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Alimentary Tract

pVAX1-A20 alleviates colitis in mice by promoting regulatory T cells

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ABSTRACT

Aim: To investigate whether the intrarectal administration of the ubiquitin E3 ligase A20 (A20) attenuates intestinal inflammation and influences regulatory T cells in experimental colitis.
Methods: A dextran sulfate sodium induced chronic colitis mouse model was established. The symptoms and manifestations of colitis and the severity of colonic mucosal inflammation were evaluated. The protective role of A20 expression in the intestine was analyzed after the administration of a pVAX1-A20 recombinant eukaryotic vector, which was encapsulated into poly(L-lactide-co-glycolide) as a nanoparticle.
Results: pVAX1-A20 administration markedly ameliorated colonic tissue damage and reduced intestinal inflammation via the suppression of the mucosal mitogen-activated protein kinase and nuclear factor (NF)-κB signaling cascade. Furthermore, pVAX1-A20 promoted the splenic regulatory T cell population and forkhead box P3 expression in colonic tissue.
Conclusion: A20 plays a key role in the regulation of intestinal inflammation and that the overexpression of A20 in the intestine protects mice from dextran sulfate sodium induced chronic colitis.

1. Introduction

Inflammatory bowel disease (IBD) is a group of chronic inflammatory conditions of the colon and small intestine, including Crohn's disease (CD) and ulcerative colitis (UC), that are characterized by periods of activation and remission, sometimes with severe complications [1]. The pathological characteristics involve inappropriate immune cell activation and pro-inflammatory cytokine overproduction, causing intestinal mucosa damage [2]. As an immune disease, IBD is currently incurable, and the goal of treatment is to induce remission. Medications for IBD, including anti-inflammatory drugs, immune-modulators and biologic agents, are employed according to the extent of disease, and those agents are ineffective for some refractory IBD [3]. Novel, effective, therapeutic strategies for IBD are required.

The ubiquitin E3 ligase A20, also known as tumor necrosis factor alpha induced protein 3 (TNFAIP3), is a cytoplasmic protein that is involved in the negative feedback regulation of NF-κB activation. A protective role for A20 was observed in inflammation models, such as atherosclerosis and collagen induced arthritis [4]. Genetic studies have revealed that genes encoding A20 polymorphisms and mutations are strongly related to the pathogenesis of inflammatory autoimmune diseases, including systemic lupus erythematosus, rheumatoid arthritis, type 1 diabetes and IBD [5]. A20 is expressed in various cell types and regulates multiple immune cell functions, and the expression of A20 in intestinal epithelial cells plays a role in the response to intestinal microorganism derived molecules [6]. A20 ablation increased mice hypersensitivity to dextran sulfate sodium (DSS) induced colitis and tumor necrosis factor (TNF)-α induced intestinal inflammation [5]. Thus, A20 should be an attractive agent for the treatment of colitis.

The immune homeostasis in the intestine is tightly regulated, which protects the intestine from being invaded by pathogenic organisms and antigens, and prevents skewed immune response-induced inflammation [7]. Deficiencies in immune regulatory factors and excessive inflammatory responses may lead to chronic inflammation in the intestine, such as IBD [8]. Regulatory T cells

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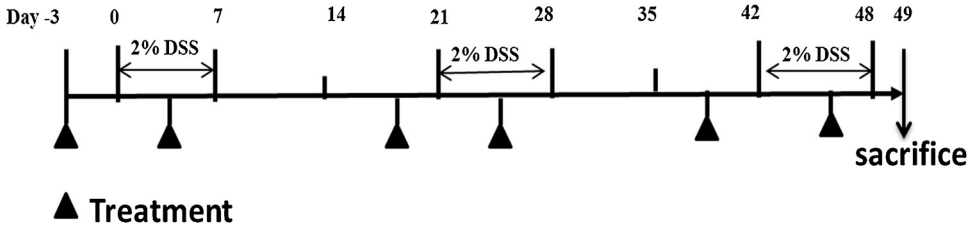

Fig. 1. Schematic outline of chronic colitis induction and treatment. C57BL/6 mice were given 3 cycles of seven-day 2% DSS treatment with two 14-day intervals to induce chronic colitis, which served as the DSS group. For the evaluation of the therapeutic effects of pVAX1-A20 on DSS-induced colitis, the DSS + pVAX1-A20, DSS + pVAX1 and DSS + sulfasalazine groups were used, with the mice in each group receiving pVAX1-A20 (20 μg per mouse), pVAX1(vehicle control, 20 μg per mouse), and sulfasalazine (as a positive control, 0.9 mg per mouse) treatment, respectively, via intrarectal instillation on days −3, 4, 18, 39, and 46 of chronic colitis establishment. DSS: Dextran sulfate sodium.

(Tregs) play important roles in maintaining intestinal homeostasis through the downregulation of effector T cell activation and the induction of immune tolerance to self-antigens [9]. The Treg population is insufficient in colitis and the way to increase Tregs in the intestinal mucosa still keeps challengeable [10].

The chemically induced murine colitis models reflect some key immunological and histopathological features of human IBD [11]. Trinitro-benzene-sulfonic acid (TNBS) induced acute colitis can only provide limited information regarding human colitis and is suitable for studying the physiology of acute inflammation and wound healing in the intestinal mucosa. The long-term oral administration of DSS via drinking water induces severe colitis in mice, showing symptoms of weight loss, bloody diarrhea, ulcer formation and inflammatory cell infiltration, that well mimics the chronic course of human IBD and the features of inflammation in colitis [11,12]. By establishing a DSS induced chronic colitis mouse model and constructing a eukaryotic expression vector, pVAX1-A20, this study has been designed to investigate whether the administration of A20 attenuates intestinal inflammation and influences the Treg response in experimental colitis.

