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Baishideng Publishing Group Inc
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Pleasanton, CA 94588, USA
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Fax: +1-925-2238243
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ORIGINAL ARTICLE

Basic Study

Sodium selenite ameliorates dextran sulfate sodium-induced chronic colitis in mice by decreasing Th1, Th17, and γδT and increasing CD4(+)CD25(+) regulatory T-cell responses

Li-Xuan Sang, Bing Chang, Jun-Feng Zhu, Fang-Li Yang, Yan Li, Xue-Feng Jiang, Da-Nan Wang, Chang-Long Lu, Xun Sun

Li-Xuan Sang, Jun-Feng Zhu, Fang-Li Yang, Yan Li, Xue-Feng Jiang, Da-Nan Wang, Chang-Long Lu, Xun Sun, Department of Immunology, China Medical University, Shenyang 110122, Liaoning Province, China

Li-Xuan Sang, Department of Geriatrics, First Affiliated Hospital, China Medical University, Shenyang 110001, Liaoning Province, China

Bing Chang, Department of Gastroenterology, First Affiliated Hospital, China Medical University, Shenyang 110001, Liaoning Province, China

Author contributions: Wang DN and Lu CL are the co-corresponding authors; Sang LX, Chang B and Sun X performed the experiments, analyzed and interpreted the data, and wrote the manuscript; Jiang XF, Yang FL, and Li Y performed animal experiments and molecular biology experiments; Wang DN and Lu CL conceived the study; all authors approved the final manuscript.

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Correspondence to: Dr. Chang-Long Lu, Professor, Department of Immunology, China Medical University, No. 77, Puhe Road, Shenyang North New Area, Shenyang 110122, Liaoning Province, China. cllu@mail.cmu.edu.cn
Telephone: +86-24-83283764
Fax: +86-24-83283765

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Abstract

AIM

To assess the effect of sodium selenite on the severity of dextran sulfate sodium (DSS)-induced colitis in C57BL/6 mice.

METHODS

Mice were randomly divided into four groups (n = 10/group): normal group, selenium (Se) group, chronic colitis group, and Se + chronic colitis group. The

mice were sacrificed on day 26. Survival rates, clinical symptoms, colon length, and histological changes were determined. The percentages and absolute numbers of immune system cells in the lamina propria lymphocytes (LPL) of the colon, the expression of mRNA in colon tissue, and the concentrations of Th1, Th17, and Treg cytokines in LPL from the large intestine, were measured.

RESULTS

Se significantly ameliorated the symptoms of colitis and histological injury ($P < 0.05$ each), increasing the proportions of neutrophils and $CD4^+$ $CD25^+$ T cells ($P < 0.05$ each) and decreasing the proportions of γδT cells, $CD4^+$, $CD4^+CD44^+$, and $CD4^+$ $CD69^+$ T cells in LPL ($P < 0.05$ each). Moreover, Se reduced the expression of IL-6, IFN-γ, IL-17A, IL-21, T-bet, and RORγt ($P < 0.05$ each), but enhanced the expression of IL-10 and Foxp3 ($P < 0.05$ each).

CONCLUSION

These results suggest that Se protects against DSS-induced chronic colitis perhaps by increasing the number of CD4(+)CD25(+) Tregs that suppress the secretion of proinflammatory cytokines and populations of Th1, Th17, and γδT cells.

Key words: Sodium selenite; Dextran sulfate sodium; Chronic colitis

Core tip: Se significantly ameliorated the symptoms of colitis and histological injury, increasing the proportions of neutrophils and $CD4^+$ $CD25^+$ T cells and decreasing the proportions of γδT cells, $CD4^+$, $CD4^+CD44^+$, and $CD4^+$ $CD69^+$ T cells in LPL. Moreover, Se reduced the expression of IL-6, IFN-γ, IL-17A, IL-21, T-bet, and RORγt, but enhanced the expression of IL-10 and Foxp3. The study suggests that Se protects against DSS-induced chronic colitis perhaps by increasing the number of CD4(+)CD25(+) Tregs that suppress the secretion of proinflammatory cytokines and populations of Th1, Th17, and γδT cells.

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INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic, remittent-relapsing intestinal inflammatory condition. It consists of two major forms, ulcerative colitis and Crohn's disease, which are more common in developed countries than in developing countries. The major symptoms of IBD include abdominal pain, diarrhea, weight loss, and intestinal bleeding. Although the pathogenesis of IBD has not been definitively elucidated, factors including genetic mutations, immunological disorders, environmental exposure, oxidative stress, and intestinal flora have been suggested to be involved in this process[1-5]. In addition, there is sufficient evidence linking IBD to the over-response of the mucosal immune system[6]. T cells are important components of the adaptive immune response. The activation and proliferation of colonic lamina propria (LP) T lymphocytes during immune responses are important in maintaining intestinal immune homeostasis. $CD4^+$ T cells are especially important in regulating intestinal inflammation. $CD4^+$ T cells have been divided into subsets, based on the cytokines they produce; these subsets include Th1, Th2, Th17, and Treg cells. Th1 and Th17 cells are involved in the pathogenesis of IBD[7,8], whereas Treg cells ameliorate intestinal inflammation by suppressing Th17 cells[9].

