at 95° C). Results were analysed with the sequence detection software ABI 7900HT SDS2.4. For quality control purposes all samples' dissociation curves were acquired and amplification products were visualized by 2 % agarose gel electrophoresis. Primer sequences are listed in table 2. The comparative $\Delta\Delta C_t$ method was applied for relative gene expression quantification. (C_t: threshold cycle).

Isolation of cecal lamina propria mononuclear cells (LPMC) and intestinal epithelial cells (IEC)

Cecal LPMC and IEC were isolated as previously described (Weigmann*, et al.*, 2007), with some modifications. Briefly, the dissected mucosa was washed with Ca^{+}- and Mg^{+}-free PBS, the cecal fold was removed and discarded. The tissue was cut and incubated in medium containing 20mM EDTA (Sigma-Aldrich) for 30 min at 37° C on a shaking platform (150 rpm). After incubation, the suspension of IECs, villus cells, subepithelial cells and intestinal epithelial lymphocytes was detached by vortexing and passing through a 70 µm cell strainer (BD Biosciences, Erembodegem, Belgium). The epithelial cells were washed twice, pelleted, resuspended in RTL buffer (Qiagen, Hilden, Germany), snap-frozen in liquid nitrogen, and stored at -80° C for later analysis. The remaining tissue containing LP with muscle layer was collected and incubated in medium containing 1 µg/ml collagenase type I CLS (Worthington Biochemical Corp., Freehold, New Jersey, USA) at 37° C on a shaking plate (300rpm). After 15 minutes incubation, the suspension was vortexed and filtered through a 70 µl strainer. The filtered cells were resuspended in 5 % BSA in PBS to stop the enzymatic digestion. The undigested tissue was incubated with fresh collagenase solution for additional 15 minutes. The collagenase digestion was repeated three times and the washed LPMC were pooled. LPC were pelleted twice and resuspended in DMEM supplemented with 5 % FCS. LPMC were purified using Ficoll-Paque PLUS (GE Healthcare Europe GmbH, Freiburg Germany) gradient centrifugation for 40 min at 1200 rpm. The viability of the cells was confirmed by tryptan blue staining. Cells were resuspended in RTL buffer (Qiagen, Hilden, Germany), snap-frozen in liquid nitrogen, and stored at -80° C for later analysis.

RNA isolation and genome wide mRNA expression analysis

Total RNA was isolated with the Qiacube system using the RNeasy Mini Kit with DNase digestion (Qiagen, Hilden, Germany) to eliminate genomic DNA. RNA integrity and quantity was determined on the Agilent 2100 Bioanalyzer (Agilent; Palo Alto, CA, USA). Samples with an integrity score ≥ 6.8 were sent to the Functional Genomic Centre Zurich (FGCZ) for sequencing on the Illumina® platform. The fold change (FC) was used to express the changes

206 in average gene expression between studied groups. FC was normalized against the control
207 group (rabbits receiving fennel tea only). The ENSEMBLE IDs were annotated using
208 BetterBunny augmented annotation and analysis of rabbit genes
209 (http://cptweb.cpt.wayne.edu)(Craig, *et al.*, 2012). MetaCore™ (Thomson Reuters,
210 http://portal.genego.com) was used to perform network and pathway analyses. The following
211 cut-offs were applied to select differentially expressed genes for further analysis: pValue $p \leq$
212 0.01 and fold change $\geq |2.0|$. The pathways (groups of genes belonging to the same pathway
213 map in MetaCore™ database) and gene families were considered significant with a pValue $\leq$
214 0.05 and were further selected on the basis of their relevance to inflammatory bowel diseases
215 pathology. Additional gene expression data sets for comparison were obtained from GEO
216 DataSets (NCBI) of previously published studies in colon pinch biopsies from UC and CD
217 patients (Granlund, *et al.*, 2013).
218 *Validation of the genome wide mRNA expression analysis*
219 The expression profiling results were confirmed by qPCR of selected genes involved in the
220 highlighted pathways. cDNA synthesis was performed using a High-Capacity cDNA Reverse
221 Transcription Kit (Life Technologies Ltd). Real-time PCR was performed using TaqMan
222 Gene Expression Assays (Life Technologies Ltd) and TaqMan Fast Universal PCR Master
223 Mix No AmpErase UNG (Life Technologies Ltd) on a 7900 HT Fast Real-Time PCR System
224 with SDS 2.2 Software (Life Technologies Ltd). TaqMan gene expression assays were
225 performed for COX-2 (Ptgs2, Oc03398293_m1), IL-6 (Oc04097051_m1), MMP-1
226 (Oc04250656_m1) and the housekeeping gene GAPDH (Oc03823402_g1) as an endogenous
227 control. Measurements were performed in triplicates; relative expression was calculated using
228 the $\Delta\Delta$Ct method.
229 *Histopathological evaluation of colitis*
230 After careful dissection and fixation, tissues were routinely embedded in paraffin. Serial
231 sections of 5 μm were cut using a microtome (Carl Zeiss AG, Feldbach, Switzerland) and