2. Materials and methods

2.1. Animals and the induction of the chronic colitis mouse model

Female age-matched C57BL/6 mice (6–8 weeks; with an average weight of 18 g) were purchased from the Guangzhou Experimental Animal Center and were housed under pathogen free conditions. C57BL/6 mice were chosen for they are susceptible for DSS colitis induction [11]. Animal suffering and the number of animals used in this study were minimized. The experimental procedures were approved by the Animal Ethics Committee at the ENT Institute of Shenzhen. Mice were acclimated for 7 days before experiments and were monitored daily for signs of distress and advanced colitis during experiments. Chronic colitis was induced by the oral administration of dextran sulfate sodium (DSS) (molecular weight 36,000–50,000 Da, MP biochemical, Irvine, CA, USA), according to published procedures [11]. Briefly, mice were exposed to 3 cycles of seven-day 2% DSS administration (DSS in tap water), with fourteen-day DSS free tap water intervals (Fig. 1).

2.2. In vivo experimental design

Fifty female C57BL/6 mice were randomly divided into five groups (n = 10 per group). The control group received drinking water only. Mice in the DSS group were exposed to 3 cycles of seven-day 2% DSS administration (DSS in tap water) with fourteen-day DSS free tap water intervals for the induction of chronic colitis. For the evaluation of the therapeutic effects of pVAX1-A20 on DSS-induced colitis, the DSS + pVAX1-A20, DSS + pVAX1, DSS + sulfasalazine groups were used, and mice in each group received pVAX1-A20 (20 μg per mouse), pVAX1 (vehicle control, 20 μg per mouse), and sulfasalazine (Tong Yao Jituan, Shanxi, China) (as a positive control, 0.9 mg per mouse) treatment, respectively, via intrarectal instillation on days −3, 4, 18, 39, 46 (Fig. 1) during the chronic colitis induction procedures. Body weight, stool consistency and colonic hemorrhage were inspected daily. Mice were sacrificed by cervical dislocation under isoflurane (Youcheng Biotech, Shenzhen, China) anesthesia at day 49. Colons, from the ileocecal junction to the anal verge, were collected for colonic shortening calculations and morphology observations. The disease activity index (DAI) score was assessed for each animal as a cumulative score for the severity of colitis, according to the stool consistency (score: 0, normal; 1 and 2, loose stools; 3 and 4, diarrhea), rectal bleeding (score: 0, normal; 1 and 2, bloody stool; 3 and 4, gross bleeding), and body weight loss (score: 0, none; 1, 1%–5%; 2, 5%–10%; 3, 10%–20%; 4, >20%) [13].

2.3. Tissue processing

The distal colon from each mouse was excised and fixed in 4% buffered paraformaldehyde for 12 h. After washing in phosphate-buffered saline (PBS), colon tissues were dehydrated in alcohol and embedded in paraffin at 56 °C for 24 h. Sections (4 μm) were prepared and stained with hematoxylin and eosin (H&E). Images were taken by microscope (Nikon, Tokyo, Japan) (magnification: 100×) for the evaluation of colon microscopic mucosa damage and inflammation. Histopathological changes were analyzed as a cumulative score, based on inflammation severity (0, none; 1, slight; 2, moderate; 3 severe), inflammation extent (0, none; 1, mucosa; 2, mucosa and submucosa; 3, transmural), and crypt damage (0, none; 2, basal 1/3 damaged; 3, basal 2/3 damaged; 4, entire crypt and epithelium lost), according to the previously reported scoring system [14].

2.4. Western blot analysis

Total protein was extracted from the colon tissue with a protein extraction buffer [M-PER™ Mammalian Protein Extraction Reagent (cat. no. 78503); Thermo Fisher, Shanghai, China], supplemented with a protease inhibitor cocktail. After denaturing, the protein samples (10 μg) were loaded in duplicate onto a 10% sodium dodecyl sulfate polyacrylamide gel; the proteins were separated by electrophoresis and transferred onto a nitrocellulose membrane. The membrane was blocked by 5% skim milk for 30 min and incubated with one of the following primary antibodies (50–100 ng/ml): rabbit anti-mouse Foxp3 (BD Biosciences, New Jersey, USA), rabbit anti-mouse A20, p-Erk, Erk, p-p38, p38, p-JNK, JNK, NF-κB, p-NF-κB, and β-actin (Cell Signaling Technology, Inc, Beverly, MA, USA). After washing, the nitrocellulose membranes were incubated with the corresponding peroxidase labeled goat anti-rabbit IgG (Abcam, Shanghai,