To date, treatment options for IBD have mainly focused on controlling symptoms by suppressing inflammatory responses. Infliximab, a monoclonal antibody against tumor necrosis factor (TNF)-α, has proven effective in IBD by inducing the apoptosis of mucosal T cells[10,11], suggesting that strategies targeting lamina propria lymphocytes (LPL) in the intestinal mucosa may be effective in treating IBD. However, although immunomodulatory therapies are effective in many patients, many become refractory, suggesting the need to develop new agents to treat IBD.

Selenium (Se) is an important micronutrient for human health and has antioxidative properties. Se helps maintain the catalytic function of selenoproteins that indirectly alleviate oxygen-rich free radicals[4,5]. In addition, Se is necessary for the expression of glutathione peroxidase (GPX)[12] that protects organisms from oxidative damage. Se levels are inversely correlated with cancer risk and IBD[12-14]. Clinical studies have shown that Se[15] and Se protein P[16] contents are significantly lower in patients with IBD than in healthy controls, and that Se supplementation can reduce intestinal symptoms in patients with IBD[17]. Se can also protect against experimental colitis and human ulcerative colitis (UC)[18,19].

Little is known about the mechanism of action of Se in IBD. Activation of NF-κB and AP-1 is important in the pathogenesis of IBD[19,20]. Although Se has been shown to affect NF-κB and AP-1 activation[21-24], the effect of Se on mucosal LPL in the colon remains unclear.

Experimental colitis can be induced in mice by oral administration of dextran sulfate sodium (DSS).

Table 1 Disease activity index score chart

Fecal property	Fecal occult blood	Body weight decrease (%)	Integral
Normal	Normal	0	0
		1-5	1
Relaxed	Positive fecal occult blood	> 5-10	2
		> 10-15	3
Loose stools	Naked eye fecal occult blood	> 15	4

Normal stool: shaped stool; Relaxed stool: pasty, unformed stools not attached to the anus; Loose stools: unshaped stools attached to the anus.

The pathogenesis and clinical features of this animal model of IBD resemble those of human UC. This study assessed the effect of sodium selenite on DSS-induced colitis in mice, revealing that sodium selenite pretreatment protected against chronic colitis by reducing the levels of Th1, Th17, and γδT type cytokines and increasing those of Treg cytokines.

MATERIALS AND METHODS

Experimental animals

Eight-week-old male C57BL/6 mice, weighing 20 ± 1 g, were purchased from the Animal Care Facility of China Medical University. The mice were maintained and bred under specific pathogen-free conditions (temperature 24-25 ℃, humidity 70%-75%, and a 12-h light/12-h dark lighting regimen). Mice were fed chow, which had a basal selenium content of 0.1 μg/g diet. The study protocol was approved by the Animal Ethics Committee and Animal Care Committee of China Medical University.

Induction of chronic DSS colitis

Mice received oral 1.5% DSS (molecular mass 36-50 kDa; MP Biomedicals, Solon, OH, United States) on days 0-5, 10-15, and 20-25 d and tap water on the other days.

Experimental design

The mice were randomly divided into four groups (n = 10/group): control group, Se group, chronic colitis group, and Se + chronic colitis group. The control group was fed a normal diet (0.1 μg Se/g diet) and tap water + once-daily gavage of 0.2 mL PBS for 25 d. The Se group was fed a normal diet (0.1 μg Se/g diet) and tap water + once-daily gavage of 2 μg Se/g body weight for 25 d. The chronic colitis group was subjected to chronic colitis induction and fed a normal diet (0.1 μg Se/g diet) + once-daily gavage of 0.2 mL PBS for 25 d. The Se + chronic colitis group was subjected to chronic colitis induction and fed a normal diet (0.1 μg Se/g diet) + once-daily gavage of 2 μg Se/g body weight for 25 d. Body weight and disease activity index were observed daily. Each mouse was weighed at the same time daily.

Disease activity index and histopathology

The severity of colitis was assessed using the disease activity index (DAI) based on weight loss, hemoccult or rectal bleeding, and stool consistency; the scores are described in Table 1. After sacrifice, colon tissue was fixed in 4% paraformaldehyde and embedded in paraffin, and sections 4 μm thick were stained with hematoxylin and eosin to evaluate colonic histology, with histological scores determined in a blinded fashion by two independent pathologists (Table 2).