stained with haematoxylin-eosin to investigate epithelial damage and cellular infiltration. The histological changes in the cecum were quantified in a blinded manner by two investigators with a scoring system (range 1 - 24) for morphological features and infiltration of immune cells according to the scoring system described in table 3 (Kojouharoff*, et al.*, 1997)·(Cooper*, et al.*, 1993)·(Day*, et al.*, 2008).

Analysis of myeloperoxidase activity

MPO activity was measured in different regions of the gastrointestinal tract as previously described. MPO activity was calculated as mean absorbance (460 nm) per incubation time per protein content of the sample in grams (indicated as arbitrary units U/g·s)(Bozeman*, et al.*, 1990)

Statistical analysis

The results of the 0.1 % DSS colitis were obtained in two different experiments (n = 8 and n = 4). As the experimental protocol was identical for both experiments results were pooled together. The data obtained from this study was analysed using IBM-SPSS statistic 21. The majority of the examined parameters were asymmetrically distributed. For the comparison of the treatment groups the non-parametric Mann - Whitney *U*-test for two independent samples was used.

Results

Clinical symptoms of DSS exposure in rabbits

Since rabbits have a more sensitive digestive tract in comparison to mice, the concentration of DSS to induce colitis had to be drastically decreased. We observed a reduction of daily beverage intake that we ascribed to the unpleasant taste of DSS. To overcome this problem, DSS was dissolved in organic fennel tea that successfully masked the taste of the DSS and restored a normal beverage intake during DSS exposure.

A pilot study (data not shown) showed that administration of 0.1 % DSS in fennel tea for 3 days reduced the normal weight gain from day 5 on in the treated animals. This effect was no longer present from day 14 on indicating a restitution of the colitis. Other clinical symptoms were not evident. Histological analysis of HE stained intestinal samples showed no clear signs of inflammation; only a slight reduction in the number of goblet cells in the cecum at day 7 and 10 was observed. Subsequently, the duration of the DSS phase was increased from 3 to 5 days. 12 white Himalayan rabbits were fed for 5 days with 0.1 % DSS in the daily beverage (fennel tea), whilst control rabbits (n = 5) housed in the same facility were given fennel tea without DSS. The earliest symptoms manifested at day 4, when the rabbits started to gradually diminish the daily food and beverage intake from the initial 120 g/day pellet and 210 ml/day beverage intake at day 1 down to 40 g/day and 100 ml/day at day 7 (figure 1 A, B).

Exposure to 0.1 % DSS markedly reduced the weight gain (figure 1 C). Further symptoms included the presence of loose and smeared stool (figure 1 D), behavioural abnormalities such as apathy or aggressiveness and unclean fur. A combinatorial index of disease, (figure 1 E, disease activity index DAI, described in the methods section) was used to quantify the severity of the monitored clinical symptoms. We found that whilst the DAI of control rabbits remained at baseline (DAI < 0.5), the DAI of rabbits receiving DSS increased significantly

starting from day 5 (DSS: 0.47 ± 0.32, n = 12; control: 0.08 ± 0.10. n = 5) and rose up to 2.1 at day 12.

