International Immunopharmacology 40 (2016) 1–10

Contents lists available at ScienceDirect

International Immunopharmacology

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

High fat diet exacerbates dextran sulfate sodium induced colitis through disturbing mucosal dendritic cell homeostasis

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ARTICLE INFO

Article history:
Received 20 April 2016
Received in revised form 16 August 2016
Accepted 17 August 2016
Available online xxxx

Keywords:
Inflammatory bowel disease
High fat diet
Butyrate
Dendritic cell
Mucosal immunity
Retinoic acid

ABSTRACT

Epidemiological studies have shown that fat rich western diet contributes to the high incidence of inflammatory bowel disease (IBD). Moreover, accumulated data indicated that fat dietary factor might promote the change of the composition and metabolism in commensal flora. But, the exact mechanisms for fatty diet in gut inflammation are not well demonstrated. In this study, we found that high fat diet (HFD) promoted inflammation and exacerbated the disease severity of dextran sulfate sodium (DSS) induced colitis in mice. Compared with low fat diet (LFD)/DSS mice, shorter colon length, more epithelial loss and crypt destruction and more Gr-1$^+$ myeloid inflammatory cells infiltration in colons were observed in HFD/DSS cohorts. Interestingly, such HFD mediated inflammation accompanied with the dys-regulation of hematopoiesis, and more hematopoiesis stem and progenitor cells were detected in colon and spleen. We further analyzed the effects of HFD and DSS treatment on mucosal DC subsets, and found that DSS treatment in LFD mice mainly dramatically increased the percentage of CD11c$^+$CD103$^-$CD11b$^+$ DCs in lamina propria (LP). While, in HFD/DSS mice, HFD pre-treatment not only increased the percentage of CD11c$^+$CD103$^-$CD11b$^+$ DCs, but also decreased CD11c$^+$CD103$^+$CD11b$^+$ in both LP and mesenteric lymph nodes (MLN) in mice with colitis. This disequilibrium of mucosal dendritic cells in HFD/DSS mice may depend on the reduced levels of buytrate and retinoic acid. Thus, this study declared the effects of HFD on gut microenviroment, and further indicated its potential role in the development of DSS induced colitis.

1. Introduction

Inflammatory bowel disease (IBD) is a multi-factorial complex disorder, including Crohn's disease and ulcerative colitis [1–4], which is characterized with intestinal chronic inflammation of gastrointestinal tract resulted from genetic susceptibility, immune system dysfunction and environmental factors [5,6]. What we have known about the pathogenesis of inflammatory bowel disease (IBD) suggests that nutrition and dietary factors play important roles in IBD by modulating immune function [7–9]. Actually, the incidence of IBD is high, especially in western country, with up to 1 in 1000 people suffers from this disease [10,11], and the incidence of IBD has largely risen in developing countries which have changed dietary habits [12]. However, litter is known about the mechanisms involved in IBD and fat rich western diet.

As the site of digestion and nutrient absorption, the mammalian gastrointestinal tract is induced tolerance to resident commensal microorganisms and food antigen, but responses to pathogenic microorganisms [13]. Such immune homeostasis in gut depends on the immune regulation mediated by resident immune cells in gut-associated lymphoid tissue and its resident microenvironment. Recently, multiple innate and adaptive immune cells in gut have been identified, includes regulatory T cells (Tregs) [14], CD103$^+$ dendritic cells [15], intestinal CD11b$^+$-CD11c$^-$ macrophages [16]. Indeed, these cells play vital roles in maintaining immune tolerance and mediating immune response in gut. More recently, many studies further showed that the homeostasis of colonic Tregs or mucosal CD103$^+$ DC cells regulated by short chain fatty acids or retinoid acid (RA) [17–19]. Thus, any changes in the composition of these metabolites may interfere the crosstalk between the gut microenvironment and immune cells, and then may destroy immune homeostasis and promote inflammation in gut. Here, we confirmed this hypothesis that high fat diet feeding exacerbated intestinal inflammation and tissue damage in DSS induced colitis, which through

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http://dx.doi.org/10.1016/j.intimp.2016.08.018
1567-5769/

altering the composition of gut metabolites, and then destroyed colonic dendritic cell homeostasis.