Cell preparation, culture, and stimulation

The large intestine of each mouse was cut into 1-2 mm pieces. The pieces were stirred twice for 15 min each in PBS containing 3 mmol/L EDTA and twice for 20 min each in RPMI1640 (Hyclone), containing 1 mmol/L EGTA, all at 37 ℃, to eliminate epithelium. The remaining pieces were stirred for 90 min at 37 ℃ in RPMI 1640 (Hyclone) containing 20% fetal bovine serum, 100 U/mL collagenase (C2139; Sigma-Aldrich Corp., St. Louis, MO, United States), and 5 U/mL DNase1 (Sigma-Aldrich Corp). The suspensions were centrifuged, and the pellets were washed. LPL were isolated from the lamina propria (LP)-cell preparations by centrifugation through a 45%-66.6% discontinuous Percoll (Solarbio) gradient at 2500 rpm for 20 min.

LPL (1×10^5/well in 0.2 mL RPMI1640 containing 10% fetal bovine serum, 1% penicillin, and 1% streptomycin) were cultured for 48 h in 96-well plates coated with anti-CD3 (10 μg/mL e-Bioscience, San Diego, CA, United States) and soluble anti-CD28 (1 μg/mL, e-Bioscience) mAb at 37 ℃ in an atmosphere containing 5% CO_2[25]. After 48 h, the supernatants were collected and cytokine concentrations assayed by enzyme-linked immunosorbent assay.

Enzyme-linked immunosorbent assay

Supernatants of cell cultures were collected after centrifugation at 1000 rpm for 10 min, and cytokine concentrations were measured using mouse immunoassay kits (R&D Systems Inc., Minneapolis, MN, United States), according to the manufacturer's protocol.

The levels of IL-6, IL-23, IL-1β, IL-12p70 and TNF-α were measured in supernatants without anti-CD3/anti-CD28 mAbs stimulations. The levels of IFN-γ, IL-17A, IL-21, IL-22 and IL-10 were measured in supernatants with or without anti-CD28/anti-CD3 mAbs stimulations.

RNA extraction and real-time polymerase chain reaction

Total RNA was extracted from colon tissue using Trizol reagent (Takara, Dalian, China), according to the manufacturer's protocol. RNA was reverse transcribed to cDNA using reverse transcriptase (Takara), followed by PCR assays using primers for β-actin (forward, 5′-TTCCAGCGTTCCTTCTTGGGTAT-3′; reverse, 5′-GTTGGCATAGAGGTGTTTACGG-3′). IL-

Table 2 Histology injury score chart

Grade	0	1	2	3	4
Inflammation	None	Mild	Moderate	Severe	-
Mucosal damage	None	Mucous layer	Submucosa	Muscularis and serosa	-
Crypt damage	None	1/3	2/3	100%	100% with epithelium loss
Pathological change range	None	0%-25%	26%-50%	51%-75%	76%-100%

17F (forward, 5′-TCCCACGTGAATTCCAGAAC-3′; reverse, 5′-ATGGTGCTGTCTTCCTGACC-3′), IL-21 (forward, 5′-TCAGAAGGCCAAACTCAAGC-3′; reverse, 5′-TCACAGGAAGGGCATTTAGC-3′), IL-22 (forward, 5′-GACAGGTTCCAGCCCTACAT-3′; reverse, 5′-CTGGATGTTCTGGTCGTCAC-3′), IL-23 (forward, 5′-TGCCCAGCCTGAGTTCTAGT-3′; reverse, 5′-AGTCAGAGTTGCTGCTCCGT-3′); IL-6 (forward, 5′-GAGGATACCACTCCCAACAGACC-3′; reverse, 5′-AAGTGCATCATCGTTGTTCATACA-3′), IL-10, (forward, 5′-GGTTGCCAAGCCTTATCGGA-3′; reverse, 5′-ACCTGCTCCACTGCCTTGCT-3′), IL-17A (forward, 5′-GCTCCAGAAGGCCCTCAGA-3′; reverse, 5′-AGCTTTCCCTCCGCATTGA-3′), IFN-γ (forward, 5′-AAAGACAATCAGGCCATCAG-3′; reverse, 5′-TGGGTTGTTGACCTCAAACT-3′), T-bet (forward, 5′-CCAGGGAACCGCTTATATGT-3′; reverse, 5′-CTGGGTCACATTGTTGGAAG-3′), Foxp3 (forward, 5′-GGCCCTTCTCCAGGACAGA-3′; reverse, 5′-GCTGATCATGGCTGGGTTGT-3′), and RORγt (forward, 5′-CCACTGCATTCCCAGTTTCT-3′; reverse, 5-CGTAGAAGGTCCTCCAGTCG-3′). The amplification protocol consisted of an initial denaturation at 95 ℃ for 30 s, followed by 40 cycles of denaturation at 95 ℃ for 15 s and annealing and extension at 60 ℃ for 34 s, on an ABI PRISM 7500 Sequence Detection System (Applied Biosystems, Foster City, CA, United States). The level of each gene was normalized relative to that of β-actin mRNA, and relative expression was calculated using the $2^{-\Delta\Delta CT}$ formula.