DSS induces a cecum localized pathology

Histological evaluation of the intestinal tract revealed a cecum localized pathology, whereas no clear signs of tissue damage were observed in other regions of the intestinal tract (colon and ileum, figure 2).

Histopathology of the cecum was characterized by infiltration of immune cells into the epithelial layer and the lamina propria and by morphological changes such as villous stunting, crypt distortion and villous epithelial injury (figure 3 a, c, d). The global histology score was increased from day 4 onward (figure 3b). The severity of the damage increased progressively even after the removal of DSS from a baseline value of 7.7 ± 0.9 in control rabbits (n = 5) to an average score of 13.3 ± 5.0 at day 10 (n = 4) and further increased until day 14.

Expression analysis of genes involved in the immune response

To evaluate the inflammatory response in DSS treated rabbits, we analysed the mRNA expression of inflammation related genes by q-rtPCR. iNOS, IFNγ and IL-12 p35 were chosen as they have been implicated in IBD and have been shown to be overexpressed in inflamed rabbit tissue(Schnupf and Sansonetti, 2012). Gene expression analysis of inflammatory markers in the cecum showed a transient increase of IL-12 p35 in DSS treated rabbits (figure 4), although, owing to the limited sample number in the control group, the difference did not reach statistical significance. No difference between the groups was observed for iNOS and IFNγ.

Genome wide gene expression analysis in LPMC and IEC by RNAseq

Genome wide gene expression analysis identified 470 differentially expressed genes in IECs and 215 differentially expressed genes in LPMCs ($FC \geq |2|$, $p \leq 0.05$). The process networks that were significantly over-represented in the MetaCore™ analysis were 58 in LMPCs and

299 49 in IECs, respectively. Among the most relevant process networks, there was an
300 overrepresentation of genes involved in inflammation, immune response and chemotaxis
301 (figure 5).
302 Furthermore, the diseases (by biomarkers) ontology in MetaCore™ was used to assess the
303 similarity between the gene expression in our rabbit DSS colitis model with the gene
304 expression known to be associated with selected human diseases. In both LPMC and EC we
305 found an enrichment of differentially expressed genes associated with inflammation, IBD, CD
306 and UC (figure 6).
307 To further confirm the results of the disease enrichment analysis we compared genes
308 differentially regulated in our DSS colitis model with a gene set from a genome wide gene
309 expression analysis in human CD and UC patients(Granlund, Flatberg, Ostvik, Drozdov,
310 Gustafsson, Kidd, Beisvag, Torp, Waldum, Martinsen, Damas, Espevik and Sandvik, 2013).
311 Overall, the majority of the differentially expressed genes were involved in immune response,
312 cell adhesion, cytoskeleton reorganization and chemokine signalling (figure 4; table 5).

313 Finally, the sequencing results for mRNA expression were exemplarily validated for COX-2,
314 IL-6 and MMP1 by qPCR (figure 7). In accordance with our transcriptome results the
315 expression of the selected genes was higher in both LPMC and EC cells isolated from DSS
316 treated rabbits in comparison to the non colitic controls.

317

318 *DSS transiently increases neutrophil infiltration in the rabbit cecum*
319 The neutrophil infiltration into inflamed tissues was monitored by analysis of
320 myeloperoxidase (MPO) activity (Bradley*, et al.*, 1982). MPO activity in the cecum of DSS
321 treated rabbits transiently increased at day 5-7 before returning to baseline levels at day 14
322 (figure 8 a), due to the low number of animals the change did not reach statistical
323 significance. Analysis of MPO in the ileum and the colon showed no significant differences

between colitis animals and the control group (figure 8 b,c). In the ileum, the basal MPO activity in untreated rabbits was higher than in the cecum but no changes occurred upon exposure to DSS. Overall, our results suggest that the DSS induced infiltration of neutrophils predominantly localizes in the cecum.

