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Characterisation of enterocolitis in the piroxicam-accelerated interleukin-10 knock out mouse — A model mimicking inflammatory bowel disease☆

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Abstract

Background: In inflammatory bowel disease a defective mucosal barrier, a dysregulated immune response and an excessive reactivity against the gut microbiota are assumed to cause a breakdown of the intestinal homeostasis and lead to chronic inflammation. Piroxicam treatment is a method for induction of colitis in IL-10 k.o. mice, which integrates a dysfunction of both the intestinal barrier and the immune system. However, the translational value of this model has not been thoroughly clarified.
Aim: To characterise the piroxicam-accelerated colitis (PAC) IL-10 k.o. model with respect to clinical features, pathogenic mechanisms and its ability to respond to existing therapies.
Methods: The PAC IL-10 k.o. model was established on a C57BL/6 J background and the clinical manifestations, immunological mechanisms and efficacy of ampicillin and anti-IL-12/23p40 treatment were assessed.

Abbreviations: COX, cyclooxygenase; IL-10 k.o, interleukin-10 knock out; mAb, monoclonal antibody; Meics, murine endoscopic index of colitis severity; NSAID, non-steroidal anti-inflammatory drug; PAC, piroxicam-accelerated colitis; WT, wild type.

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Results: The PAC IL-10 k.o. mice developed weight loss and diarrhoea, and colonoscopy revealed a thickened granulomatous mucosa. Histological evaluation of ileum and colon showed Crohn's disease-like changes with pronounced hyperplasia and focal transmural inflammation. Ileitis was also observed in piroxicam treated wild type mice. The total number of neutrophils, monocytes and natural killer cells was elevated in the blood compared to IL-10 k.o. and wild type mice, indicating a role of the innate immune system in the pathogenesis. These findings were supported by analyses of the intestinal cytokine profile. Ampicillin and anti-IL-12/23p40 treatment significantly suppressed disease in the model.
Conclusion: The PAC IL-10 k.o. model resembles several features of Crohn's disease and could be a useful in vivo model in preclinical research.

1. Introduction

Despite years of intensive research within the field of inflammatory bowel disease (IBD) the aetiology and pathogenesis are still unknown and a distinct need for new efficient therapeutics exists. IBD encompasses two disorders of chronic, relapsing gastrointestinal inflammation defined by symptoms, anatomical location and histopathological appearance. Crohn's disease (CD) is characterised by a focal transmural inflammation that can affect the entire gastrointestinal tract, whereas ulcerative colitis (UC) is a continuous mucosal inflammation restricted to the colon and rectum.[1–4] Both CD and UC appear to be multifactorial disorders; genetic and environmental factors play interrelated roles, leading to a breakdown of the intestinal homeostasis and an excessive immune response against the commensal microbiota.[5,6] Colectomy can be curative for some UC patients; by contrast none of the current treatments for CD are curative and only about one-tenth of CD patients remain in clinical remission of 10 years after diagnosis.[1,3,4,7] Disease-relevant animal models are an indispensable part of preclinical research and a variety of colitis mouse models have emerged including several that involve genetic manipulations.[8,9] One example is the interleukin-10 knock out (IL-10 k.o.) mouse developed by Kühn et al.[10] IL-10 is an immunoregulatory cytokine with potent anti-inflammatory properties.[11–13] The central position of IL-10 in intestinal homeostasis is stated by human genetic studies, which has identified mutations in the IL-10 and IL-10 receptor genes, as well as a low-producing IL-10 promoter allele in IBD patients.[14–16] Mice deficient of IL-10 develop a chronic colitis under conventional conditions.[10,17–19] However, the incidence of spontaneous colitis in IL-10 k.o. mice on a colitis resistant C57BL/6J inbred background is low and prolonged, which limits its usage in medical research.[17,20] Colonic pathology can be accelerated and promoted by administration of piroxicam in the chow.[21] Piroxicam is a non-steroidal anti-inflammatory drug (NSAID), which inhibits the cyclooxygenase (COX) enzymes[22] and reportedly suppresses the colonic level of prostaglandin E2 in wild type and IL-10 k.o. mice by approximately 75%.[21] Since prostaglandins are essential for the epithelial growth and maintenance of the mucosal barrier, piroxicam treatment leads to impaired mucosal integrity and penetration of luminal bacteria.[21,23] Moreover, NSAIDs cause direct damage to enterocytes and induce apoptosis of epithelial cells in the IL-10 k.o. mice.[23,24] NSAIDs are an established risk factor for IBD and associated with de-novo colitis and increasing risk of relapse.[24,25]

The piroxicam-accelerated colitis (PAC) IL-10 k.o. mouse model resembles human IBD with regard to its heterogeneous pathogenesis and the importance of IL-10 deficiency and decreased epithelial integrity in disease development. However, published data on the PAC IL-10 k.o. model is insufficient to clarify its translational value and the characterisation of its clinical features (*face* validity), underlying pathogenic mechanism (*construct* validity) and its ability to respond to exciting therapeutics (*predictive* validity) needs to be extended.[26] Hence, in this study we characterised and quantified the disease manifestations of the PAC IL-10 k.o. model, including the inflammatory response in ileum and colon. Moreover, we demonstrated that the disease could be prevented and treated with either an antibody neutralising IL-12/23p40 or the broad-spectrum antibiotic, ampicillin. The model was established on a C57BL/6J background, which enables congenic crossing of transgenes and targeted mutations from this frequent background strain.

2. Materials and methods

All experiments were conducted in accordance with the European Communities Council Directive 86/609/ECC for the protection of animals used for experimental purposes and approved by the Danish Animal Experiments Inspectorate, Ministry of Justice, Denmark as well as the internal Ethical Review Council at Novo Nordisk A/S.

2.1. Experimental design

IL-10 k.o. mice backcrossed to C57BL/6J background and control C57BL/6J mice were purchased from The Jackson Laboratory (Bar Harbor, USA) in accordance with a licence agreement with MCG (Munich, Germany). 9–12 week old female mice were used in the experiments. At Novo Nordisk (Maaloev, Denmark) the mice were kept under barrier protected conditions, free of agents listed in FELASA guidelines,[27] with 10 mice per cage in a room with 12-hour light/dark cycle. The bedding was changed weekly and dirty cage bedding was transferred between cages to ensure a homogenous microbial environment. The mice had free access to water and Altromin 1324 standard diet (Brogaarden, Gentofte, Denmark) or piroxicam (Sigma Aldrich, Broendby, Denmark) 200 ppm homogenized in 1324 Altromin diet (Altromin, Lage, Germany). Piroxicam chow was administrated to IL-10 k.o. or wild type (WT) mice ad libitum orally from day