2. Materials and methods

2.1. Animal and high fat diet fed model

High fat diet fed mice were prepared as previously described with modification [20]. In brief, male C57BL/6 mice were purchased from the animal facility of Yangzhou University at age of 6–12 weeks. After being fed with normal diet for one week, mice were separated into two groups, one was fed with low fat diet (LFD, 2.9 kcal/g, 13% kcal from fat, Teklad 2914), another group was fed with high fat diet (HFD, 5.24 kcal/g, 60% kcal from fat, Bioserv F3282) for 6–8 weeks followed by further experiments. All animal experimental protocols were approved by the Scientific Investigation Board of Jiangsu University in China.

2.2. Dextran sulfate sodium (DSS) induced colitis

Acute colitis was induced as described before [21]. Briefly, dextran sulfate sodium (DSS, 36–50 kDa, MP Biomedicals) was dissolved in drinking water, and colitis was induced with 2% DSS in drinking water for 7 days. The DSS solutions were changed every day to maintain fresh until the end of the experiment. The severity of colitis in clinic was determined as disease activity index (DAI) score every two days by scoring the changes in animal weight loss, rectal bleeding and presence of diarrhea. Base on the sum of the body weight score, the diarrheal score and the bloody stool score, DAI score was cored for 12 grades. On day 7, mice were sacrificed, the entire of colon (from the cecum to the anus) was quickly excised, and the colon length was measured as one of markers of inflammation. As DSS treatment mainly causes severe mucosal injury in proximal colon, the proximal colons from the middle were collected for further experiments. In some experiments, colon samples were stored at −80 °C until used in cytokines profiles analysis or fixed in 10% buffered formalin for histological examination.

2.3. Single cell suspension preparation

The lamina propria (LP) cells isolation was prepared as described with modificaiton [22]. Firstly, the entire of colon was harvested carefully. After fat tissue and Peyer's patches were removed, colon was further opened longitudinally and cleaned away luminal contents with PBS. Next, cut colon into small pieces of 0.5 cm in length, and epithelial cells and mucus were removed by incubating colon pieces in PBS with 5% FCS and 2 mM EDTA at 37 °C in shaking water bath for two sequential 20 min. The media containing epithelial cells and cell debris were discarded, and colon pieces were further minced into smaller 2 mm^2 pieces and digested in PBS with 5% FCS, 1 mg/mL collagenase IV (Invitrogen), and 40 U/mL DNase I (Takara, Nanjing, Jiangsu, China) for 20 min at 37 °C in shaking water bath. The digested cell suspensions were then filtrated with 100-μm strainers and pelleted by centrifugation at 300*g* for 15 min. After being washed with PBS, then carefully added on 40% Percoll solution, and further separated by centrifugation at 1400*g* for 20 min at 23 °C. To isolate bone marrow (BM) cells, femurs and tibias were collected from mouse. After cleaning muscle, bone marrow cells were flushed out using ice-cold PBS. To prepare splenocytes, spleens were grinded and cell suspensions were passed through strainers. Red blood cells in bone marrow cells and splenocyte suspension were removed using ACK lysis buffer.

2.4. Antibodies and flow cytometer analysis

All fluorescence-conjugated antibodies for flow cytometer analysis, including anti-CD11b, anti-Gr-1, anti-CD45, anti-Lineage (containing B220, CD4, CD8, Gr-1, Mac-1, Ter-119), anti-CD117, anti-Sca-1 were purchased from eBioscience (San Diego, CA). For cell surface markers staining, cells were firstly incubated with rat serum for Fc receptors blocking at 4 °C for 15 min, and then incubated with fluorescence-conjugated antibodies at 4 °C for 20 min. After being washed with PBS for three times, cells were analyzed on the FACSCalibur flow cytometer (B.D. Biosciences). Data were analyzed using CellQuest software (B.D. Biosciences) or the FlowJo (Tree Star Inc.).

