

Mouse models for Inflammatory Bowel Disease

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Figure 1. Recurrent TNBS-induced colitis in BALB/c mice: HE staining of distal colon tissue with numerous infiltrating immune cells, areas with depleted goblet cells, and irregular crypts.

Inflammatory bowel diseases (IBD) are chronic inflammatory diseases including ulcerative colitis and Crohn's disease. Mouse models of colonic inflammation are invaluable tools for the pre-clinical evaluation of the potency of novel drug candidates for the prevention or treatment of IBD. TNO now offers the chronic colitis model based on repeated TNBS administration in BALB/c mice and the chronic colitis model in IL-10 knockout mice, in addition to models of acute colitis induced by TNBS, DSS, or oxazolone. These chronic models better reflect human IBD and allow us to evaluate prolonged prophylactic or therapeutic treatment with drug candidates. The models are also valuable tools to evaluate the gut health promoting effects of food components.

Background

IBD is caused by a dysbalanced immune response to normal bacterial components of the intestine, which is normally in a state of tolerance. Disease is characterized by a chronic relapsing-remitting course. During exacerbations patients experience abdominal pain, diarrhoea, and bloody stools. Currently, therapy aims to suppress inflammation with non-specific anti-inflammatory drugs like corticosteroids, classical immunosuppressives and antibiotics. Many therapeutics in development are at immunomodulatory.

For the pre-clinical evaluation of the potency of novel drug candidates, pharma and biotech companies largely depend on animal models for IBD. The classical animal colitis models are based on TNBS-, DSS- and oxazolone-induction of acute inflammation in the colon. Although they offer rapid screening tools, these models are characterized by severe acute tissue damage, and they lack aspects of chronic inflammation. Furthermore, these models are highly variable in disease induction and response to treatment. Therefore, TNO emphasizes models with chronic aspects of inflammation that better represent chronic IBD in humans.

Animal models reflecting the chronic aspects of intestinal inflammation are based on the spontaneous development of colitis due to a genetic defect or the induction of colitis by repeated chemical triggering of chronic processes. They have the advantage of evaluating the efficacy of prolonged prophylactic or therapeutic treatment of chronic colitis. These models can also be used as intestinal challenge models to evaluate the intestinal health effects of nutritional components.

Recurrent TNBS-induced colitis

The recurrent TNBS-induced colitis model in the BALB/c mouse is an animal model for human IBD. Colitis is induced by repeated intra-rectal administration of 2,4,6-trinitrobenzene sulfonic acid (TNBS), which leads to the development of various aspects of chronic intestinal inflammation. Both T_H1 and T_H17 cells, and associated cytokines play a role in the underlying inflammatory response¹.

