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IL-33 induces both regulatory B cells and regulatory T cells in dextran sulfate sodium-induced colitis

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ABSTRACT

Interleukin (IL)-33 is a member of the IL-1 family. Serum levels of IL-33 are increased in inflammatory bowel diseases (IBD), suggesting that IL-33 is involved in the pathogenesis of IBD, although its role is not clear. In this study, we investigated the role of IL-33 in the regulation of T-helper (Th) cell and B cell responses in mesenteric lymph nodes (MLN) in mice with dextran sulfate sodium (DSS)-induced colitis. Here, we showed that IL-33-treated mice were susceptible to DSS-induced colitis as compared with PBS-treated mice. The production of spontaneous inflammatory cytokines production by macrophages or dendritic cells (DC) in MLN significantly increased, and the responses of Th2, regulatory T cells (Treg) and regulatory B cells (Breg) were markedly upregulated, while Th1 responses were significantly downregulated in MLN of IL-33-treated mice with DSS-induced colitis. Our results demonstrate that IL-33 contributes to the pathogenesis of DSS-induced colitis in mice by promoting Th2 responses, but suppressing Th1 responses, in MLN. Moreover, IL-33 treatment increased Breg and Treg responses in MLN in mice with DSS-induced colitis. Therefore, modulation of IL-33/ST2 signaling is implicated as a novel biological therapy for inflammatory diseases associated with Th1 responses.

1. Introduction

Inflammatory bowel disease (IBD) is a chronic form of inflammation of the gastrointestinal tract encompassing ulcerative colitis (UC) and Crohn's disease (CD) [1]. Although the precise etiology of IBD remains unclear, imbalanced cytokine production and T cell dysfunction are considered to be key factors in the pathogenesis of IBD [2]. Less than a decade ago, CD was designated as a T-helper (Th) 1-mediated condition based on elevated production of interleukin (IL)-12 and interferon gamma (IFN-γ), whereas T cells in UC produce excessive amounts of Th2-type cytokines, such as IL-5 and IL-13. However, the Th1/Th2 paradigm was revised with the discovery of Th17 and regulatory T cells (Treg) [3].

IL-33 (also known as IL-1F11) is a recently discovered IL-1 family member [4]. IL-33 is produced by endothelial cells, epithelial cells, fibroblastic cells, lymphoid cells and nerve cell [5]. IL-33 is a ligand for the IL-1 receptor-related protein ST2 [6] (also known as T1, IL-1RL1, DER4), which is a member of the TLR/IL1R superfamily. There are at least three isoforms of ST2: a soluble form (sST2), a membrane-bound form (ST2L), and a constitutively active variant (ST2 V) [7]. sST2 negatively regulates IL-33 signaling, acting as a decoy receptor, whereas ST2L is a functional component of IL-33 receptor [8]. ST2L is expressed predominantly in immune cells, including eosinophils, basophils, Th2 cells, dendritic cells (DC), natural killer (NK) cells, mast cells, and keratinocytes [9,10]. Binding of IL-33 to the ST2L/IL-1RAcP complex leads to the recruitment of myeloid differentiation primary-response 88 (MyD88) and activation of IL-1R associated kinase (IRAK) 1 and 4, which induce two independent pathways, involving mitogen-activated protein kinases (MAPKs) and nuclear factor κB (NF-κB), causing gene expression [11].