Flow cytometry

Briefly, 1 × 10^6 cells were isolated from the colon of each mouse. For surface antigen detection, the cells were labeled with monoclonal antibodies against Gr-1, F4/80, αβTCR, γδTCR, NK1.1, CD4, CD44, CD25, and CD69 for 30 min at 4 ℃. LPL were stimulated with ionomycin (1 mg/mL; Sigma-Aldrich) and PMA (25 ng/mL; Sigma-Aldrich) for 5 h at 37 ℃, with brefeldin A (10 mg/mL; Sigma-Aldrich) added after 1 h.

For intracellular staining, cells were fixed and permeabilized with fixation/ permeabilization working solution for 20 min at 4 ℃, followed by incubation with monoclonal antibodies against IFN-γ, IL-17A, and IL-10. The cells were analyzed using a Cytofix/ Cytoperm Kit Plus (BD Biosciences, San Jose, CA, United States).

Statistical analysis

Data are expressed as mean ± SD and analyzed by one-way ANOVA or *t*-test. *P* values < 0.05 were considered statistically significant.

RESULTS

Sodium selenite ameliorates the severity of DSS-induced chronic colitis

The effect of sodium selenite on DSS-induced colitis in mice was assessed by comparing survival rates, clinical symptoms (body weight loss, diarrhea, and rectal bleeding), DAI score, colon length, and colon histology in mice that received and did not receive sodium selenite. Survival rates were similar in the chronic colitis and the Se + chronic colitis group (Figure 1A), and body weight loss, colon length, and macroscopic inflammatory score were similar in the control and Se groups (Figure 1B-G) ($P > 0.05$ each). Compared with the chronic colitis group, the Se + chronic colitis group showed significant amelioration of body weight loss (Figure 1B) and a significantly lower DAI score (Figure 1C), beginning on day 23 ($P < 0.05$ each), as well as significantly longer colon (Figure 1D and E) and a significantly lower macroscopic inflammatory score (Figure 1F and G) ($P < 0.05$ each).

Cytokine production by LPL

Assessment of cytokine concentrations in the culture supernatants of unstimulated LPL showed that the levels of IL-6, IL-23, IL-1β, TNFα, IFN-γ and IL-17A were significantly lower in the Se + chronic colitis than in the chronic colitis group. There were no significant between-group differences in the levels of IL-12p70, IL-21, IL-22, and IL-10 (Figure 2).

When cytokine concentrations were assayed in culture supernatants of LPL stimulated with anti-CD3 and anti-CD28 mAbs for 48 h, the concentrations of IFN-γ, IL-17A and IL-21 were found to be significantly lower, while the concentration of IL-10 was significantly higher, in supernatants from the Se + chronic colitis than from the chronic colitis group. The level of IL-22, however, was similar in these two groups (Figure 2).

mRNA expression in colon tissue

RT-PCR assays of mRNA levels in cells showed that the expression levels of IL-6, IFN-γ, IL-17A, IL-21, IL-23, T-bet, and RORγt mRNAs were significantly lower, while the levels of IL-10 and Foxp3 mRNAs were significantly higher, in the Se + chronic colitis group than in the chronic colitis group. There was no significant between-group differences in the expression levels of IL-22 mRNA (Figure 3).

