International Immunopharmacology 43 (2017) 219–226

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Dextran sulphate sodium colitis in C57BL/6J mice is alleviated by *Lactococcus lactis* and worsened by the neutralization of Tumor necrosis Factor α

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ARTICLE INFO

Article history:
Received 19 September 2016
Received in revised form 8 December 2016
Accepted 21 December 2016
Available online 29 December 2016

Keywords:
Lactococcus lactis
TNFα neutralization
DSS model
Colitis
Mice

ABSTRACT

TNFα has a well-established role in inflammatory bowel disease that affects the gastrointestinal tract and is usually manifested as Crohn's disease or ulcerative colitis. We have compared *Lactococcus lactis* NZ9000 displaying TNFα-binding affibody with control *Lactococcus lactis* and with anti-TNFα antibody infliximab for the treatment of mice with dextran sulphate sodium (DSS)-induced colitis. *L. lactis* NZ9000 alleviated the colitis severity one week after colitis induction with DSS, more effectively when administered in preventive fashion prior to, during and after DSS administration. TNFα-binding *L. lactis* was less effective than control *L. lactis*, particularly when TNFα-binding *L. lactis* was administered in preventive fashion. Similarly, an apparently detrimental effect of TNFα neutralization was observed in mice that were intraperitoneally administered anti-TNFα monoclonal antibody infliximab prior to colitis induction. The highest concentrations of tissue TNFα were observed in groups without DSS colitis that were treated either with TNFα-binding *L. lactis* or infliximab. To conclude, we have confirmed that *L. lactis* exerts a protective effect on DSS-induced colitis in mice. Contrary to expectations, but in line with some reports, the neutralization of TNFα aggravated disease symptoms in the acute phase of colitis and increased TNFα concentration in colon tissue of healthy mice. Nevertheless, we have demonstrated that oral administration of bacteria with surface displayed TNFα-binding affibody can interfere significantly with TNFα signaling and mimic the infliximab response in the given animal model of colitis.

1. Introduction

Inflammatory bowel disease (IBD) is an inflammatory disease that typically affects the gastrointestinal tract. Exacerbations and remissions are characteristic clinical features. IBD usually manifests itself as Crohn's disease (CD) or ulcerative colitis (UC). In Europe there are 2.5–3 million people affected, and the incidence of CD and UC is 0.5–10.6 and 0.9–24.3 cases per 100,000 people, respectively [1]. The etiology of the disease is complex and not completely elucidated; however the combination of the patient's genetics, microbiota, immune response and the environment are thought to contribute to disease initiation and progression [2].

In the last decade there has been a substantial effort to investigate the role of microbiota and probiotics in IBD, including its possible treatment strategies, such as fecal microbiota transplantation [3] or probiotic intervention [4]. The therapeutic effects of various probiotics on experimental colitis have been studied extensively. The most frequently studied are lactic acid bacteria (LAB), and beneficial health effects have already been established for several of these [5]. LAB from the genera *Lactobacillus* and *Bifidobacterium* have been particularly successful in the treatment of experimental (dextran sulphate sodium (DSS)-induced) colitis [6]. *Lactococcus lactis*, on the other hand, has been mostly regarded as a model LAB with industrial importance [7]. In recent years however, there have been several reports confirming its ameliorating effect in experimental colitis, particularly for the strains *L. lactis* subs. *lactis* S-SU2 [8], *L. lactis* NCDO 2118 [9], *L. lactis* subsp. *cremoris* FC [10] and Lactococcus lactis I-1631 [11]. The beneficial effects of *L. lactis* have been further improved by genetic engineering of *L. lactis* for the delivery of therapeutic recombinant proteins to the intestine [12]. Among other delivered proteins, IL-10 [13], anti-TNFα nanobody [14], trefoil factor [15], LcrV [16] and protease inhibitors [17] have demonstrated improvement of symptoms of DSS-induced colitis. Non-immunoglobulin scaffold-based cytokine binding proteins, such as engineered derivatives of immunoglobulin-binding domain of protein

Abbreviations: TNFα, Tumor Necrosis Factor α; DSS, dextran sulphate sodium; IBD, inflammatory bowel disease; CD, Crohn's disease; UC, ulcerative colitis; LAB, lactic acid bacteria; GM-17, M-17 medium with 0.5% glucose.

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http://dx.doi.org/10.1016/j.intimp.2016.12.027
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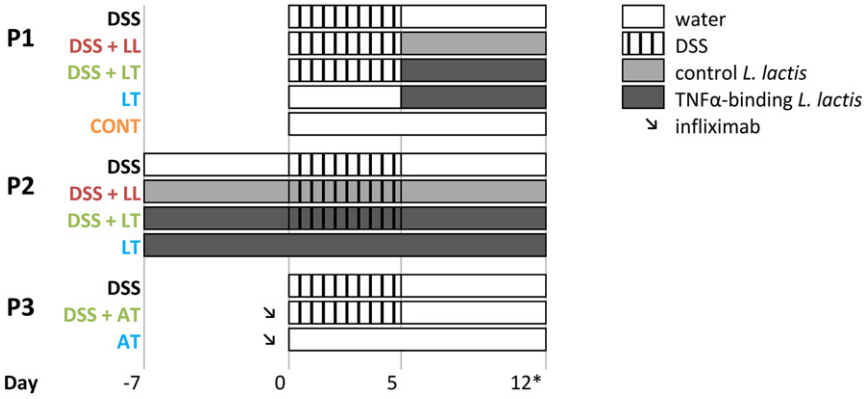

Fig. 1. Schematic representation of protocols of induction and treatment of DSS colitis. P1 represents a curative setup in which mice were treated with bacteria after colitis induction. P2 represents preventive setup in which mice were treated before, during and after colitis induction. P3 represents infliximab control setup. At the end of the protocols (day 12) mice were euthanized (asterisk). DSS: mice with DSS induced colitis. DSS + LL: mice with DSS induced colitis treated with control *L. lactis*. DSS + LT: mice with DSS induced colitis treated with TNFα-binding *L. lactis*. DSS + AT: mice with DSS induced colitis pretreated with infliximab. LT: healthy mice treated with TNFα-binding *L. lactis*. AT: healthy mice pretreated with infliximab. CONT: healthy control mice.