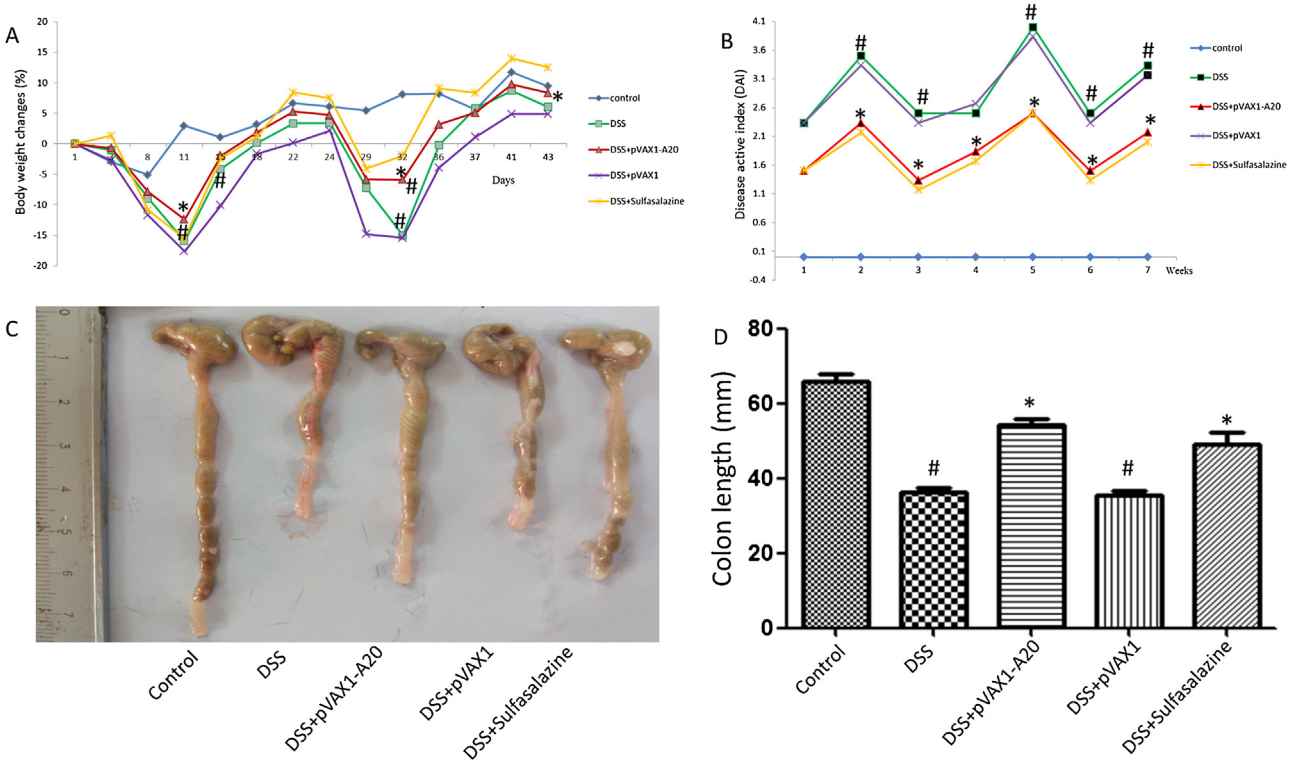

Fig. 2. pVAX1-A20 ameliorates the manifestations of dextran sulfate sodium induced chronic colitis. Chronic colitis was induced in C57BL/6 mice with 2% DSS administration, and mice were treated with either pVAX1-A20, pVAX1 or sulfasalazine, following the procedures shown in Fig. 1. The colitis manifestations in each group were monitored, and mice were sacrificed on day 49 for the further evaluation of colitis. (A) Body weight changes were recorded from days 1 to 43. (B) Disease activity index (DAI). (C-D) Colon morphology and length in each group. Data are from three independent experiments and are presented as the mean ± SD. #P < 0.05 versus the control group, *P < 0.05 versus DSS group or DSS + pVAX1 group. DSS: Dextran sulfate sodium.

China). The immune complex on the membrane was developed by enhanced luminol-based chemiluminescence (BioSpectrum, Upland, CA, USA), and the results were photographed using the UVP BioSpectrum Imaging system (BioSpectrum, Upland, CA, USA). Densitometry analysis of the protein relative expression was measured by Image J (National Institutes of Health, USA).

2.5. Reverse transcription-quantitative polymerase chain reaction (RT-qPCR).

Total RNA from colon samples was extracted by the RNeasy mini kit (Qiagen, Inc., Valencia, CA, USA). A total of 1 μg RNA was reverse-transcribed into cDNA with the IScript™ cDNA synthesis kit (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The resulting complementary DNA was then subjected to qPCR using the 7500 Real-Time PCR Systems platform (Thermo Fisher Scientific, MA, USA). The primers used in the experiments were as follows: Foxp3: forward: 5′-CACCCAGGAAAGACAGCAACC-3′ and reverse: 5′-GCAAGAGCTCTTGTCCATTGA-3′; β-actin: Forward: 5′-TACAGCTTCACCACCACAGC-3′ and reverse: 5′-TCTCCAGGGAGGAAGAGGAT-3′; IL-4: forward: 5′-TACCAGGAGCCATATCCACGGATG-3′ and reverse: 5′-TGTGGTGTTCTTCGTTGCTGTGAG-3′; IL-6: forward: 5′-CCGGAGAGGAGACTTCACAG-3′ and reverse: 5′-TCCACGATTTCCCAGAGAAC-3′; IFN-γ: forward: 5′-ATGAACGCTACACACTGCATC-3′ and reverse: 5′-CCATCCTTTTGCCAGTTCCTC-3′. The target gene expression level relative to the expression level of a housekeeping gene was analyzed using the comparative quantification cycle method.

2.6. Flow cytometry

Splenic mononuclear cells were isolated and cultured for 48 h, and then they were fixed with 1% formaldehyde and 0.1% Triton X-100 for 30 min at 4 °C, washed 3 times with 1% bovine serum albumin (BSA) in PBS, and blocked for 30 min at 4 °C with 1% BSA. Cells were incubated with APC labeled rat anti-mouse CD4, FITC rat anti-mouse CD25 and PE rat anti-mouse Foxp3 (BD Biosciences, New Jersey, USA) antibodies or isotype IgG for 1 h at room temperature. After washing with PBS, the cells were analyzed with a flow cytometer (FACSCanto II; BD Biosciences, Franklin Lakes, NJ, USA). The results were analyzed with FlowJo (FlowJo, LLC, USA), using the data of isotype IgG as a gating reference.

2.7. Statistical analysis

SPSS 13.0 software was used for statistical analysis. All values are presented as the mean ± standard deviation (SD) of a minimum of three independent experiments. The data was analyzed using a one-way analysis of variance, followed by Tukey's test for multiple comparisons. P < 0.05 was considered a statistically significant difference.