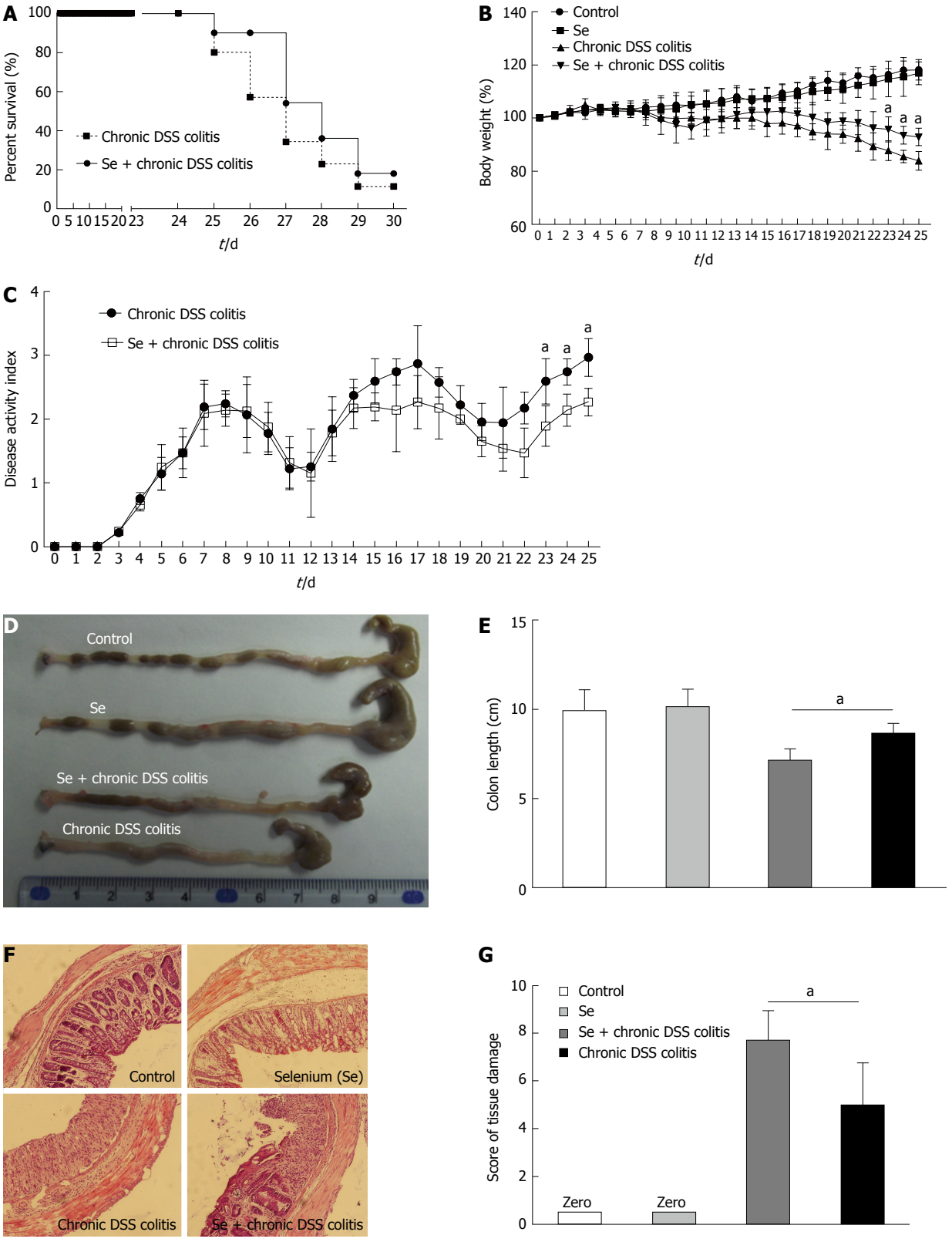


Figure 1 Sodium selenite ameliorates chronic dextran sulfate sodium-induced colitis in the C57BL/6 mice. A: Survival rate; B: Changes in body weight (%); C: Changes in the disease activity index (DAI); D and E: Colon length; F and G: Colon histopathological injury scores. The data are presented as the mean ± SD (Se + chronic DSS colitis vs chronic DSS colitis, $^{a}P < 0.05$) ($n = 10$). DSS: Dextran sulfate sodium.