A – affibodies, can also be delivered by recombinant LAB [18–20]. Anti-TNFα affibody non-covalently attached to the lactococcal surface was able to bind recombinant TNFα *in vitro* [19].

TNFα is a cytokine with a well-established role in IBD, and its neutralization by monoclonal antibodies has become a vital approach in IBD therapy in humans [21]. TNFα is also abundant in the feces of IBD patients [22] and oral administration of avian antibodies against TNFα improved symptoms of experimental colitis in rats [23], these two facts providing a rationale for intraluminal neutralization of TNFα in IBD. Our aim was thus to compare short-term (one week) effects of oral administration of *L. lactis* with surface-displayed small protein binder anti-TNFα affibody [19,20] with control *L. lactis* and anti-TNFα antibody infliximab on acute phase of colon inflammation in a mouse model of DSS-induced colitis. The latter is a well-established and frequently used animal model for testing new strategies for the treatment of IBD [24–27], including probiotic interventions [6,8,28].

2. Materials and methods

2.1. Bacterial growth and culture conditions

L. lactis NZ9000 [29,30] was grown in M-17 medium (Merck, Darmstadt, Germany) supplemented with 0.5% glucose (GM-17) and 10 μg/mL chloramphenicol at 30 °C without aeration. Electroporation of *L. lactis* with pSDZ-TNF (surface display of TNFα-binding affibody) [19] or pNZ8148 (control) [29,30] was performed as described [31], using a Gene Pulser II apparatus (Biorad, Hercules, USA). Overnight cultures of *L. lactis* NZ9000 harboring the appropriate plasmid (pSDZ-TNF or pNZ8148) were diluted (1:100) in 500 mL of fresh GM-17 medium, grown to an A_{600} of 0.80 and supplemented with 25 ng/mL nisin (Fluka AG, Buchs, Switzerland) to induce recombinant protein expression. Three hours after induction, the culture was centrifuged at 5000 ×*g* for 20 min and the supernatant decanted. The pellet was resuspended in the growth medium at a concentration of 2.5–3.0 × 10^{10} cells/mL and stored at 4 °C until used (max. 3 days).

2.2. Animals and experimental protocol

Seven to eight week old female C57BL/6JOlaHsd mice (Medical experimental centre, Ljubljana, Slovenia) were used. The mice were housed 5 per cage (Ehret, Germany; 825 cm^2 floor space) under standard controlled environmental conditions, with a 12-h light/dark cycle on bedding material (Lignocel, Germany). Mice received drinking water and standard diet for laboratory mice (Altromin 1324, Germany) *ad libitum*.

Colitis was induced by the addition, over 5 consecutive days, of DSS (3% DSS; TdB Consultancy AB, Uppsala Sweden, molecular mass 40 kDa) dissolved in drinking water, provided *ad libitum*. Fresh DSS solutions were made every morning. Mice were treated by adding bacterial culture with confirmed bacterial count and viability daily to the fresh drinking water to a final concentration of 2.0–2.5 × 10^9 cells/mL, or by intraperitoneal administration of 10 mg/kg anti-TNFα monoclonal antibody. Water consumption was monitored daily and, on average, mice consumed 3–5 mL of water per mouse daily.

Three different experimental protocols were designed to assess the treatment of colitis (Fig. 1). Mice were treated for one week after the exposure to DSS (curative setup, protocol P1), for one week before, during and after the exposure to DSS (preventive setup, protocol P2), or by administration of single dose of anti-TNFα monoclonal antibody prior to DSS exposure (protocol P3; according to [32]). Mice were divided into groups of 10 animals in each protocol. Groups were treated with DSS (or water) and administered with TNFα-binding *L. lactis*, control *L. lactis*, monoclonal antibody against TNFα (Infliximab; Merck & Co - gift of Kemofarmacija, Slovenia), or were used as controls, according to the scheme shown in Fig. 1.

The clinical picture, body weight of mice, and water/DSS consumption were monitored on a daily basis during the experiment. The weight of each mouse was normalized to the average mouse weight on the first day of the experimental protocol. The clinical picture was evaluated by determining the state of mice, consistency of feces and gross blood loss in feces.

Table 1
Scoring of DSS-induced histological changes.

Grade	Extent of inflammation	Neutrophil infiltration	Mononuclear cell infiltration	Alteration in crypts	Epithelial regeneratory atypia
0	None	None	Normal	None	None
1	Mucosa	Scarce	Scarce	Cryptitis	Hyperplasia
2	Mucosa + submucosa	Mild	Mild	Crypt abscess	Indefinite dysplasia
3	Mucosa + submucosa + muscle layer	Moderate	Moderate		Low grade dysplasia
4	Transmural	Severe	Severe		High grade dysplasia