3. Results

3.1. pVAX1-A20 protects mice from developing chronic colitis

The recombinant pVAX1-A20 expression vectors were constructed and encapsulated into poly(L-lactide-co-glycolide) (PLGA) (Sigma, Shanghai, China) to form nanoparticles before rectal administration (see Supplementary data and Fig. S-1). To investigate the therapeutic effects of pVAX1-A20 on chronic colitis, we

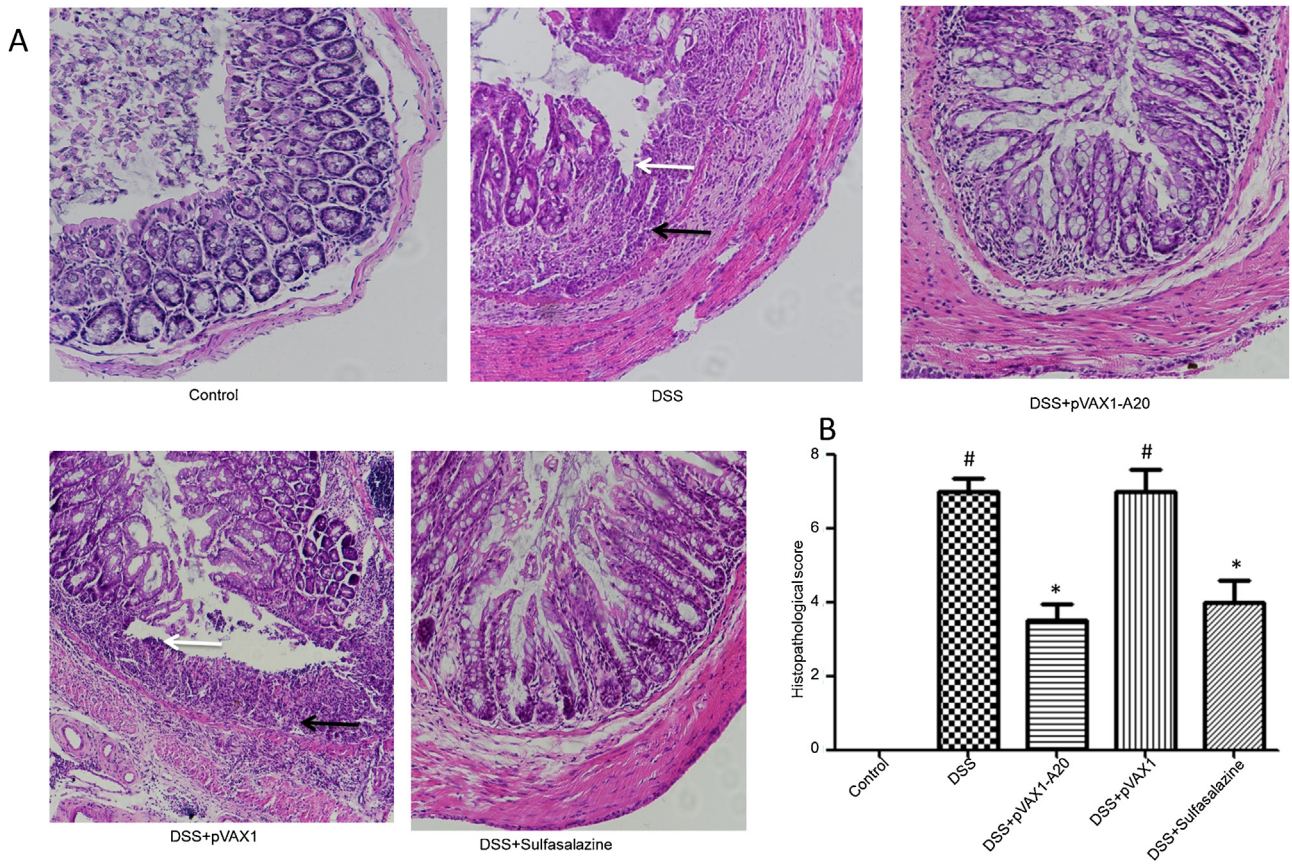

Fig. 3. pVAX1-A20 protects mice from dextran sulfate sodium induced colonic damage. (A) Representative HE staining (magnification 100×) of colonic sections from each group. (B) Histogram represents the histopathological evaluation of the colonic damage from each group. Black arrow, area of transmural inflammatory cells inflammation with loss of crypt structure and goblet cells; white arrow, ulceration. Data are from three independent experiments and are presented as the mean ± SD. #P < 0.01 versus the control group, *P < 0.01 versus DSS group or DSS + pVAX1 group. DSS: Dextran sulfate sodium.

induced chronic colitis in C57BL/6 mice by the administration of 2% DSS and challenged the mice with pVAX1-A20, pVAX1 (vehicle control) or sulfasalazine, through rectal instillation. The colitis manifestations in each group were evaluated (Fig. 2). Compared with the control group, which showed a steady weight increase and no diarrhea, bloody stools or shortened colons, the DSS group and the DSS + pVAX1 group developed severe colitis, including rapid body weight loss from days 3 to 18, after the first administration of 2% DSS, which recovered during days 18–24, followed by further body weight loss from days 24 to 36, after the second challenge with 2% DSS (Fig. 2A). The DAI score increased weekly throughout the induction procedure in the DSS group and the DSS + pVAX1 group compared to that in control group ($P < 0.05$) (Fig. 2B), and shortened colons were observed in the DSS group and the DSS + pVAX1 group at day 49 when compared with the control group (Figs. 2C and D). The groups that received rectal treatments with pVAX1-A20 (20 μg per mouse) and sulfasalazine (0.9 mg per mouse) showed alleviated body weight loss and more rapid recovery than that DSS group and the DSS + pVAX1 group ($P < 0.05$). The colitis manifestations, such as diarrhea, bloody stools, colon length shortening and DAI scores, were also ameliorated after the pVAX1-A20 treatment compared with those of the DSS group ($P < 0.05$) (Fig. 2). These results suggest that pVAX1-A20 protects mice from developing 2% DSS induced chronic colitis.