The TNO model of recurrent TNBS-induced colitis combines published induction

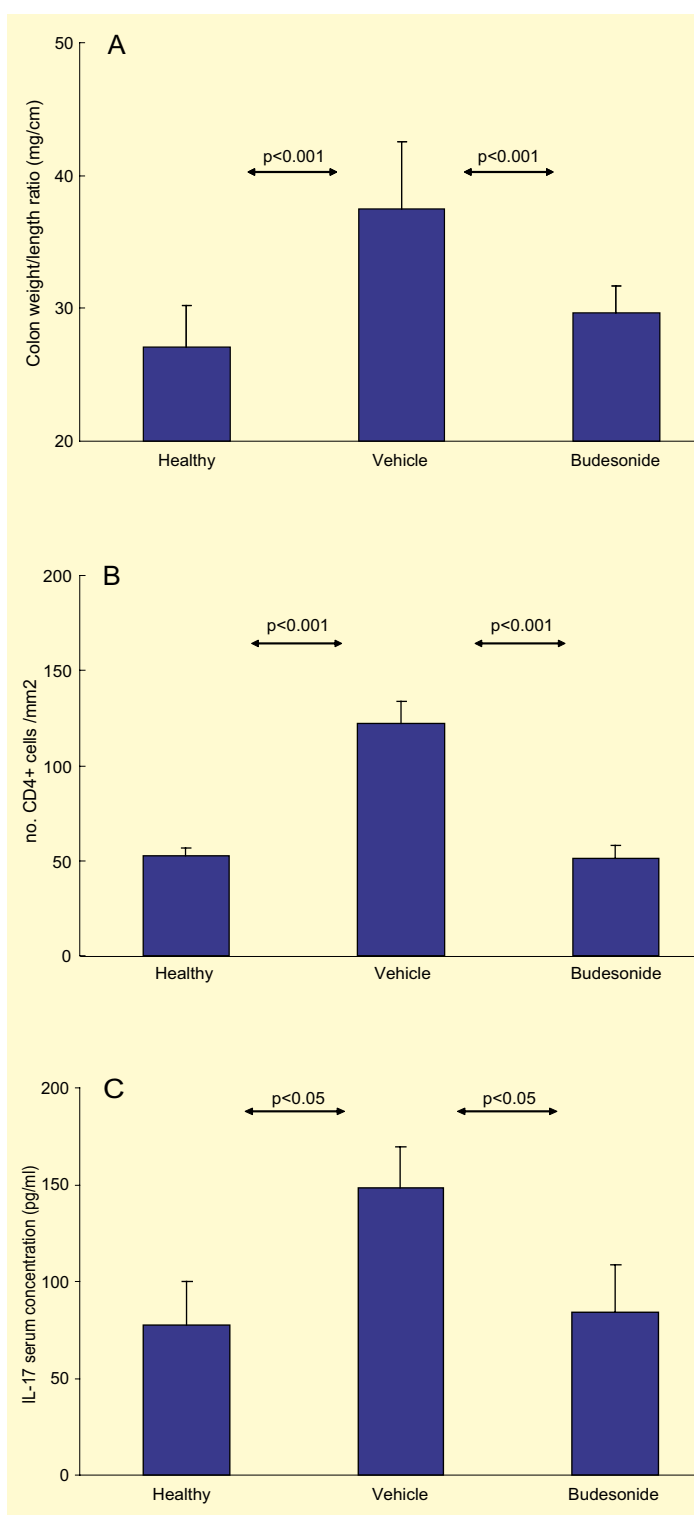


Figure 2. Effect of budesonide treatment on selected inflammation read-outs. A. Colon weight/length ratio; B. Number of infiltrating CD4+ T cells; C. Serum concentration of IL-17.

protocols with skin sensitization with TNBS prior to the rectal TNBS administrations². This induction protocol results in high reproducibility and low mortality. In this model colitis is characterized by prolonged inflammation and relatively mild tissue destruction. At end-point, the inflamed colon shows increased weight and increased thickness, especially in the distal part. Histopathologically, TNBS-induced inflammation is primarily associated with

increased inflammatory cellular infiltration, irregular crypts and loss of Goblet cells (Figure 1). The cellular infiltrate consists of CD4+ and CD8+ T cells, macrophages, granulocytes, and mast cells. Multiplex analysis revealed elevated serum levels of pro-inflammatory cytokines, including IL-17, IFN- γ , IL-1 β , and MIP-1 α , implicating the involvement of the Th1/Th17 axis of the immune response, in line with the processes thought to be involved in Crohn's Disease.

TNO has validated the model with local corticosteroid treatment. Budesonide effectively inhibits TNBS-induced colitis, as is shown by its effects on colon weight (Figure 2A) and thickness, an improved histological appearance of the colon as reflected by the number of CD4+ T cells (Figure 2B), and normalization of serum levels of the cytokines that are induced by colitis induction, such as IL-17 (Figure 2C) and IFN- γ .

Recently, we have shown that prophylactic treatment with probiotics improved resistance to subsequent induction of colitis in this model. These effects were reflected by reduced mucosal infiltration of immune cells and by serum cytokine levels that were comparable to those detected in healthy mice. Transcriptional profiling on colon tissue has revealed that probiotic treatment inhibits many of TNBS-induced gene expression changes (Figure 3). The majority of these genes are involved in immune response and antimicrobial response pathways that may contribute to control of intestinal homeostasis.

Advantages of recurrent TNBS-induced colitis model compared to acute models:

- Model with aspects of mild to moderate chronic inflammation
- Prolonged window of treatment: up to 5 weeks
- Limited acute tissue damage
- Validated with budesonide and probiotics
- Low mortality
- Limited variability

Chronic colitis in IL-10 knockout mouse

An alternative mouse model for chronic IBD is the interleukin-10 deficient (IL-10^{-/-}) mouse model. Mice with a targeted disruption in the IL-10 gene spontaneously develop chronic intestinal inflammation. The onset and severity of the colitis is strongly influenced by the husbandry conditions of the mice, specially the commensal flora, and the genetic background of the mouse strain. The spontaneous onset of gastrointestinal inflammation is reported to become evident between 6 weeks and 6 months of age. At TNO, the development of colitis in IL-10^{-/-} mice is synchronized by feeding them piroxicam³. This compromises the intestinal barrier function and, as such, facilitates the induction of an inflammatory response to intestinal microbiota.

In this model, colon inflammation is characterized by marked cellular infiltration of the colon, increased inflammatory cytokine production, and colonic epithelial proliferation. These changes occur within 2 weeks of piroxicam treatment in young IL-10^{-/-} mice, and the inflammation becomes chronic after removal of piroxicam. Colitis can be prevented or treated by administering IL-10, confirming a central role of this cytokine in piroxicam-synchronized colitis. This model is characterized by overproduction of pro-inflammatory cytokines such as IL-1, IL-6, and TNF- α . The induction of IL-12 and IFN- γ release is indicative of a strong Th1-biased inflammatory response. This mouse model is therefore a model for chronic colitis with a Th1 skewed immune response.

