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The application and relevance of *ex vivo* culture systems for assessment of IBD treatment in murine models of colitis

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ABSTRACT

The aim of this study was to investigate the relevance of mouse *ex vivo* cultures as a first screening model for new therapeutic agents of Inflammatory Bowel Disease (IBD). Two murine models (dextran sodium sulphate (DSS)-induced colitis and Gαi2-deficient mice) and two anti-inflammatory agents (methyl-prednisolone and the proteasome inhibitor MG132) were evaluated. The *in vivo* effects of methyl-prednisolone were assessed in both models. *Ex vivo* colonic tissue from both mouse models were cultured in the presence or absence of the drugs and TaqMan Low-Density arrays were used to assess the regulation of inflammatory genes before and after drug treatment. Colitis induced a similar inflammatory gene profile in both mouse models in *in vivo* studies and in *ex vivo* cultures. The differences encountered reflected the different phases of colitis in the models, e.g. innate cytokine/chemokine profile in the DSS model and T cell related markers in Gαi2-deficient mice. After steroid treatment, a similar pattern of genes was suppressed in the two mouse models. We confirmed the suppression of inflammatory gene expression for IL-1β, IL-6 and iNOS in *ex vivo* and *in vivo* colons from both mouse models by quantitative RT-PCR. Importantly, the inflammatory responses in the murine *ex vivo* culture system reflected the *in vivo* response in the inflamed colonic tissue as assessed by changes in inflammatory gene expression, suggesting that the murine culture system can be used for validation of future IBD therapies.

1. Introduction

Human Inflammatory Bowel Disease (IBD), which traditionally is divided in two entities, Crohn's disease (CD) and Ulcerative colitis (UC) is manifested as a chronic inflammation, with relapsing and remitting phases of unknown origin. In an attempt to understand the pathogenesis of IBD, a diverse variety of animal models have been developed with intestinal inflammation sometimes being the only common denominator of all models. The two major groups of animal models are chemically induced colitis and genetically engineered models spontaneously developing colitis (reviewed in [1]). Gαi2-deficient ($G\alpha i2^{-/-}$) mice and colitis induced by dextran sodium sulphate (DSS) are generally considered as models of UC based on clinical symptoms and histopathological changes. Clinical symptoms in the models are body weight loss, diarrhoea, and hunched posture. The histopathological characteristics of the DSS model include distorted epithelial and crypt architecture, infiltration of neutrophils and macrophages early in disease progression accompanied by T and B cell infiltration later in the inflammation [2,3]. Infiltration of inflammatory cells induces production of pro-inflammatory mediators, e.g. IL-1, IL-6 and IL-12 [4,5]. In contrast, the $G\alpha i2^{-/-}$ mice develop a spontaneous colitis at five to seven weeks of age, the inflammation leading to death of the animal within a few weeks from the onset of colitis. The histological changes in these mice are distorted crypt architecture, infiltration of neutrophils and lymphocytes, crypt abscesses and loss of goblet cells. Similar to the DSS model, an elevation in pro-inflammatory and Th1 cytokine mediators is observed in these mice [6]. In addition the $G\alpha i2^{-/-}$ mouse model of colitis has an associated predisposition to development of adenocarcinomas [7]. Currently, assessment of new therapeutic agents for IBD is performed *in vivo* using IBD rodent models. We have recently evaluated the effects of short chain fatty acids in *ex vivo* cultures from DSS-induced colitic mice [8]. To further extend those results, in the

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present study, we evaluated the relevance of mouse colon *ex vivo* cultures as a first screening model for new therapeutic agents. Specifically, we assessed the effects of a well-characterised anti-inflammatory agent, methyl-prednisolone, both *in vivo* and in *ex vivo* cultures of colon obtained from DSS-induced and Gαi2$^{-/-}$ colitic mice. A steroid was chosen as they represent an important class of anti-inflammatory drugs and are the mainstay treatment in IBD patients, especially during acute flares [9]. In addition, the potential anti-inflammatory effect of the proteasome inhibitor MG132, was evaluated in the *ex vivo* cultures. Treatment responses in *ex vivo* cultures were determined by mRNA and protein analysis of inflammatory markers in colonic tissue and supernatants, respectively. In addition to the mRNA analysis, efficacy of treatment *in vivo* was judged by the combination of clinical symptoms, mRNA and protein analysis of inflammatory markers in the colon and histology.

The direct comparison of human and mouse systems brings further understanding on how to translate findings from *in vivo* animal models to the human diseases. Although *in vivo* experiments on animal models of IBD will continue to be a crucial component in the evaluation of new drug candidates, culture systems as described herein, may substantially reduce the number of mice necessary for such studies. In addition, they provide a useful screening method for the assessment of novel anti-inflammatory agents that may be beneficial for the treatment of IBD.