Discussion

The helminth parasite *T. suis* has shown promising results for the treatment of IBD in human studies. Unfortunately, efficacy and safety (in particular in immune-compromised subjects) as well as the underlying mechanisms cannot be studied in the well-established mouse and rat models of IBD since the parasite's ova fail to hatch in the intestine of these rodents. *T. suis* ova (TSO) hatch in pig (the natural host), men, and rabbits. Since the life cycle of *T. suis* in human and in rabbits appears similar, a rabbit model of colitis would represent an adequate model for investigations into TSO therapy. The aim of the present study was to develop an IBD model in rabbits by administration of dextran sodium sulphate (DSS) in the daily beverage. This study shows that administration of 0.1 % DSS for 5 days is sufficient to induce a clear acute inflammation that is localized in the cecum. Localization of the pathology in the cecum makes the DSS model particularly suitable to study the effects of *T. suis* ova (TSO) treatment as the cecum is the site of *T. suis* colonization in rabbits.

In accordance with the disease manifestation in other species, the clinical symptoms observed in rabbits were reduced weight gain, reduced food and beverage intake, loose stools and unclean fur(Wirtz, Neufert, Weigmann and Neurath, 2007). The strong reduction in liquid intake began after five days, only, hence the daily intake of DSS remained constant throughout the whole induction phase.

The reduction in food intake reflects the response to abdominal discomfort and the disturbances in feeding behaviour that are seen in patients with inflammation of the gastrointestinal tract(Rigaud*, et al.*, 1994) and are also commonly observed in mouse and rat models of gastrointestinal inflammation(McHugh*, et al.*, 1993),(McDermott*, et al.*, 2006). To facilitate the evaluation of the disease outcome we developed a disease activity index (DAI) based on the monitoring of the different clinical parameters. Starting from day 4 after DSS administration, rabbits manifested clear symptoms of pathology that gradually worsened and a

peak of disease activity was reached at day 9. Afterwards, the DAI decreased and stabilized until the last analysed time point at day 14.

Macroscopical analysis of the internal organs following euthanasia showed no abnormalities. In contrast, histopathological analysis of the intestinal tract revealed that DSS causes an inflammation predominantly localized in the cecum. The other sections of the large intestine and the small intestine remained unaffected.

The cecum localization of the DSS induced inflammation is also observed in guinea pigs and in the Mongolian gerbil model(Iwanaga*, et al.*, 1994)·(Bleich*, et al.*, 2010). These species possess a functional cecum that is particularly enlarged and provides a niche for the microbial fermentation of cellulose(Snipes, 1982)·(Snipes, 1997). The cecum localization of the DSS induced inflammation might be due to an increased permeability of the intestinal barrier to DSS in this particular section of the intestine(Hoshi*, et al.*, 1996). The localization of the lesions in gerbils has been linked to the increased absorption of sulphated polysaccharides in this particular section of the gerbil intestine and absorption of DSS in the cecum has also been reported in rabbits(Sharratt*, et al.*, 1971) and might explain our observations. In accordance with DSS models in other species, DSS treatment induced both a disruption of the mucosal morphology and an infiltration of immune cells (Melgar, Karlsson and Michaelsson, 2005). In particular, the histopathology of the cecum displays crypt loss*,* epithelial damage and infiltration of immune cells. These manifestations reproduce characteristic traits commonly observed in ulcerative colitis(Okayasu*, et al.*, 1990). Despite the progressive amelioration of the clinical symptoms after their peak at day 9, the histological damage persists longer and displays some characteristics of chronic intestinal inflammation such as the atypical branching of the crypts.

The initial pathology (day 4 - 9) presents classical features of an acute inflammation. From day 7 to day 10, we observe a transient increase of neutrophil infiltration into the cecal mucosa accompanied by an increased expression of the pro-inflammatory cytokine IL-12 p35.