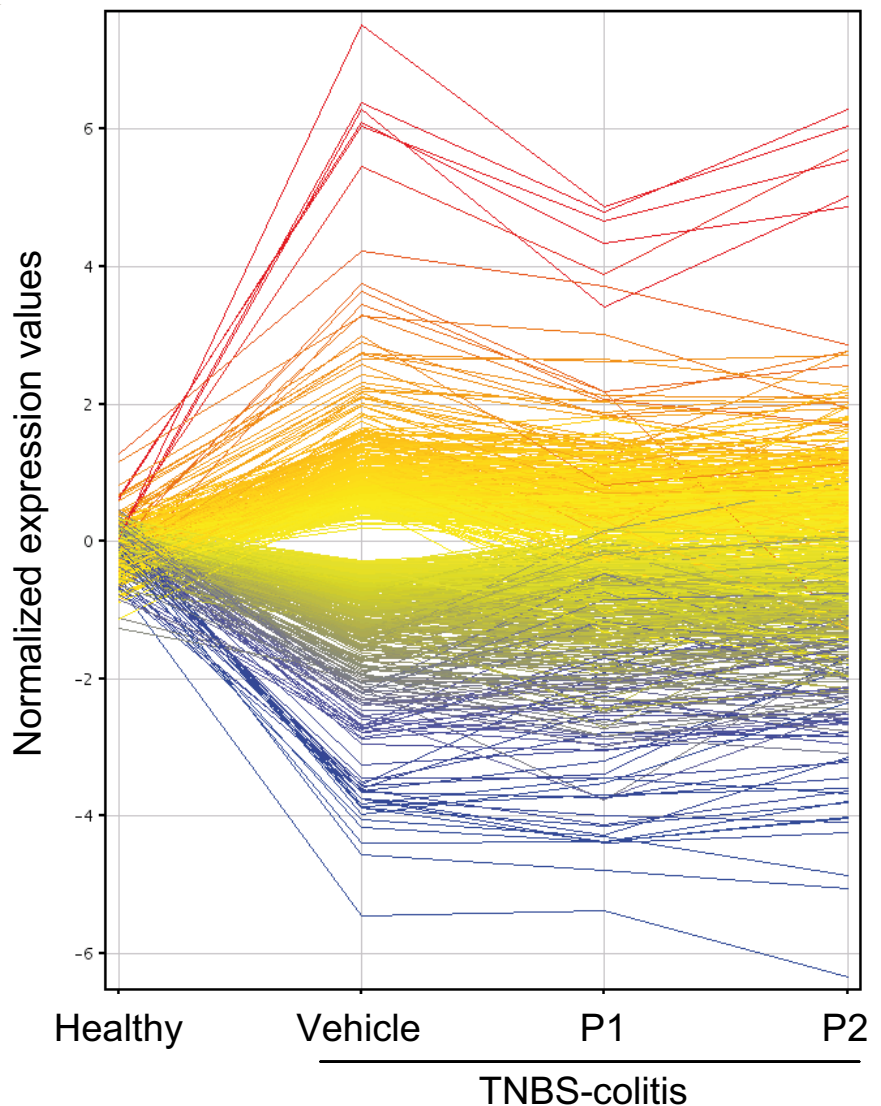


Figure 3. Effect of probiotic treatment on expression of genes that are affected by recurrent TNBS-induced colitis. Upon treatment with probiotic preparations P1 and P2, the expression of genes that are significantly affected in vehicle treated mice becomes more similar to those in healthy mice.

Advantages of IL-10 knockout mouse model of IBD:

- Model based on spontaneously developing chronic colitis
- Prolonged window of treatment: up to 5 weeks
- No chemically induced tissue damage
- Low mortality
- Limited variability

Applications of chronic colitis models

- Evaluation of efficacy of pharmaceuticals and biologicals aimed at suppression of IBD
- Evaluation of health effects of nutritional components
- Investigation of underlying mechanisms

Primary read-out parameters

- Body weight
- Macroscopical evaluation of colon length, weight, and thickness
- Histopathology scores based on semi-quantitative scoring of cellular infiltrate, epithelial integrity, and presence of Goblet cells
- Immunohistochemical detection of cellular infiltration of the colon tissue

Optional analyses

- Cytokine analysis in serum or colon tissue by ELISA or Multiplex assay
- Transcriptional profiling of specific gene sets or whole genome
- Microbiota profiling by genomic analysis
- Flowcytometry and cytokine production on isolated lymphocytes

References

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3. Berg DJ, *et al.* Rapid Development of Colitis in NSAID-Treated IL-10-Deficient Mice. *Gastroenterology* 2002; 123: 1527-42.

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