3.2. pVAX1-A20 attenuates colonic damage on microscopic evaluations

Colonic tissues were sectioned and stained by HE for histopathological analysis. The colonic tissues in the DSS group and the DSS + pVAX1 group showed severe damage compared with those from the control group: The mucosa and submucosa showed fibrosis and collagen deposits, distorted crypts, ulceration, disruption of the epithelium, and loss of goblet cells, with large numbers of infiltrating inflammatory cells and thickened muscularis propria (Fig. 3A). Colonic destructions were ameliorated after treatment with pVAX1-A20 or sulfasalazine in the DSS + pVAX1-A20 group and the DSS + sulfasalazine group, respectively (Fig. 3A), and the histopathological damage score was obviously relieved after treatment when compared with the DSS group (Fig. 3B) ($P < 0.05$).

3.3. pVAX1-A20 promotes the Treg population

To elucidate whether the protective effects of pVAX1-A20 administration on 2% DSS induced chronic colitis were related to the induction of Tregs, we further assessed the Tregs in the spleen by flow cytometry. The results showed that the frequency of CD4 + T cells from splenic mononuclear cells was approximately 13% in each group (Fig. 4A). The frequency of CD4 + CD25 + Foxp3 + T cells in the pVAX1-A20 treatment group increased sharply (18.1%) compared with those in the DSS group (7.09%) and the DSS + pVAX1 group (7.79%) ($P < 0.01$) (Fig. 5B and C). CD4 + CD25 + Foxp3 + T cells in the DSS induced colitis group also slightly increased compared with the control group (5.18%) ($P < 0.05$). In addition, after the administration of sulfasalazine, CD4 + CD25 + Foxp3 + T cells (11.3%) in the DSS + sulfasalazine group were significantly increased compared with the DSS group ($P < 0.05$) (Fig. 4B and C). The results suggest that pVAX1-A20 treatment promotes Treg proliferation in the mouse spleen under conditions of DSS induced chronic colitis.

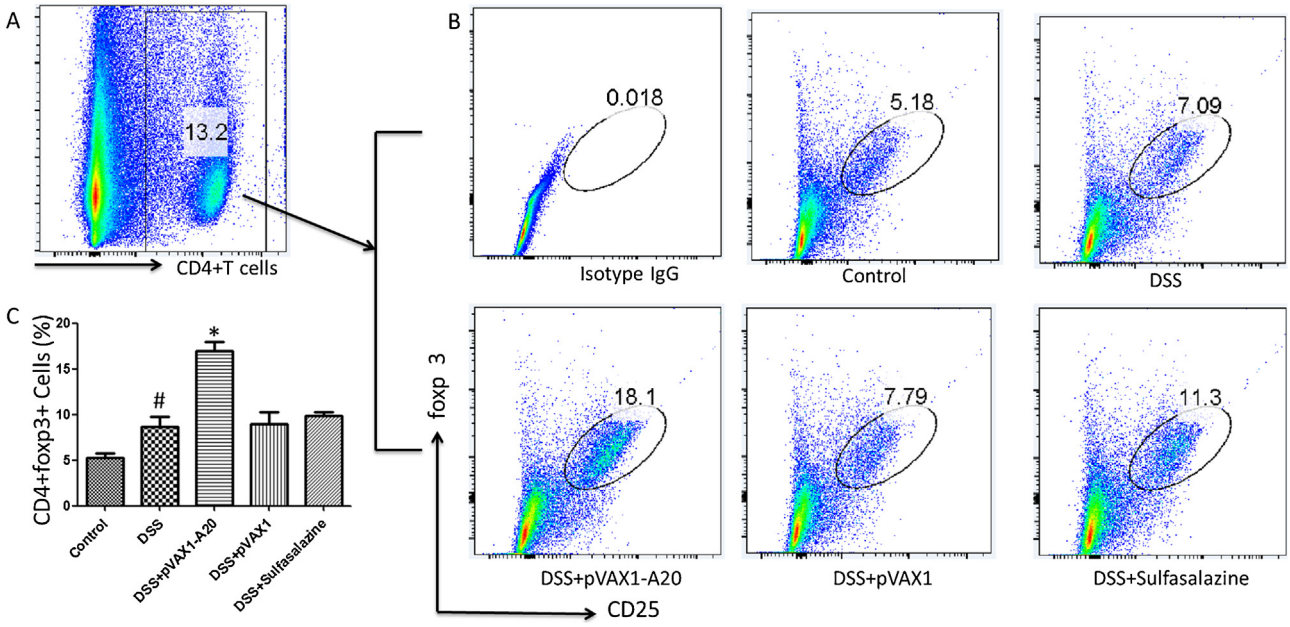

Fig. 4. pVAX1-A20 increases the regulatory T cell population in the spleen. Mice splenic mononuclear cells from each group were isolated and cultured for 24 h, and the cells were then stained with fluorescent labeled anti-CD4, anti-CD25 and anti-Foxp3 antibodies and analyzed by flow cytometry. (A) The gated cells indicate the frequency of CD4 + T cells. (B) CD4 + T cells in each group were further analyzed for the frequency of CD25 + Foxp3 + T cells (groups are annotated below the dot plots). (C) The bars indicate the summarized frequencies of (B). The data are presented as the mean ± SD. #P < 0.05 versus control group, *P < 0.01 versus DSS group or DSS + pVAX1 group. Treg: regulatory T cell; DSS: dextran sulfate sodium.