Several lines of evidence support a clear role for IL-33 in promoting the production of Th2-associated cytokines and specifically inhibiting IFN-γ production in vivo and in vitro [12–14]. In contrast, a few studies show that IL-33 amplifies both Th1- and Th2-type responses [15,16]. Several studies have shown that IL-33 plays a pathogenic role in experimental colitis [17–20]. However, several recent studies suggest that IL-33 attenuates mucosal inflammatory responses in the gut [21–23]. Recent studies have shown that IL-33 levels are significantly increased in the peripheral blood and intestinal tissues of patients with IBD [20,24,25], Furthermore, a few studies have shown increased IL-33 mRNA levels in human biopsy specimens from untreated or active UC patients compared to controls [19,26–29]. Thus, the effects of IL-33 are either

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pro- or anti-inflammatory depending on the disease and the model [30]. These results suggest that IL-33 is involved in the pathogenesis of IBD. Despite some knowledge of the roles of IL-33 in IBD, the roles of IL-33 in the regulations of Th cell and B cell differentiation and functions in IBD are highly intricate and remain to be fully elucidated. Moreover, effector cells from the mesenteric lymph nodes (MLN) act as an effector tissue in gastrointestinal inflammation [31]. Therefore, we conducted the current study to elucidate the roles of IL-33 in the regulation of MLN immune responses during intestinal inflammation.

2. Materials and method

2.1. Mice

Seven-week-old male C57BL/6 mice weighing 18–22 g were purchased from Beijing HFK Bioscience Co., LTD (Beijing, China). The mice were maintained under special-pathogen-free (SPF) conditions at China Medical University for at least one week before being used in experiments. All the animal procedures were approved by the China Medical University Animal Care and Use Committee.

2.2. Expression and purification of recombinant IL-33

The cDNA fragment encoding mature IL-33 protein was amplified from mouse spleen cDNA using specific primer pairs: 5′-GCTGAATTCATGAGCATCCAAG GAAC-3′ (forward) and 5′-CCGCCTCGAGGATTTTCGAGAGCTTAAACA-3′ (reverse). The resulting amplified fragment was inserted into the expression vector pET21a (+) at the *EcoR*I–*Xho*I sites to yield the construct pET21a-IL-33. This construct was transformed into *Escherichia coli* strain BL21DE3, and the recombinant IL-33 protein was induced by isopropyl-D-thiogalactoside (IPTG) and Ni-agarose purified using the 6× His-Tagged Protein Purification Kit (Beijing CoWin Biotech, Beijing, china), followed by the removal of endotoxin that might have come from the host cells. Endotoxin removal was carried out with ToxinEraser™ Endotoxin Removal Kit (GenScript, Nanjing, China). The purity of recombinant IL-33 exceeded 95%. Endotoxin from the host cells was removed using the ToxinEraser™ Endotoxin Removal Kit (GenScript). The level of endotoxin was less than 1EU/mg of protein, as determined using the ToxinSensor™ Chromogenic LAL Endotoxin Assay Kit (GenScript). Bioactivity was checked by titration of recombinant IL-33 to lymphocytes of MLN and assaying IL-5 production by enzyme-linked immunosorbent assay (ELISA). The biological activity of recombinant IL-33 was comparable with commercially obtained protein (Abcam, Cambridge, UK).

2.3. Antibodies

Fcγ receptor-blocking mAb (CD16/32; 2.4G2), anti-Gr-1(RB6-8C5), anti-CD4 (RM4-5), anti-CD44 (IM7), anti-IL-17A (TC11-18H10.1), anti-IL-10 (JES5-16E3), anti-IFN-γ (XMG1.2), purified anti-CD3 and anti-CD28 (37.51) were purchased from BD Biosciences (San Diego, CA, USA). Anti-F4/80 (BM8), anti-B220 (RA3-6B2), anti-TCRβ (H57-597), anti-NK1.1 (PK136), anti-TCRγδ (GL3) and anti-CD25 (PC61), anti-CD19 (MB19-1), were purchased from Biolegend (San Diego, CA, USA).

