International Immunopharmacology 59 (2018) 361–368

Contents lists available at ScienceDirect

International Immunopharmacology

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

Fecal microbiome from patients with ulcerative colitis is potent to induce inflammatory responses

Shaoxuan Wang[a,*], Li Yao[b,**], Yanyan Liu[c]

[a] Department of Gastroenterology, The First People's Hospital of Jining, Jining, Shandong, China
[b] Department of Gastroenterology, Ningxia People's Hospital, Yinchuan, Ningxia, China
[c] Outpatient Clinic of Internal Medicine, The First People's Hospital of Jining, Jining, Shandong, China

ARTICLE INFO

Keywords:
Fecal microbiome
Monocyte
Ulcerative colitis

ABSTRACT

Ulcerative colitis (UC) is a chronic idiopathic disease affecting the colon. Patients with UC display a number of alterations in immune-related molecules and cells, as well as dysbiosis in the intestinal microbiota. It remains unclear whether the alterations in the patients' immune systems are initiating factors of UC or a result from external insults. Also, precisely how these intestinal microorganisms affect UC development is not completely understood. To answer these questions, fecal bacteria were collected from UC patients during the active phase (UC-active), UC patients during the remission phase (UC-remission), and healthy controls. The fecal bacteria were then used to stimulate monocytes from three additional healthy subjects. We found that fecal bacteria from both UC-active and UC-remission patients presented higher capacity than fecal bacteria from healthy controls, resulting higher expression of MHC class I and MHC class II molecules, as well as higher expression of costimulatory molecules CD80 and CD86. The production of multiple cytokines, including IL-6, TNF-α, IL-10, and IL-12, were higher following stimulation with fecal bacteria from UC-active and UC-remission patients. Notably, when fecal bacteria were diluted to lower concentration, the bacteria from UC-active patients was clearly more effective at activating monocytes than those from UC-remission patients and controls. Collectively, our results revealed that the fecal bacteria from UC patients could cause stronger inflammatory responses than fecal bacteria from healthy controls.

1. Introduction

Ulcerative colitis (UC) is a chronic inflammation affecting the colon, and may result in bloody diarrhea, abdominal pain, weight loss, and developmental delays. Though not directly life threatening, UC is often debilitating and can produce dangerous complications [1]. Several risk factors, such as familial history, smoking, the use of certain drugs, and the presence of risk loci, are associated with UC development [2–5], but the immediate pathogenic factors remain unknown.

Patients with active UC display a number of alterations in immune-related molecules and cells. Elevated TLR-2 and TLR-4 expression was observed in the lamina propria and the colonocytes [6]. Dendritic cells from UC patients presented higher levels of costimulatory molecules [7]. Antibodies against colonic proteins were detected s at increased frequency in UC patient than in Crohn's disease patients and normal controls [8]. A number of T cell-related dysregulations are characterized in UC patients. It was found that UC patients presented exacerbated inflammation of modified Th2 cells, with increased IL-4, IL-5, and IL-13 mRNA and/or protein expression in the T cells and the rectal biopsies [9–11]. Recent investigations also implicated IL-9 and Th9 cells in UC development [12]. It remains unclear whether the alterations in the patients' immune systems were initiating factors of UC or a result from external insults.

UC patients also display dysbiosis in the intestinal microbiota, characterized by decreased biodiversity, higher proportions of gammaproteobacteria, sulfite-reducing deltaproteobacteria, and enterobacteriaceae, and lower proportions of firmicutes and bacteroidetes [13–16]. In recent years, fecal microbiota transplantation, a strategy to alter and repopulate the intestinal tract of patients with microorganisms from healthy individuals, is being developed for the treatment of UC. The pooled estimate of UC patients who achieved clinical remission after fecal microbiota transplant was approximately 40% across various studies [17]. These lines of evidence suggest that dysbiosis in the intestinal tract is critical to UC development, but precisely how these intestinal microorganisms promoted UC pathogenesis is not completely understood.

* Correspondence to: S. Wang, Department of Gastroenterology, The First People's Hospital of Jining, Jining, Shandong, China.
** Correspondence to: L. Yao, Department of Gastroenterology, Ningxia People's Hospital, 301 ZhengYuan North Road, Yinchuan, Ningxia, China.
E-mail addresses: wang632217318@sina.com (S. Wang), yaoli06240624@sina.com (L. Yao).

https://doi.org/10.1016/j.intimp.2018.04.014
Received 28 December 2017; Received in revised form 31 March 2018; Accepted 9 April 2018