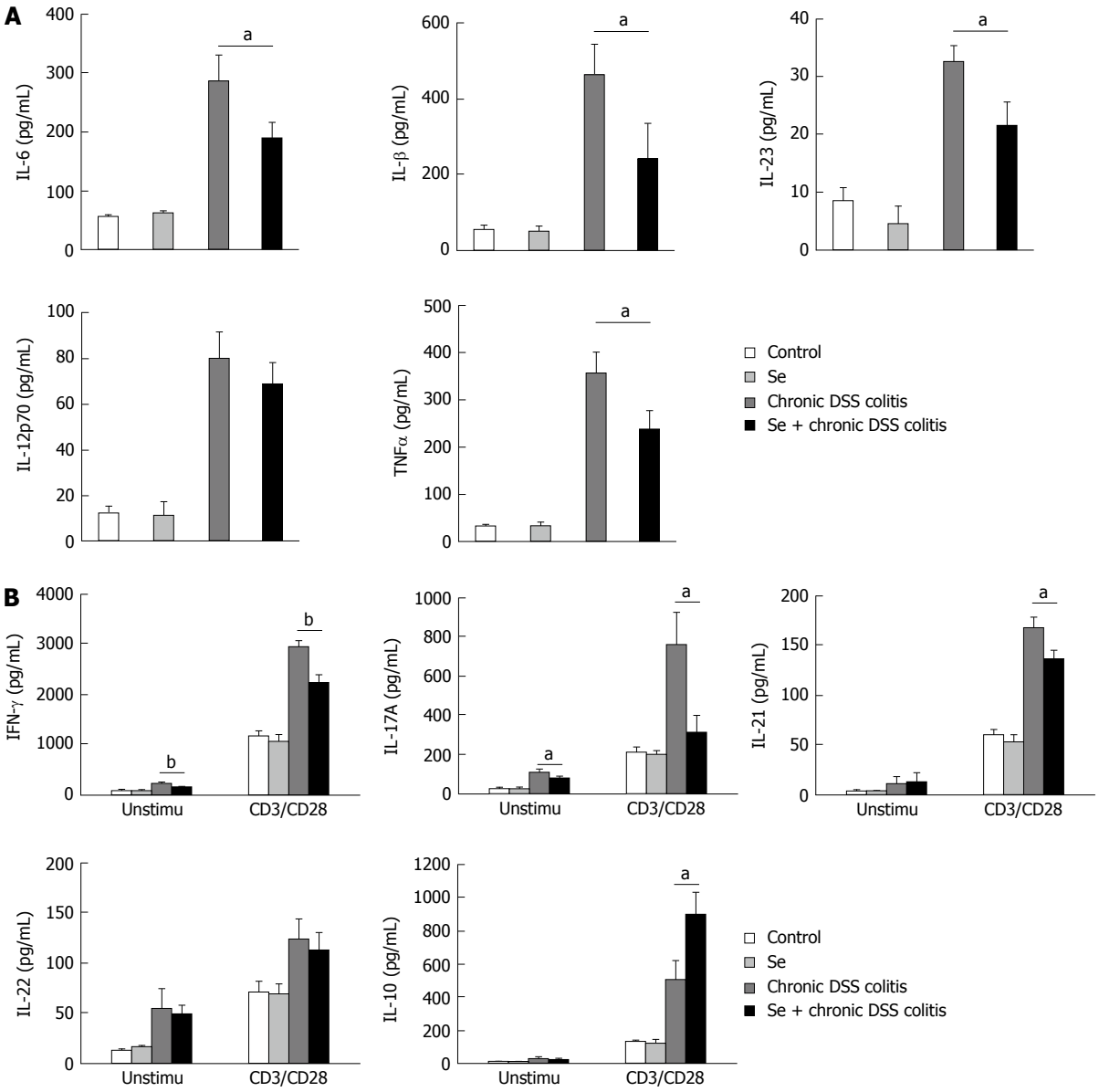


Figure 2 Cytokine production of LPL cells analyzed by ELISA. A: Unstimulated cells; B: LPL cells with or without anti-CD3 and anti-CD28 mAbs (CD3/CD28) stimulations. Each group consisted of three mice. Values represent mean ± SD ($^aP < 0.05$; $^bP < 0.01$) ($n = 3$).

Flow cytometry analysis of LPL populations in mouse colon

There were no significant differences in the percentages and absolute numbers of neutrophils ($CD11b^+Gr1^+F4/80^-$), macrophages ($CD11b^+Gr1^-F4/80^+$), γδT cells, NK cells, NKT cells, $CD4^+$, $CD4^+CD44^+$, $CD4^+CD25^+$, and $CD4^+CD69^+$ T cells in the colons of control and Se group mice. The percentages and absolute numbers of neutrophils and $CD4^+CD25^+$ T cells in LPL were significantly higher, while the percentages and absolute numbers of γδT and $CD4^+CD44^+$ and $CD4^+CD69^+$ T cells in LPL were significantly lower, in the Se+ chronic colitis group than in the chronic colitis group. These two groups did not differ in the percentages and absolute numbers of macrophages ($CD11b^+Gr1^-F4/80^+$), NK cells, and NKT cells (Figure 4).

Cytokine production by T-LPL cells in mice

There were no significant differences in the percentages and absolute numbers of $CD4^+IL\text{-}17A^+$, $CD4^+IFN\text{-}\gamma^+$, and $CD4^+IL\text{-}10^+$ cells in LPL of the control and Se groups. The percentages and absolute numbers of $CD4^+IL\text{-}17A^+$ and $CD4^+IFN\text{-}\gamma^+$ cells in LPL were significantly lower, while the percentages and absolute numbers of $CD4^+IL\text{-}10^+$ cells in LPL were significantly higher, in the Se + chronic colitis group than in the chronic colitis group (Figure 5).

DISCUSSION

Many diseases have been associated with lack of the essential trace element Se in the body, including asthma, rheumatoid arthritis, and cancer[26-28]. In these diseases, Se deficiency is abnormal, making