379 This transient inflammatory activity correlates well with the peak of the DAI and with the
380 histological findings and suggests an initial T helper 1 driven acute response. The increased
381 neutrophil activity in the cecal mucosa is a common feature with the guinea pig colitis model.
382 However, the described model in guinea pigs was performed with high concentration of DSS
383 (3 %) and had a fulminant outcome, with 96 % of the animals dying within 96 h (Iwanaga et
384 al., 1994a).
385 Genome wide mRNA expression profiling in cecum LPMCs and ECs at day 10 showed an
386 enrichment of genes involved in chemotaxis and immune response. In particular, the immune
387 response was characterized by genes involved in Th17 signalling, particularly in epithelial
388 cells. An activation of the innate immune response is a feature shared by both CD and UC
389 patients. In contrast, Th17-associated cytokines are usually observed in the inflamed mucosa
390 of CD patients, only. Our analysis further showed enrichment for IL-4 related cytokines that
391 would rather suggest a Th2 type response. This type of response correlates well to the atypical
392 Th2 response (mediated by natural killer cells producing IL-13) observed in UC patients
393 (Fuss*, et al.*, 2004). A switch into a Th2 type response has been observed as the colitis
394 matures from an acute toward a chronic phase (Alex*, et al.*, 2009). The features that appear at
395 later stages of the rabbit colitis might indicate that after an acute phase characterized by
396 severe clinical symptoms, mucosal damage and acute inflammation, the pathology acquires a
397 certain degree of chronicity with a shift toward a Th12 immune response. A long-term
398 analysis is necessary to investigate these preliminary observations and to clarify if the disease
399 resolves after the acute phase or if it progresses to chronicity.
400 In summary, we report the development and characterization of a novel DSS induced colitis
401 model in rabbits. The initial pathology has an acute nature and is characterized by specific
402 clinical symptoms, histopathological changes and higher mRNA expression of inflammatory
403 markers. Our model provides a safe and reliable induction of colitis in rabbits that is
404 particularly suitable to study the effects and mechanisms of TSO treatment in IBD.

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IL performed the animal experiments, collected and analyzed the samples, performed the genetic analysis and drafted the manuscript. FN performed the animal experiments and collected the samples. KA was involved in sample preparation. AC scored the histology specimens. AA performed the pilot animal experiment. BT and RG contributed to the conception of the study. GR contributed to the interpretation of data; study concept and design; critical revision of the manuscript for important intellectual content; study supervision. IFW contributed to the interpretation of data; statistical analysis, study concept and design; writing and revision of the manuscript; study supervision.

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Figure legends

Figure 1: Manifestation of clinical symptoms upon DSS exposure. Response to colitis induction was monitored daily according to a detailed score sheet. Food intake (a), beverage intake (b), weight change (c), stool consistency (d. representative pictures for control and DSS rabbits at day 10) were summarized into a disease activity index (e. DAI, 0-4). DSS rabbits (●, n = 12) were fed with 0.1 % DSS in the daily beverage (fennel tea) for 5 days. The control group (○, n = 5) was maintained under the same conditions with fennel tea as beverage. Data represent mean ± SD; Mann Whitney Test, ** $p \leq 0.005$, * $p \leq 0.05$.

Figure 2: Absence of histopathology in the colon and ileum. Representative HE stained colon (A) and ileum (B) sections in control and in DSS exposed rabbits at day 10. Scale: 200 µm.

Figure 3: Histopathological changes of the cecum at different time points after colitis induction. HE stained cecum sections were scored (A, 1 - 4) for markers of inflammation (infiltration of lamina propria eosinophils, lamina propria lymphocytes, intraepithelial lymphocytes) and for the distortion of morphological features (villous stunting, villous epithelial injury, crypt distortion) . Single parameters were summarized to a global score (B). Black dots represent DSS treated rabbits (●); white dots represent control rabbits (○). Horizontal lines represent the arithmetical mean; Mann Whitney Test, ** $p \leq 0.05$, * $p \leq 0.1$. Representative HE stained cecal sections of control rabbits (C) and of DSS exposed rabbits at different time points after colitis induction (D). Scale: 200 µm.

Figure 4: mRNA expression of pro-inflammatory cytokines during colitis induction. mRNA expression of IL-12 p35 in cecum from DSS treated (●) or control (○) rabbits. Results are shown as mean expression relative to GAPDH using the $2^{-\Delta\Delta Ct}$ method. Dots represent single animals, each sample was analyzed in triplicate and data are expressed as mean ± SE.