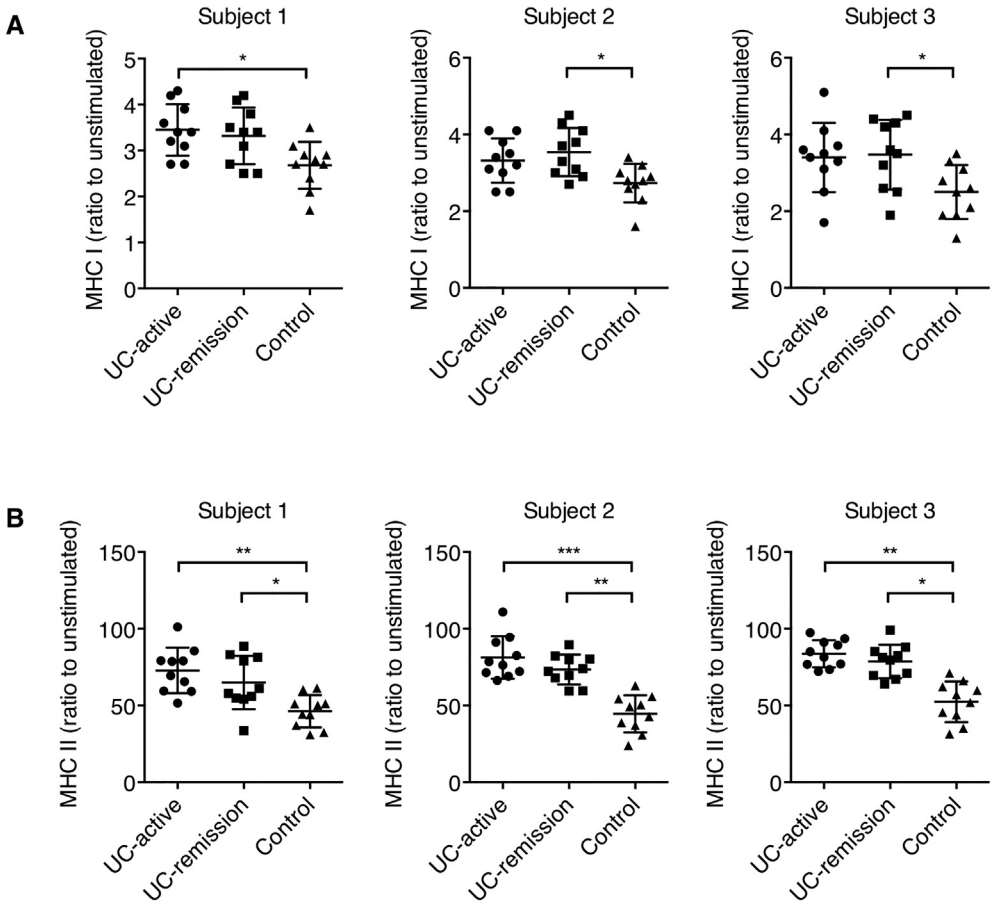

Fig. 1. Expression of MHC molecules by monocytes/macrophages following incubation with fecal bacteria.
Fecal bacteria from ten UC-active, ten UC-remission, and ten control subjects were added to monocytes from three healthy subjects at 1 μL stock to 5×10^4 monocytes. The expression of (A) MHC class I molecules and (B) MHC class II molecules by monocytes/macrophages was examined 72 h after stimulation, and the values were expressed as the ratio of MHC expression level after 72-h stimulation to MHC expression level before stimulation. Kruskal-Wallis ANOVA and Dunn's comparisons. $^{*}P < 0.05$. $^{**}P < 0.01$. $^{***}P < 0.001$.

In this study, we collected fecal specimens from UC patients with active disease, UC patients during remission, and normal controls. The effect of fecal microorganisms on the immune cells from three healthy individuals was investigated.

2. Materials and methods

2.1. Study participants

UC-active and UC-remission patients were diagnosed and recruited at the First People's Hospital of Jining. The UC-active patients included five men and five women between 22 and 48 years of age. The UC-remission patients included six men and four women between 25 and 47 years of age. In addition, ten healthy individuals without inflammatory bowel disease, autoimmune diseases, infections in the gastrointestinal tract within the past year, diabetes, obesity, or other systemic inflammations were recruited as controls. The control group included five men and five women between 20 and 49 years of age. No study participant was taking antibiotic treatment in the three months prior to sample collection. Fecal samples were collected from all subjects in a sterile stool container. Immediately following collection, the stool samples were frozen and stored at −80 °C until use. Peripheral blood specimens were collected from three additional healthy individuals (not included in the ten healthy controls that provided stool). The PBMCs were harvested by standard Ficoll-Paque (Sigma) centrifugation. The study was approved by the Ethics Board of the First People's Hospital of Jining. All subjects provided written consent form.

2.2. Fecal bacteria collection

Thawed fecal samples were weighed and suspended in sterile PBS at 1 g stool per 10 mL PBS. The fecal samples were vortexed extensively, and then centrifuged at 1500 rpm for 5 min at 4 °C. The solid pellet was discarded, and the supernatant was transferred to a clean sterile tube and centrifuged for two additional rounds at the same conditions, with the solid pellet discarded after each round. The remaining solution was transferred to 2-mL sterile tubes and centrifuged at 12,000 rpm for 30 min at 4 °C. After discarding the supernatant, the pellet containing the bacteria was resuspended in sterile PBS, vortexed and pelleted again at the same conditions, and resuspended in sterile PBS to $OD_{600} = 0.05$. This fecal bacteria-containing solution was considered the 1× stock solution.

2.3. Monocyte processing

Monocytes from healthy PBMCs were isolated *via* magnetic negative selection by applying the Human Monocyte Enrichment Kit (Stemcell) following the manufacturer's protocol. The monocytes were then seeded at 5×10^4 monocytes per well in a 24-well plate and incubated in 100% humidity, 5% CO_2, and 37 °C. 1 μL of fecal bacteria at 1 μL of 1×, 0.33×, and 0.1× stock solution were added to the monocytes in antibiotic-free RPMI 1640 supplemented with 15% FBS and 1× GlutaMax (Thermo Fisher) for 6 h, at which time the monocytes were firmly attached to the bottom of the plates. The culture supernatant containing excess bacteria was then removed by tilting the plate and aspiration, and was replaced by RPMI 1640 supplemented with 15% FBS, 1× GlutaMax, and 100 U/mL penicillin-streptomycin (Thermo Fisher) for an additional 66 h incubation. Monocytes without any bacterial stimulation were processed in the same way and maintained as unstimulated controls. All experiments were performed under sterile conditions.