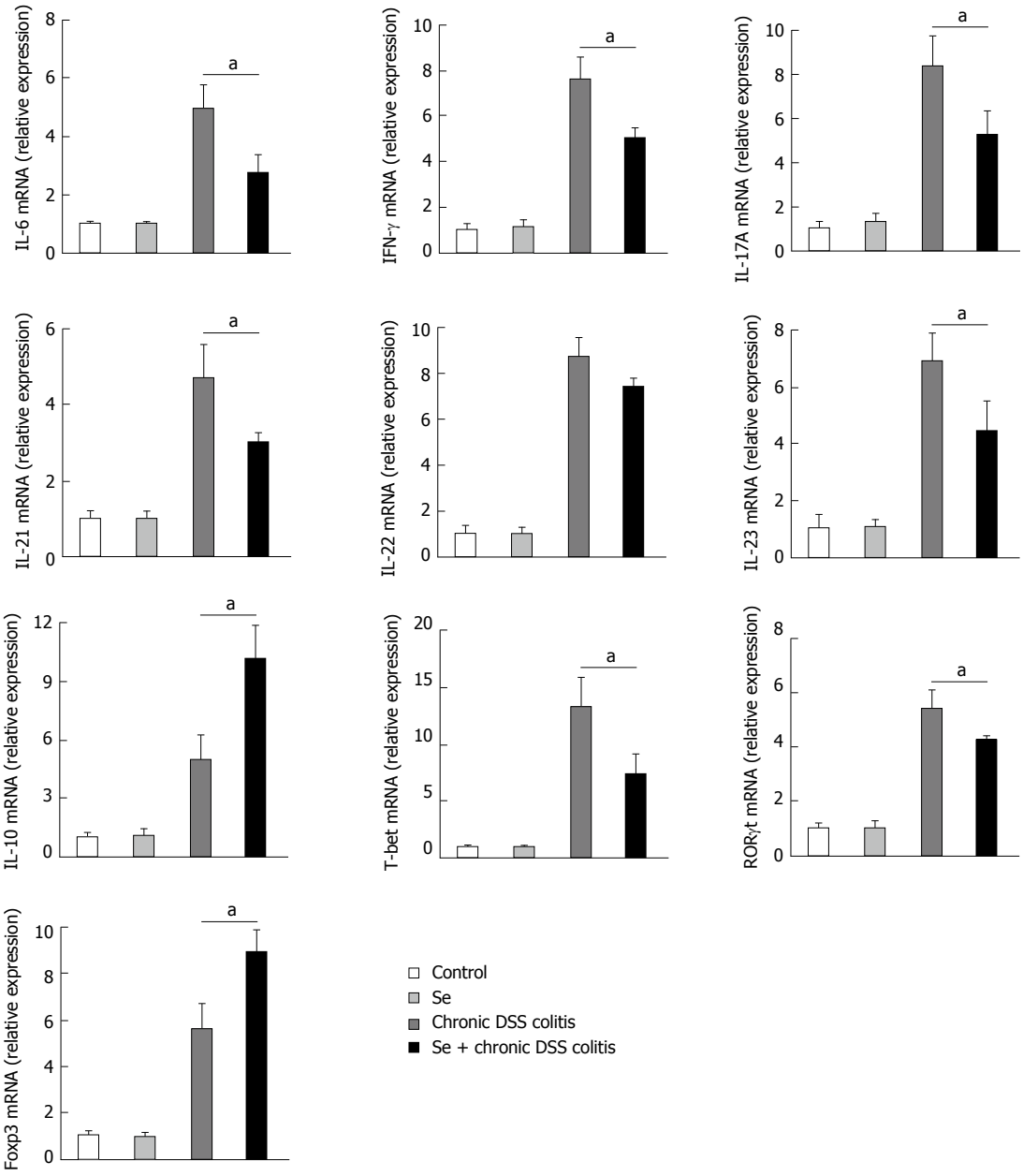
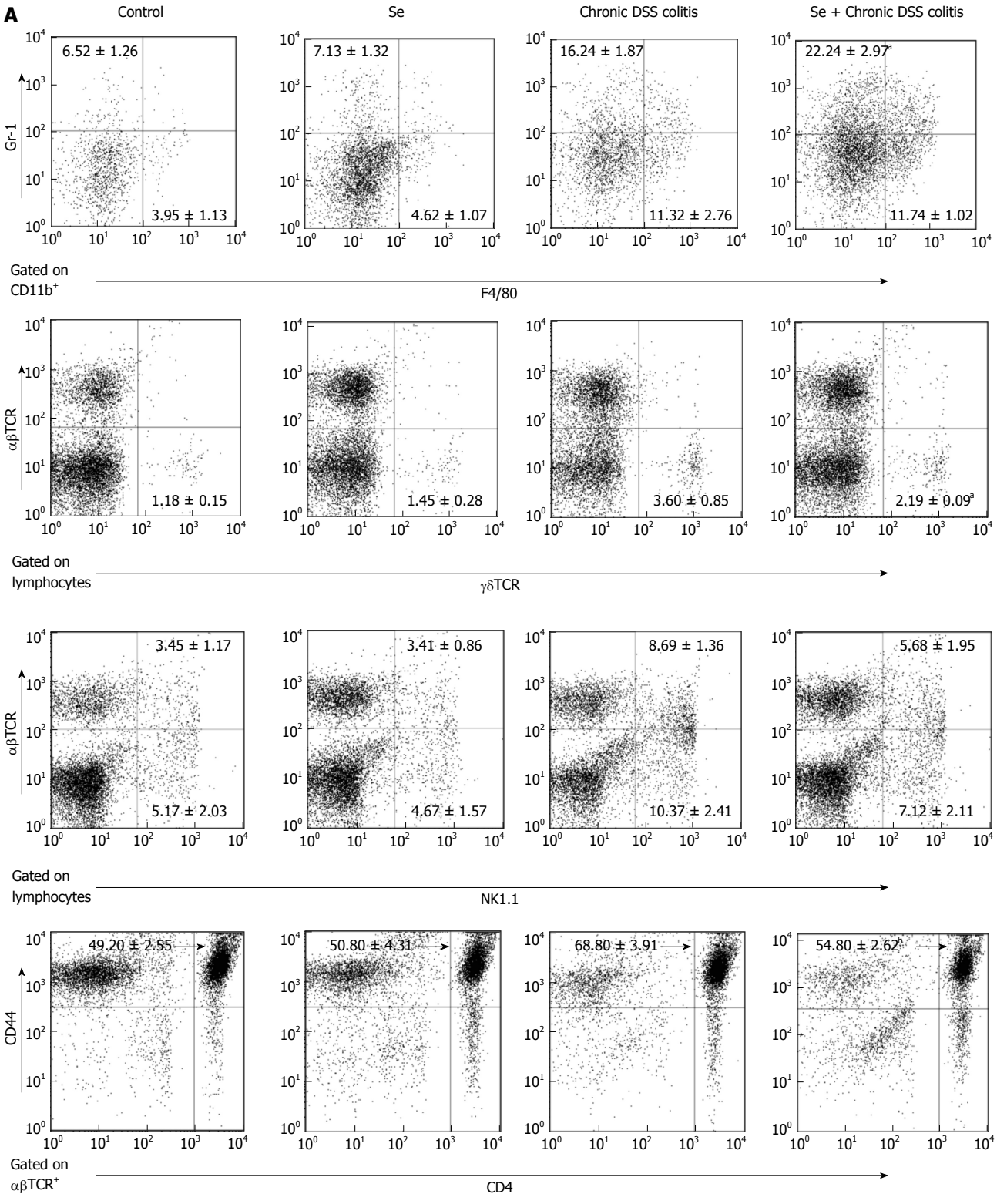