573

574 Figure 5: Process network analysis of differentially expressed genes in epithelial cells (EC,
575 black bars and line) and lamina propria mononuclear cells (LPMC, grey bars and line). This
576 analysis is based on a manually curated database of process networks, which details more
577 specific biological processes than GO annotations alone. Most prominent process networks
578 associated with the identified genes were involved in cell adhesion and chemotaxis (═) and
579 immune and immune responses (▬). Analysis was performed with MetaCore™. Bars
580 represent the Log(p-Value) of enriched pathways, whereas lines represent the ratio between
581 differentially expressed genes upon DSS exposure and the total number of genes involved in
582 the specific process network. Gene expression threshold: fold change ≥ |2.0|; p-Value ≤ 0.05.

583

584 Figure 6: Enrichment of differentially expressed genes in selected disease categories (by
585 biomarkers) in epithelial cells (IEC, white bars and line) and lamina propria mononuclear
586 cells (LPMC, grey bars and line). The Gene IDs of the orthologous genes assigned to the
587 differentially expressed rabbit mRNAs were analysed for enrichment in selected disease
588 categories using MetaCore™. Terms relevant for IBD and experimental colitis are displayed.
589 Bars represent the Log(p-Values) of enriched pathways, whereas lines represent the ratio
590 between the differentially regulated genes upon DSS exposure and the total number of genes
591 involved in the specific process network. IBD, inflammatory bowel disease, UC, ulcerative
592 colitis, CD, Crohn's disease. Gene expression threshold: fold change ≥ |2.0|; p-Value ≤ 0.05.

593

594 Figure 7: Quantitative RT-PCR showing expression of COX-2 (A, D), IL-6 (B, E) and MMP-
595 1 (C, F) in intestinal epithelial cells (IEC, upper panel) and lamina propria mononuclear cells
596 (LPMC, lower panel) of DSS and control rabbits at day 10 post colitis induction. Expression
597 is shown relative to *gapdh* in the distal colon, n = 4–9. Values are given as mean ± SD and
598 difference between groups was tested by two-tailed Student's t test.

599

600 Figure 8: Myeloperoxidase (MPO) was determined as a marker for neutrophil infiltration in
601 the gastrointestinal epithelium. Values for MPO activity in cecum (A), duodenum (B) and
602 ileum (C) samples were normalized for the total protein concentration as determined by BCA
603 assay and for the incubation time (values are represented in arbitrary units $U/g^{-}s$). Dots
604 represent single animals.

605

Table 1: Scoring system for the daily monitoring of the disease activity index.

Score	weight loss	stool appearance and cecotrophs	reduction in food intake	reduction in beverage intake	fur appearance
0	None	well-formed solid pellets, 0 cecotrophs	none	none	clean, bright fur
1	0-2 %	easy to smear and loose stool, ≤1 cecotrophs	0-30 %	0-30 %	dim fur
2	2-5 %	loose stool, 2-3 cecotrophs	30-60 %	30-60 %	shagged fur
3	5-10 %	loose smeared stool in cage, 4-5 cecotrophs	60-90 %	60-90 %	smudgy, unclean fur
4	> 10 %	loose smeared stool in cage, > 5 cecotrophs	> 90 %	> 90 %	smudgy, stool-stains, smeared anus

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607

Table2: Primers for qPCR

Marker	Forward primer Backward primer	Primer location within CDS	Target size	NCBI Accession
IL-12p35	AAGGCCAGACAAACTCTAGAATTC TTGGTTAACTCCAGTGGTAAACAGG	Exon 3/4 and 4/5 from ~8	116 nts	XM_002716291
iNOS	GACGTCCAGCGCTACAATATCC GATCTCTGTGACGGCCTGATCT	Undetermined	102 nts	XM_002718780
IFNγ	TGCCAGGACACACTAACCAGAG TGTCACTCTCCTCTTTCCAATTCC	Exon 1 and 2/3 from 4	127 nts	NM_001081991
GAPDH	TGACGACATCAAGAAGGTGGTG GAAGGTGGAGGAGTGGGTGTC	Exon 1 of 1	120 nts	NM_001082253

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Table 3: Scoring system for DSS-induced histological changes in the cecum.