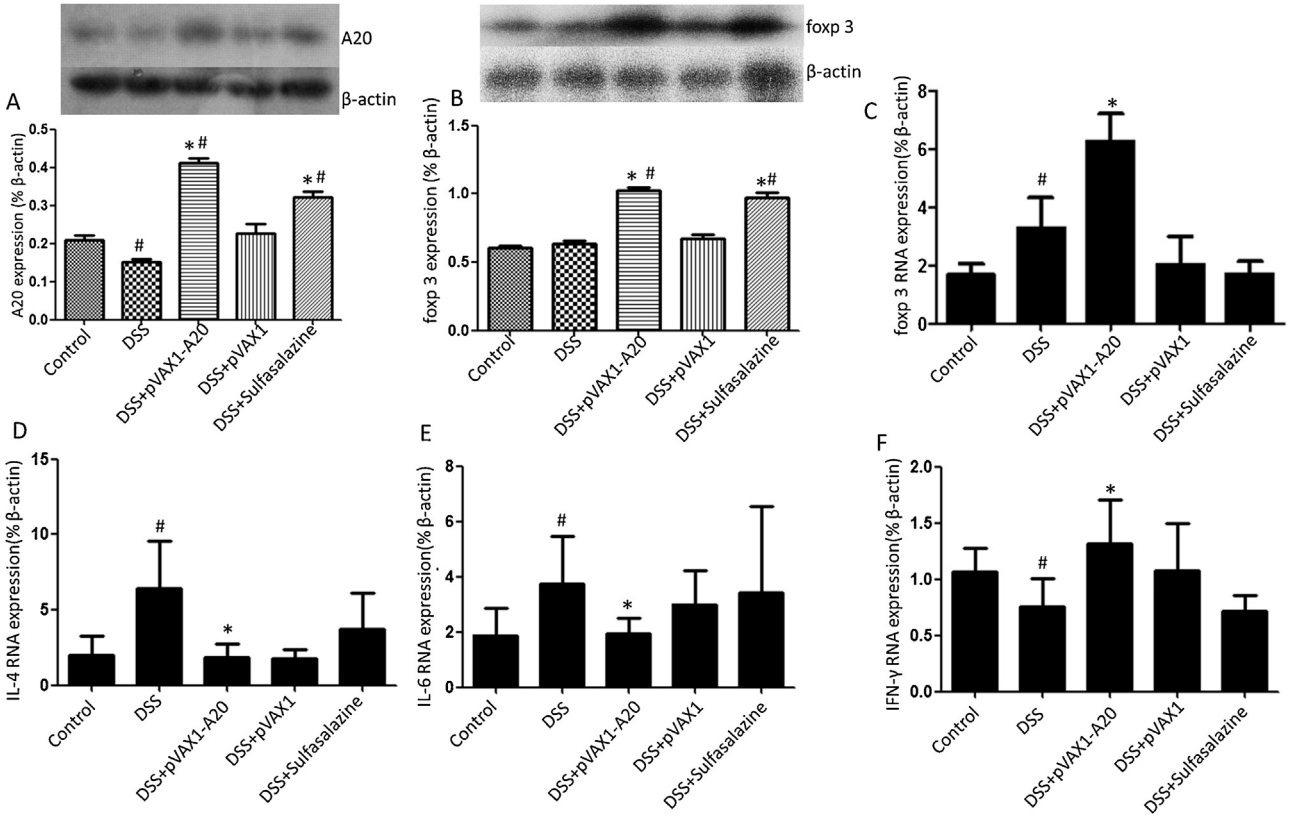

Fig. 5. pVAX1-A20 increases A20 and Foxp3 expression in the intestinal tissue. Colonic tissues from each group were collected and analyzed by real time PCR and Western blot. (A) The immune blots indicate the colonic A20 protein expression in each group, and the bars below the blots indicate the integrated densities of the immune blots. (B) Foxp3 protein levels in the colonic tissue. (C) The bars represent Foxp3 mRNA levels in the colonic tissue. (D-F) The bars represent IL-4, IL-6 and IFN-γ mRNA levels in the colonic tissue. The data are presented as the mean ± SD. #P < 0.05 versus control group, *P < 0.05 versus DSS group or DSS + pVAX1 group. Foxp3: forkhead box P3.

