Biochemistry and Biophysics Reports 12 (2017) 29–39

Contents lists available at ScienceDirect

Biochemistry and Biophysics Reports

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

Transcriptional modulation of pattern recognition receptors in chronic colitis in mice is accompanied with Th1 and Th17 response

Bin Zheng[a], Mary E. Morgan[a], Hendrik J.G. van de Kant[a], Johan Garssen[a,b], Gert Folkerts[a], Aletta D. Kraneveld[a,*]

[a] Division of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Faculty of Science, Utrecht University, Utrecht 3586 CG, The Netherlands
[b] Nutricia Research, Utrecht 3508 TB, The Netherlands

ARTICLE INFO

Keywords:
Inflammatory bowel disease
Toll like receptors
Th cell responses

ABSTRACT

Pattern recognition receptors (PRRs) may contribute to inflammatory bowel diseases (IBD) development due to their microbial-sensing ability and the unique microenvironment in the inflamed gut. In this study, the PRR mRNA expression profile together with T cell-associated factors in the colon was examined using a chronic colitis mice model. 8–12 week old C57BL/6 mice were exposed to multiple dextran sodium sulfate (DSS) treatments interspersed with a rest period to mimic the course of chronic colitis. The clinical features and histological data were collected. The mRNA expressions of colonic PRRs, T cell-associated components were measured. Finally, the colons were scored for Foxp3+ cells. During chronic colitis, the histological data, but not the clinical manifestations demonstrated characteristic inflammatory symptoms in the distal colon. In contrast to acute colitis, the expression of all *Toll-like receptors (Tlrs)*, except *Tlr5* and *Tlr9*, was unaffected after repeated DSS treatments. The expression of *Nod1* was decreased, while *Nod2* increased. After third DSS treatment, only the expressions of *Tlr3* and *Tlr4* were significantly enhanced. Unlike other PRRs, decreased *Tlr5* and increased *Tlr9* mRNA expression persisted during the chronic colitis period. As the colitis progress, only the mRNA expression of *Ifn*γ and *Il17* staid increased during chronic colitis, while the acute colitis-associated increase of *Il23,* and *Il10* and *Il12* was abolished. Finally, increased histological score of Foxp3+ cell in colon was found during the chronic colitis period. This study provides an expression pattern of PRRs during chronic colitis that is accompanied by a Th1- and Th17 cell-mediated immune response.

1. Introduction

Inflammatory bowel disease (IBD) is a chronic gastrointestinal disorder and is characterized by a relapse-remitting course that is caused by recurrent intestinal inflammation [1]. The two major forms of IBD, Crohn's disease (CD) and ulcerative colitis (UC), often have an onset during early adulthood and significantly affect the quality of the life [2]. The established high prevalence of IBD population in US and Europa indicate the requirement for an efficient treatment for IBD [2]. However, the existing treatments are mainly focus on relieving of symptoms and are often accompanied with unwanted side effects [3]. To elucidate the disease pathogenesis and develop more efficient treatment, there is growing interest for targeting pattern recognition receptors (PRRs). The unique microenvironment in the gut, where abundant microorganisms co-exist and the microbial-sensing ability of PRRs suggest that PRRs could contribute to both maintaining and breakdown of the intestinal homeostasis [4,5]. The development of gut dysbiosis and imbalances in host-microbiome interaction has been demonstrated to contribute to the extent, severity and chronicity of intestinal inflammation [2]. Furthermore, an aberrant immune response towards gut bacteria has been suggested to be the major contributor in the inflammatory response of IBD [6].