	Morphological features			Inflammation		
	Villous stunting	**Villous epithelial injury**	**Crypt distortion**	**Intraepithelial lymphocytes**	**LP lymphocytes and plasma cells**	**LP eosinophils**
1	Normal mucosa	Normal mucosa	Normal mucosa	5–10/50 IEL/epithelial cells	25 % of the villous lamina propria	2–3 cells per × 40 field
2	Mild villous stunting	Mild villous epithelial injury	Mild crypt distension, hyperplasia and distortion	11–30 IEL/50 epithelial cells.	25–50 % of the villous lamina propria	5 -10 per × 40 field.
3	Moderate villous stunting	Moderate villous epithelial injury	Moderate crypt distension, hyperplasia and distortion.	31–50 IEL/ 50 epithelial cells may be focally clustered.	50–75 % of the villous lamina propria.	10 - 20 per × 40 field.
4	Marked villous stunting	Marked villous epithelial injury	Marked crypt distension, hyperplasia and distortion	51–100 IEL/ 50 epithelial cells, may be clustered and at all levels of the epithelium	75 – 100 % of the villous lamina propria.	> 20 per × 40 field

610

Table 4: **Genes concordantly up-regulated in LPMCs of DSS colitis rabbits and IBD biopsies.**

Cell adhesion and Cytoskeleton reorganization	**Metabolism and biosynthesis**
CD38	PTGS2(COX2)
PLEK	SLC6A14[a]
S100A9	TCN1
SELL[a]	
Cytokine and Cytokine R genes	**Development**
IL1A	EGR2[a]
IL6	
IL8	
IRF1	
Chemokine and Chemokine R genes	**Tissue remodelling Genes**
CCR7[a]	MMP3
CXCL10	
CXCL11	
CXCR4	
ENA-78[a]	
Immune response	
Innate Immune defence	*BCR and TCR signalling*
TMEM173	CD19
DMBT1	LAX1
FAM65B[a]	
SLC11A1[a]	

List of genes concordantly up-regulated in LPMC from the cecum of DSS treated rabbit and colonic biopsies of inflamed tissue from IBD patients. LPMC: Lamina propria mononuclear cells. [a]differentially expressed in UC patients, only.

611

612

Table 5: **Genes concordantly up-regulated in ECs of DSS colitis rabbits and IBD biopsies**.

Cell adhesion and Cytoskeleton reorganization	**Metabolism and biosynthesis**
SELL[a]	FCRLA [b]
PLEK	PLA2G7[a]
VNN1	SLC11A1[a]
S100A9	SLC2A3
CLEC4A	SLC6A14[a]
CD38	TCN1
Cytokine and Cytokine R genes	**Apoptosis**
IL1A	UBD
IL1B	IER3[a]
IL6[a]	PEA15[a]
IL8	
Chemokine and Chemokine R genes	**Cell-cell signalling**
CCR7[a]	ADM
CXCL10	TNFAIP6[a]
CXCL11	**Tissue remodelling Genes**
CXCL13	CTSK
CXCL5[a]	MMP1
CXCL6	MMP12
CXCL9	SERPINE2
Immune response	
Innate immune defence	*BCR and TCR signalling*
OAS2	CD19
TLR8 [b]	CD74
Humoral immune response	CD79B
POU2AF1	CD86[a]
CD83[a]	LYN[a]
Acute-phase response	SLAMF8[a]

SERPINA1	*inflammatory response*
Antigen processing	NFKBIZ[a]
HLA-DMA	*anti-inflammatory response*
HLA-DPA1	A1F1
HLA-DPB1[b]	

List of genes concordantly up-regulated in EC from the cecum of DSS treated rabbit and colonic biopsies of inflamed tissue from IBD patients. EC: epithelial cells. [a]genes differentially expressed in UC patients, only. [b]genes differentially expressed in CD patients, only.

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