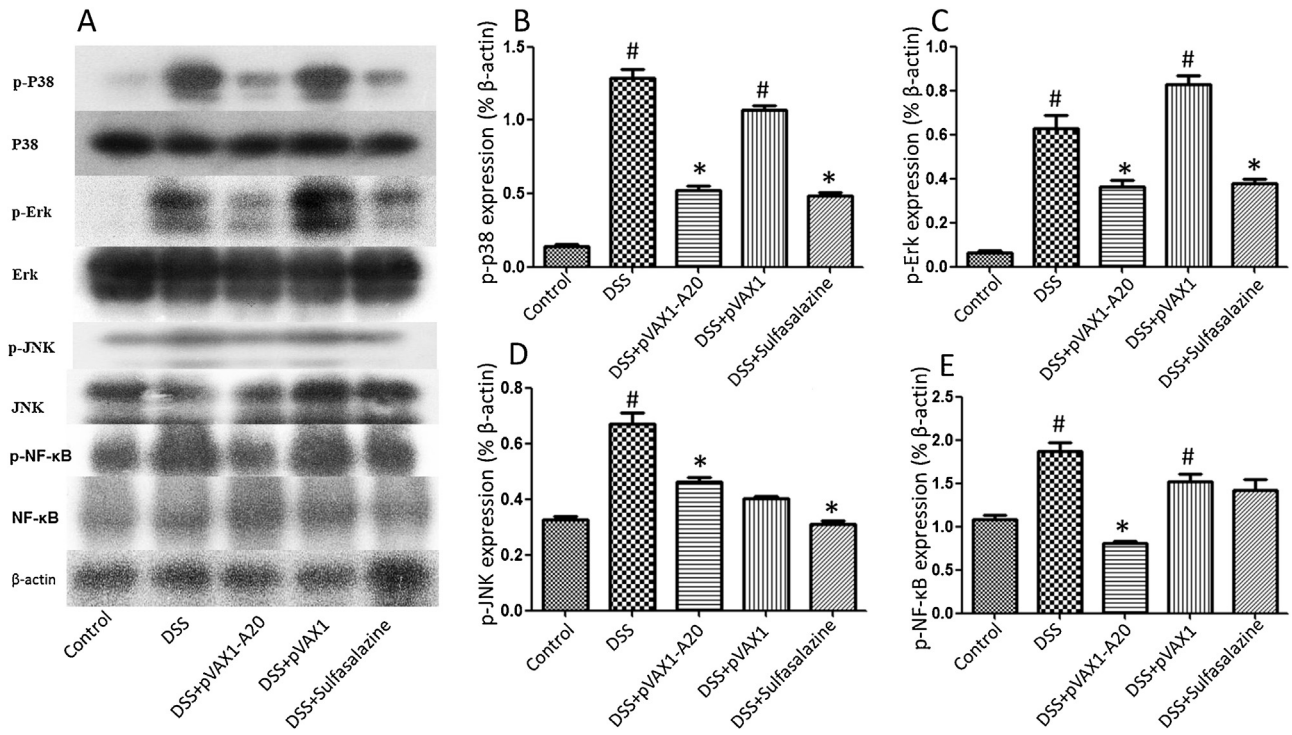

Fig. 6. pVAX1-A20 suppresses MAPK and NF-κB signaling activation. Colonic tissues were analyzed by Western blot. (A) The immune blots indicate the colonic p-p38, p-Erk, p-JNK, p-NF-κB protein expression in each group, and the bars in B, C, D, and E indicate the integrated densities of the immune blots. The data are presented as the mean ± SD. #P < 0.01 versus control group, *P < 0.01 versus DSS group. MAPK: Mitogen-activated protein kinase.

3.4. Foxp3 expression is increased in the intestinal tissue after treatment with pVAX1-A20

pVAX1-A20 administration increased mucosal A20 expression greatly (Fig. 5A). We next examined Foxp3 expression in colonic tissue after treatment with pVAX1-A20. The results showed that Foxp3 expression, at both the protein and mRNA levels, was significantly increased in the DSS + pVAX1-A20 group compared with that of the DSS group ($P < 0.05$), while only an increase in Foxp3 mRNA levels was detected in the DSS group when compared with the control group ($P < 0.05$) (Fig. 5B and C). These findings indicate that pVAX1-A20 treatment promotes the Treg population in DSS induced chronic colitis tissue, possibly through enhancing the Foxp3 expression. We further tested the effect of A20 administration on cytokine RNA expression in the colon tissue via qRT-PCR, and the results showed that IL-4 and IL-6 expression increased in the DSS group compared with the control group, and their expression was inhibited by A20 treatment in the DSS + pVAX1-A20 group. IFN-γ expression in the DSS group was decreased compared with that of the control group and was upregulated in the DSS + pVAX1-A20 group ($P < 0.05$) (Fig. 5D–F). The pro-inflammatory cytokines such as IL-4, IL-6 and IFN-γ involved in initiating the inflammation in IBD. This results indicate that A20 overexpression in the colon may regulate cytokine production.

3.5. pVAX1-A20 suppresses 2% DSS induced colonic MAPK and NF-κB signaling activation

MAPK and NF-κB signaling activation results in pro-inflammatory cytokine production. We assessed the inhibitory effects of pVAX1-A20 on the activities of MAPK and NF-κB in colonic tissue extracts from mice with colonic inflammation. Western blot analysis showed that MAPK and NF-κB signaling, including phosphorylated p38 (p-p38), p-Erk, p-JNK and p-NF-κB, were strongly activated in the colonic colitis tissue after treatment with 2% DSS (the DSS group) compared with those of the control group ($P < 0.01$) (Fig. 6A–E). The intrarectal administration of pVAX1-A20 efficiently decreased the levels of p-p38, p-Erk and p-JNK expression in colonic tissue ($P < 0.01$) (Figs. 6A–E), and the activation of phosphorylated NF-κB (p-NF-κB) in the DSS + pVAX1-A20 group was decreased significantly compared with that in the DSS group.

4. Discussion

IBD, including ulcerative colitis and Crohn's disease, is a relapsing inflammation of the gut with unknown etiology. Current therapies for IBD can only relieve inflammation but do not alter the natural course or the progression of the disease [3,15]. Therefore, more effective treatments for IBD are required. Our results in the present study showed that pVAX1-A20 administration markedly ameliorated colonic damage and the manifestations of experimental colitis, and reduced the intestinal inflammation by suppressing DSS induced activation of mucosal MAPK and NF-κB signaling. Furthermore, pVAX1-A20 promoted the splenic Treg population and Foxp3 expression in the intestine. To the best of our knowledge, the results presented here provide the first experimental evidence that the mucosal administration of A20, as a type of gene therapy, protects mice from DSS induced chronic colitis.