Toll-Like receptors (TLRs) are possibly the best studied PRRs that sense a broad spectrum of invading pathogens by recognize pathogen-associated molecular patterns (PAMPs), unique molecules on these pathogens [7]. Up to date, 13 TLRs have been discovered, from which TLR1-9 are conserved in both human and mouse. Based on the location, these TLRs can be divided into two groups; receptors expressed on the cell surface (TLR1, TLR2, TLR6, TLR4 and TLR5) and intracellular receptors (TLR3, TLR7, TLR8 and TLR9) [7]. To recognize the specific ligand, the TLRs form functional mono-dimers or a functional complex with other component such as MD-2 (for TLR4), except TLR1, TLR2 and TLR6 [7]. Both TLR1 and TLR6 form functional hetero-dimer complex with TLR2 to recognize triacyl- or diacyl-lipopeptides from bacteria,

* Corresponding author.
E-mail address: a.d.kraneveld@uu.nl (A.D. Kraneveld).

http://dx.doi.org/10.1016/j.bbrep.2017.08.009
Received 5 January 2016; Received in revised form 2 September 2016; Accepted 17 August 2017
Available online 22 August 2017

respectively [8]. Upon activation by specific ligand derived from bacteria, fungi, parasites or virus, they will induce innate immune response and strength adaptive immune response to provide protection against pathogens [9]. However, unregulated activation of TLRs could lead to extensive and chronic inflammation, which results in inflammatory disease such as IBD [4,10]. In agreement with this hypothesis, increased expression of TLRs such as TLR2, TLR3 and TLR4, are found in the colon of IBD patient [11,12]. In addition, an association was found between the polymorphism of TLRs and the susceptibility of IBD development [13–15].

Nucleotide-binding oligomerization domain-containing protein (human: NOD; mice Nod) is another PRR family. Two member of this PRR group, NOD1 and NOD2 are located exclusively intracellular and able to detect peptidoglycan, a cell wall component on bacteria [16]. There has been great interest in studying the role of NOD2 and its related receptor NOD1 in IBD development, because NOD2 encoding gene *NOD2* is the first gene that has been directly associated with CD and confer great risk for the development of IBD [17,18]. The CD risk variant *NOD2* gene causes 'loss of function' resulting in reduced autophagy induction which result in reduced bactria killing and impaired antigen presenting, which could be the trigger to the development of IBD [19].

Experimental animal models have been used to study the pathology of the IBD and clarify the underlying mechanisms [20]. Among these animal models, DSS-induced colitis in mice is most commonly used model to investigate IBD-like colitis due to its simplicity and the ability to induce predictable intestinal inflammation [21,22]. While single DSS exposure induces colitis modeling acute injury and repair mechanism, repeated DSS exposure cycles interspersed with recovery period mimic the chronic nature of IBD [23,24].

In our previous study, we have illustrated the colonic expression pattern of PRRs and the T-helper (Th) cell response in mice using a DSS-induced acute colitis model. To extent our knowledge of the expression of these PRRs and activated immune response during chronic colitis, the mRNA expression of these PRRs in the colon during chronic colitis was determined after repeated DSS exposure. In addition, the T cell development during the chronic colitis was monitored by measuring the mRNA expression of T cell-associated master transcription factors and cytokines.

2. Materials and methods

2.1. Animals

Female C57BL/6 mice were purchased from Charles River Laboratories (Maastricht, the Netherlands). All mice were used at 8–12 weeks of age and were housed under standard conditions in the animal facilities at Utrecht University. All animal experiments were approved by and were in accordance with the guidelines of the Dutch Experimental Animal Commission. The approval document is encoded with 2008.II.03.030.

2.2. Experimental colitis

Chronic colitis was induced in groups of 6 mice by administration of 3 cycles 1.5% DSS to the drinking water of the mice for 6 days with a rest period of 10 days. Colitis development was monitored by measuring the bodyweight and scoring the feces condition during the experiment and measuring the colon length/weight ration after sacrificing the mice on the end of each DSS treatment cycle (day 7, day 23 and day 39). The feces condition assessment was started from experimental day 5 until the end of the experiment (day 39). The feces condition score was determined from two parameters: stool consistency (0 = normal, 1 = soft with normal form, 2 = loss of form/diarrhea) and fecal bleeding (0 = no blood, 1 = blood observation using Colo-rectal Test kit (Axon Lab AG, Germany), 2 = blood observation without test).