2.5. Q-PCR analysis

Total RNA was extracted from murine BM, spleen, liver and colon using Trizol (SuPerfec TRI, USA). Then total RNA was reverse-transcribed into cDNA using RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific USA). Q-PCR analysis was performed using SYBR Green Premix Ex Taq™ (Takara, Dalian, Liaoning, China). The primers were designed using Primer 5 software and synthesized by Sangon (Shanghai, China). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a reference gene. The PCR mixture (10 μL), consists of 5 μL SYBR Premix Ex Taq™, 0.5 μL cDNA, 0.2 μL sense primer, 0.2 μL antisense primer and 4.1 μL ddH_2O, which pre-degeneration temperature was 95 °C for 30 s, degeneration temperature was 95 °C for 5 s, annealing temperature was 58 °C for 20 s and elongating temperature was 72 °C for 30 s. The amount of DNA is calculated from cycle numbers using the $2^{-\Delta\Delta Ct}$ method, the results are normalized to housekeeping gene GAPDH from the same sample. Primers used in q-PCR analysis are listed below:

- Murine *GAPDH*, forward 5′-GGCATTGCTCTCAATGACAA-3′ reverse 5′-TGTGAGGGAGATGCTCAGTG-3′
- Murine *IL-10*, forward 5′-ACAACATACTGCTAACCGACTC-3′ reverse 5′-TGGGGCATCACTTCTACCAG-3′
- Murine *IL-1β*, forward 5′-AGCCTTTCAGGGAATTAAGCTC-3′ reverse 5′-TCCCAAGATCAACCGATGGAC-3′
- Murine *IL-6*, forward 5′-GAGGAGACTTCACAGAGGATAC-3′ reverse 5′-GACTCTGGCTTTGTCTTTCTTG-3′
- Murine *TNF-α*, forward 5′-GAACTGGCAGAAGAGGCACT-3′ reverse 5′-GGTCTGGGCCATAGAACTGA-3′
- Murine *MCP-1*, forward 5′-ATGCAGGTCCCTGTCATG-3′ reverse 5′-GCTTGAGGTGGTTGTGGA-3′
- Murine *SDF-1α*, forward 5′-GAGAGCCACATCGCCAGAG-3′ reverse 5′-TTTCGGGTCAATGCACACTTG-3′
- Murine *CXCR4*, forward 5′-AGCATGACGGACAAGTACC-3′ reverse 5′-GATGATATGGACAGCCTTACAC-3′
- Murine *SCF*, forward 5′-TATGATAACCCTCAACTATGTCGCC-3′ reverse 5′-TTCTTTTATATTCTTCGGTGCGTTT-3′
- Murine *ALDH1a1*, forward 5′-GGGAAAGAGCCCTTGCATTGTGTT-3′ reverse 5′-GCGACACAACATTGGCCTTGATGA-3′
- Murine *ALDH1a2*, forward 5′-ACCGTGTTCTCCAACGTCACTGAT-3′ reverse 5′-TGCATTGCGGAGGATACCATGAGA-3′
- Murine *ALDH1a3*, forward 5′-TCAACAAGATAGCCTTCACCGGCT-3′ reverse 5′-TTGAAGAACACTCCCTGGTGAGCA-3′

2.6. Colony forming assay

Colony forming unit assay was performed using MethoCult™ GF M3434 (Stemcell) according to the protocol from manufacturer. Briefly, murine Splenocytes or LP cells were counted and suspended in IMDM with 2% FBS at final concentration 2×10^6/mL (splenocytes) or 3×10^6/mL (LP cells). After transferring 0.3 mL of prepared cells to 2.7 mL MethoCult™ medium, the tubes were vortexed vigorously to mix cells and medium thoroughly. Then, tubes were let stand for 2–5 min to allow bubbles to dissipate. 1.1 mL of cells-MethoCult mixture was added carefully by syringe attached with 16-gauge blunt-end needle to each 35 mm culture dishes (Corning). The dishes were then incubated with 5% CO_2 and 95% humidity at 37 °C for at least