Compared with DSS induced acute colitis, the repeated administration of lower doses of DSS for longer periods leads to chronic colitis with a mixed Th1 and Th2 cell-associated inflammation [1,11]. Previous reports showed that TNBS induced long-term intestinal inflammation, which was characterized by collagen deposition and fibrogenic extracellular matrix changes that well mimicked the relapsing features of IBD [12]. Through the oral administration of 2% DSS, we developed a model of chronic colitis in mice, with body weight loss, diarrhea, bloody stools, and increased DAI scores. In addition, the DSS induced chronic colitis mice showed shortened colon lengths, severe colonic tissue dam-

age, with mucosa and submucosa fibrosis and collagen deposition, distorted crypts, disruption of the epithelium, loss of goblet cells, large numbers of infiltrating inflammatory cells, and thickened muscularis propria.

Tumor necrosis factor alpha (TNF-α) is a principal cytokine involved in the induction of inflammation, which is emerging as a potent target for inflammatory diseases, including rheumatoid arthritis, IBD and sarcoidosis [16]. The blockade of TNF-α by a specific antibody (infliximab) or by a soluble TNF-α receptor fusion protein (etanercept) has been shown to be effective but was limited by the occurrence of neutralizing antibodies or the development of tuberculous uveitis, sarcoidosis and granulomatous thyroiditis during clinical application [17]. A20, known as tumor necrosis factor alpha induced protein 3 (TNFAIP3), is a cytoplasmic protein that is involved in the negative feedback regulation of NF-κB activation in response to TNF, IL-1, and TLR stimulation [18]. Mice lacking A20 spontaneously exhibited DC activation and T cell expansion, and the expression of A20 in DC prevented the development of colitis [4]. The intestinal epithelial expression of TNFAIP3 activated an IL-10-dependent anti-inflammatory response that is necessary to prevent colitis [19]. Our results showed that the increased A20 expression in the colon, resulting from the administration of pVAX1-A20, protected mice from DSS induced colitis. Consistent with findings of other reports, the present results indicate that A20 is important for the regulation of intestinal inflammation, and could be useful to develop future therapies for the treatment of IBD. Colonic A20 expression in IBD patients remains controversial, and increased A20 expression was reported only in active UC patients [20], while others have observed decreased A20 expression in patients with CD [21]. We showed that A20 expression in DSS induced chronic colitis tissue decreased compared with controls. This result suggests that A20 expression is insufficient in the colitis tissue. A20 may be temporarily increased in the intestinal tissue during the acute stage and may be exhausted during the chronic recurring period of IBD. TNF signaling-related NF-κB and JNK activation are inhibited by A20, which acts as an inhibitor in the negative-feedback loop [22]. A20 inhibits the MAPK signaling pathway by suppressing the activation of ERK, JNK and p38, but did not inhibit NF-κB activation in human mesangial cells [23]. Our findings indicated that NF-κB and MAPK signaling activation, including phosphorylated p38 (p-p38), p-Erk, p-JNK and p-NF-κB, is observed during DSS induced chronic colitis was efficiently inhibited by the intrarectal administration of pVAX1-A20.

T cells was reported to accumulate in the inflamed mucosa in DSS chronic colitis over time, and they may play a pathogenic role [24]. Immune-deregulation-related effector T cell overactivation is one of the immune mechanism in colitis [1,11]. Tregs play a key role in maintaining intestinal homeostasis and preventing autoimmune disease [8]. Studies have reported that decreased Treg numbers in the blood and increased Tregs in the intestinal mucosa in IBD patients; the circulating Tregs inversely correlate with colitis activity [9,25]; this result may be due to a recruitment and expansion of Tregs to the inflammatory site [26]. To further elucidate the underlying mechanism by which A20 overexpression in the intestine suppresses DSS induced inflammation, we analyzed the Treg numbers in the spleen and Foxp3 expression in the colonic tissue. We found that, compared with controls, Tregs in the spleen increased slightly in DSS colitis mice, although this increase may not be efficient enough to suppress colonic inflammation, while the administration of A20 greatly promoted Treg expression in the colitis tissue. In addition, the expression of Foxp3, as a master transcriptional regulator in the development of Tregs, also increased in the colitis tissue of the A20 treatment group. A20 is required for DC to maintain immune quiescence by preventing DC activation and T cell expansion, but A20 is not required for the intrathymic Treg development [4,27]. Our results suggest that A20 facilitates peripheral Treg proliferation, and the suppression of colonic inflammation may partly be due to the promotion of Treg development in colitis. Tregs are becoming an attractive therapeutic factor for IBD treatment, and the transfer of Tregs can prevent the development of inflammation in a murine model of IBD [8]. When autologous ovalbumin-specific Tregs were injected back into patients with refractory CD in a clinical trial, the disease activity of CD was alleviated in 40% of the patients [28]. The pro-inflammatory cytokines, including IL-4 and IL-6, play important roles in initiating inflammation in IBD. IL-6 can induce TNF, IFN and IL-1 production and prevent mucosal T cell apoptosis by binding with IL-6 receptors. Th2 cytokines, such as IL-4 and IL-5, are expressed at increased levels in UC, and decreased Treg cells and increased effector T cells were found in CD [29]. To this end, in addition to the analysis of the Tregs, we also evaluated the pro-inflammatory cytokine expression in the colonic tissue. The results showed that A20 administration inhibited colonic IL-4 and IL-6 expression and increased IFN-ϒ expression which has been shown to protect mice from colitis [30]. These results indicate that A20 overexpression in the colon may regulate cytokine production by promoting Tregs.

In conclusion, our results showed that pVAX1-A20 administration alleviated DSS-induced chronic colitis in mice by promoting regulatory T cells. The results highlight the importance of A20 in the regulation of intestinal inflammation and imply that the exogenous overexpression of A20 in colitis tissue could become an innovative strategy for the treatment of IBD.

Conflict of interest

None declared.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.dld.2018.11.005.

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