2.3. Histological evaluation of colon damage and immunohistochemical staining

After sacrificing the mice on the end of each DSS treatment cycle (day 7, day 23 and day 39), colons (n = 3) were taken out for histological evaluation and immunohistochemical staining. The colon was opened longitudinally; half of each colon was washed in the phosphate buffered saline (PBS) and placed on a piece of blotting paper. After fixing in 10% formalin for 24 h, colons were paraffin-embedded as swiss-roles and sectioned (5 μm). Two researchers assessed general inflammatory features blindly after staining sections with hematoxylin and eosin according the assessment system described before [25]. Briefly, the histological assessments included four pathological criteria: the extent of cellular infiltration (0: no infiltration, 1: infiltration between the crypts, 2: infiltration in the submucosa, 3: infiltration in the muscularis externa, 4: infiltration in entire tissue); cover area of cellular infiltration in the region (0: no infiltration, 1: < 25%, 2: 25–50%, 3: 50–75%, 4: > 75%); loss of crypts (0: no damage, 1: 30% shortening of crypts, 2: 65% shorting of crypts, 3: total loss of crypts, 4: loss of entire epithelial layer); extent of crypts loss in the region (0: no crypt loss, 1: < 25%, 2: 25–50%, 3: 50–75%, 4: > 75%). Individual scores were obtained for the whole colon from caecum to anus by two researchers who did not know the origin of the sample.

Immunohistochemistry was employed to determine the Ly-6B.2+ cells (neutrophils & some activated macrophages) and Foxp3+ cells. The sections were subjected to a heat-induced epitope retrieval step. Slides were washed with PBS and blocked with rabbit or goat serum before an overnight incubation (4 °C) with primary antibodies against Ly-6B.2 (AbD Serotec, Dusseldorf, Germany) or Foxp3 (eBioscience San Diego, CA USA). For detection, biotinylated goat anti-rat (Dako, Glostrup, DK) secondary antibodies were administered followed by incubation with peroxidase-labeled streptavidin (Vectastain EliteABC kit, Vector, Burlingame, CA USA). The peroxidase activity was visualized using the substrate, DAB (Sigma, Gillingham, UK). The cell nuclei were visualized by a short incubation with Mayer's hematoxylin (Klinipath, Duiven, the Netherlands). Background staining was determined by substituting the primary antibody with a rat IgG isotype control (Abcam, Cambrige, UK, Fig. S6). The neutrophil infiltration score and Foxp3+ cell score were determined using the following criteria: the extent of Ly6B.2+ or Foxp3+ cellular infiltration (0: no infiltration, 1: infiltration between the crypts, 2: infiltration in the submucosa, 3: infiltration in the muscularis externa, 4: infiltration in entire tissue); cover area of cellular infiltration in the region (0: no infiltration, 1: < 25%, 2: 25–50%, 3: 50–75%, 4: > 75%). Individual scores for Ly6B.2+ cellular infiltration were obtained for the whole colon from caecum to anus. Individual scores for Foxp3+ cellular infiltration were tallied for the proximal colon (characterized by bulges in the colon wall) and the distal colon (the region starting from end of proximal portion stretching to the anus). Scoring was performed by two researchers who did not know the origin of the sample

2.4. Assessment of myeloperoxidase concentration in the colon tissue

After sacrificing the mice, colons (n = 3) were taken out for myeloperoxidase (MPO) concentration assessment, marker for neutrophils. The colon was opened longitudinally; half of each colon was separated into proximal colon (characterized by bulges in the colon wall) and distal colon (the region starting from end of proximal portion stretching to the anus). Subsequently, the colons pieces were transferred into RIPA buffer (Thermo Scientific, Rockford, IL USA) and homogenized using a Precellys®24-Dual homogenizer (Precellys, Villeurbanne, France). The homogenates were centrifuged at 14,000 rpm for 10 min at 4 °C and the MPO concentration in the supernatant was measured using an ELISA kit according to the manufacturer's protocol (Hycult biotech, Uden, the Netherlands).