2.4. Induction of colitis by DSS

For induction of colitis, mice were administered 2.5% DSS (MW 36,000–50,000, MP Biomedicals, USA) in their drinking water for 7 days (day 0-day 6). Mice were administered intraperitoneally with either IL-33 (1 μg in 100 μL PBS/mouse/day) or PBS (100 μL mouse/day, as control) on daily basis. The disease activity index (DAI) was determined by scoring the loss of body weight, stool trait, and occult blood in the stool or hematochezia (over the entire period) according to the following classic scoring system by Cooper [32]. The scoring process is given as follow: body weight loss (0, none; 1, 1%–5%; 2, 5%–10%; 3, 10%–20%; 4, >20%), stool consistency (0, normal; 2, loose stools; 4, diarrhea), and stool blood (0, negative; 2, fecal occult blood test positive; 4, gross bleeding). All animals were sacrificed on day 6 following DSS-induced colitis. The colons were removed and cleaned for histological analyses. MLN lymphocytes were extracted for analysis by flow cytometry, quantitative real-time PCR and ELISA.

2.5. Histological assessment of colitis

The colon specimens were fixed with 4% paraformaldehyde, embedded in paraffin, and stained with hematoxylin-eosin (HE). Histology was scored as described previously [33]: epithelium (E): 0, normal morphology; 1, loss of goblet cells; 2, loss of goblet cells in large areas; 3, loss of crypts; 4, loss of crypts in large areas; and infiltration (I): 0, no infiltrate; 1, infiltrate around the crypt base; 2, infiltrate reaching the L muscularis mucosae; 3, extensive infiltration reaching the L muscularis mucosae and thickening of the mucosa with abundant edema; 4, infiltration of the L submucosa. The total histologic score was given as E + I.

2.6. Lymphocytes of MLN isolation

Lymphocytes of MLN were gently disrupted with a sterile syringe plunger and passed through a nylon cell strainer (40-μm mesh; BD Biosciences, San Jose, CA, USA) with PBS containing 5% FBS. After centrifugation at 1500 rpm at room temperature for 5 min, the cells were washed with 1× PBS. The number of viable cells was counted using the trypan blue exclusion method.

2.7. Cytokine analysis by enzyme-linked immunosorbent assay (ELISA)

To measure spontaneous cytokine production by lymphocytes isolated from MLN, the cells (5×10^5/well) were cultured for 24 h in vitro in 96-well flat-bottomed plates without any stimulation at 37 °C under 5% CO_2. To measure cytokine production by T cells in lymphocytes isolated from MLN, the cells were incubated for 48 h in vitro in 96-well flat-bottomed plates (Falcon; BD Biosciences) coated with anti-CD3 (10 μg/mL) and anti-CD28 (1 μg/mL) antibodies. The culture supernatants were harvested and assayed for cytokine concentration using ELISA kits (R&D Systems) according to the manufacturer's instructions.

2.8. Confirmation of transcription factor mRNA expression by quantitative real-time PCR

Total RNA was extracted from lymphocytes isolated from MLN using the RNAiso plus (Takara, Dalian, China) according to the manufacturer's instruction. Total RNA (1 μg from each sample) was reverse-transcribed to generate first strand cDNA using the PrimeScript™ RT reagent Kit with gDNA Eraser (Perfect Real-Time) (Perfect Real-Time, Takara). The PCR mixture was prepared using SYBR® Premix Ex Taq™ (Tli RNaseH Plus, Takara) and one of primers listed in Table 1 in a total volume of 25 μL. Each PCR amplification was performed using the following parameters: 95 °C for 30 s; and 40 cycles of 95 °C for 15 s and 60 °C for

Table 1
Primer sequences for real-time PCR.

Gene	Forward	Reverse
Foxp3	GGCCCTTCTCCAGGACAGA	GCTGATCATGGCTGGGTTGT
GATA-3	ACAGCTCTGGACTCTTCCCA	GTTCACACACTCCCTGCCTT
T-bet	CCAGGGAACCGCTTATATGT	CTGGGTCACATTGTTGGAAG
ROR-γt	CCACTGCATTCCCAGTTTCT	CGTAGAAGGTCCTCCAGTCG
β-actin	TTCCAGCGTTCCTTCTTGGGT	GTTGGCATAGAGGTGTTTACG