2. Materials and methods

2.1. Mice

2.1.1. DSS colitis

Specific pathogen free female C57BL/6JOlaHsD mice, 7–9 weeks old, weighing 20–24 g, were obtained from Harlan, the Netherlands. Mice were housed in groups of six mice per cage and acclimatised for at least two weeks before entering the study. Animals were kept in the animal house facilities at AstraZeneca R&D Mölndal under standard conditions of temperature and light, and were fed with standard laboratory chow and water *ad libitum*.

2.1.2. Gαi2-deficient colitis

Specific pathogen free female and male Gαi2$^{-/-}$ mice on a 129SvEv background were bred as heterozygotes at the animal facility at Department of Experimental Biomedicine at Göteborg University. Mice were kept in filter top cages with forced ventilation, standard conditions of temperature and light, and were fed with standard laboratory chow and water *ad libitum*. The offspring were genotyped through PCR analysis using tail genomic DNA, and Gαi2$^{-/-}$ mice were monitored every day from four weeks of age, Treatment started the same day as the onset of diarrhoea; generally at the age of five to seven weeks, when the mice weighed 10–16 g. The Local Animal Ethical Committee at Göteborg University approved the studies.

2.2. Induction and assessment of DSS-induced colitis

Three per cent DSS (45 kD; TdB Consultancy AB, Uppsala, Sweden) was administered for five days followed by one day of water to mice in the *in vivo* or 5% DSS for the same number of days in the *ex vivo* study, respectively, as recently described [4,8]. Fresh DSS solutions were prepared daily and mice were monitored for clinical symptoms, i.e. the general health condition of the mice including diarrhoea, rectal bleeding, hunched posture and body weight were recorded daily. The number of mice per group given DSS in the *in vivo* experiment was 11–12; three healthy control mice were given pure water. The inflammatory score reflecting the degree of inflammation in the colon at sacrifice was based on the extent of oedema (0–3), thickness (0–4), stiffness (0–2) and ulcerations (0–1), resulting in a total score of 10 [10].

2.3. Colitis development and assessment of colitis in Gαi2$^{-/-}$ mice

Gαi2$^{-/-}$ mice spontaneously develop a wasting disease at the age of five to seven weeks. The mice were considered to be colitic at the onset of diarrhoea. The general health condition of the mice was monitored daily in the same way as in DSS-induced colitic mice. Wild type littermates were used as healthy controls; the number of mice used in the study was five to six per group containing males and females. The inflammatory score reflecting the degree of inflammation in the colon was based on the same parameters as for DSS-induced colitis. Only Gαi2$^{-/-}$ mice that had documented diarrhoea and thus considered colitic were used for the *ex vivo* cultures.

2.4. In vivo treatment of DSS-induced and Gαi2$^{-/-}$ colitis

Methyl-prednisolone (Sigma–Aldrich, Stockholm, Sweden) was diluted in 0.5% hydroxypropyl methylcellusose (HPMC, Shin-Etsu, Tokyo, Japan) and administrated per-orally at 3, 10 and 30 mg/kg, respectively, once daily for six days in DSS-induced colitic mice. The highest dose of methyl-prednisolone was the most effective in the DSS model shown by the reduction of colonic inflammatory markers, although with a severe loss of body weight (as shown in Section 3). Therefore, Gαi2$^{-/-}$ colitic mice were treated per-orally with 30 mg/kg. Due to the low body weight of these mice at the start of the treatment, mice were only treated every second day with a total of five treatments. Vehicle-treated mice (DSS or Gαi2$^{-/-}$) received 0.5% HPMC. The experiments were terminated 24 h after the last dose followed by collection of plasma and colonic tissue from each animal.

2.5. Tissue and plasma sampling

At the end of the study period, mice were anaesthetized with isoflurane (Abbot Scandinavia AB, Solna, Sweden), blood was collected in EDTA-containing tubes by retro orbital puncture followed by sacrifice of the mouse by cervical dislocation. The intestines were excised and carefully rinsed with NaCl (Gibco, Invitrogen Corp., UK). For DSS colitic mice, the colon was excised in proximity to the ileocecal valve and the rectum, respectively. Half a centimetre of the distal colon was used for RNA analysis. The next three centimetres of the distal colon was used for histological analysis by rolling it as a "Swiss roll", fixing in Zinc-formalin solution (pH 7.4, Histolab Products AB, Göteborg, Sweden) and embedding in paraffin. For Gαi2$^{-/-}$ mice, the colon was excised and the three most distal centimetres of the colon was divided longitudinally. One piece was used for histological analysis as described above, whereas the other piece was directly frozen in liquid nitrogen and used for RNA analysis. The proximal colon was also prepared for histological analysis, as the inflammation in Gαi2$^{-/-}$ mice protrude along the entire colon, whereas the DSS-induced colitis is most pronounced in the distal part of the colon.

2.6. Organ cultures

The colons from mice treated with 5% DSS for five days followed by one day with regular water and colitic Gαi2$^{-/-}$ mice were collected and placed in ice-cold Dulbecco's modified Eagle's medium (DMEM). Prior to culture, the murine colons were rinsed with physiological NaCl to remove faeces and macroscopically assessed for inflammation and in some cases