Figure 3 mRNA expression changes in colonic tissue. Values represent mean ± SD ($^aP < 0.05$) ($n = 3$).

supplementation with exogenous Se a reasonable method of treatment. Se may be useful for the prevention and/or amelioration of several autoimmune diseases, including IBD[29]. In support of this concept, our results demonstrate that Se sufficiency protects against DSS-induced chronic colitis by attenuating the symptoms of colitis, as shown by measurements of DAI, body weight, and colon length and by histological assessment of mucosal injury. Se may alleviate chronic colitis by enhancing the activity of Tregs, which suppress the secretion of proinflammatory cytokines and populations of Th1, Th17, and γδT cells.

Although neutrophils have been reported to have a proinflammatory effect[30], these cells maybe play a protective role in intestinal colitis[31,32]. Depletion of neutrophils has been shown to exacerbate colonic inflammation, suggesting that neutrophils be involved in mucosal repair processes[33]. Neutrophils produce IL-22 in response to coordinated signaling by IL-23 and TNF-α[34]. IL-22 is up-regulated in chronic colitis and considered beneficial for intestinal epithelial barrier function[35-37]. Neutrophil recruitment was shown to ameliorate experimental colitis by genetic ablation of IL-21[38]. Our results showed that Se reduced IL-21 and IL-23 expression, but had no effect on the IL-22 level, suggesting that Se may enhance neutrophil populations, leading to reductions in IL-21 and IL-23 expression.

LP macrophages are activated in many animal models of experimental colitis[39]. These macrophages produced TNF-α in DSS-induced colitis. IL-10 suppresses macrophage-derived proinflamma-


A
Control
Se
Chronic DSS colitis
Se + Chronic DSS colitis
Gr-1
6.52 ± 1.26
3.95 ± 1.13
7.13 ± 1.32
4.62 ± 1.07
16.24 ± 1.87
11.32 ± 2.76
22.24 ± 2.97a
11.74 ± 1.02
Gated on CD11b+
F4/80
αβTCR
1.18 ± 0.15
1.45 ± 0.28
3.60 ± 0.85
2.19 ± 0.09a
Gated on lymphocytes
γδTCR
αβTCR
3.45 ± 1.17
5.17 ± 2.03
3.41 ± 0.86
4.67 ± 1.57
8.69 ± 1.36
10.37 ± 2.41
5.68 ± 1.95
7.12 ± 2.11
Gated on lymphocytes
NK1.1
CD44
49.20 ± 2.55
50.80 ± 4.31
68.80 ± 3.91
54.80 ± 2.62b
Gated on αβTCR+
CD4

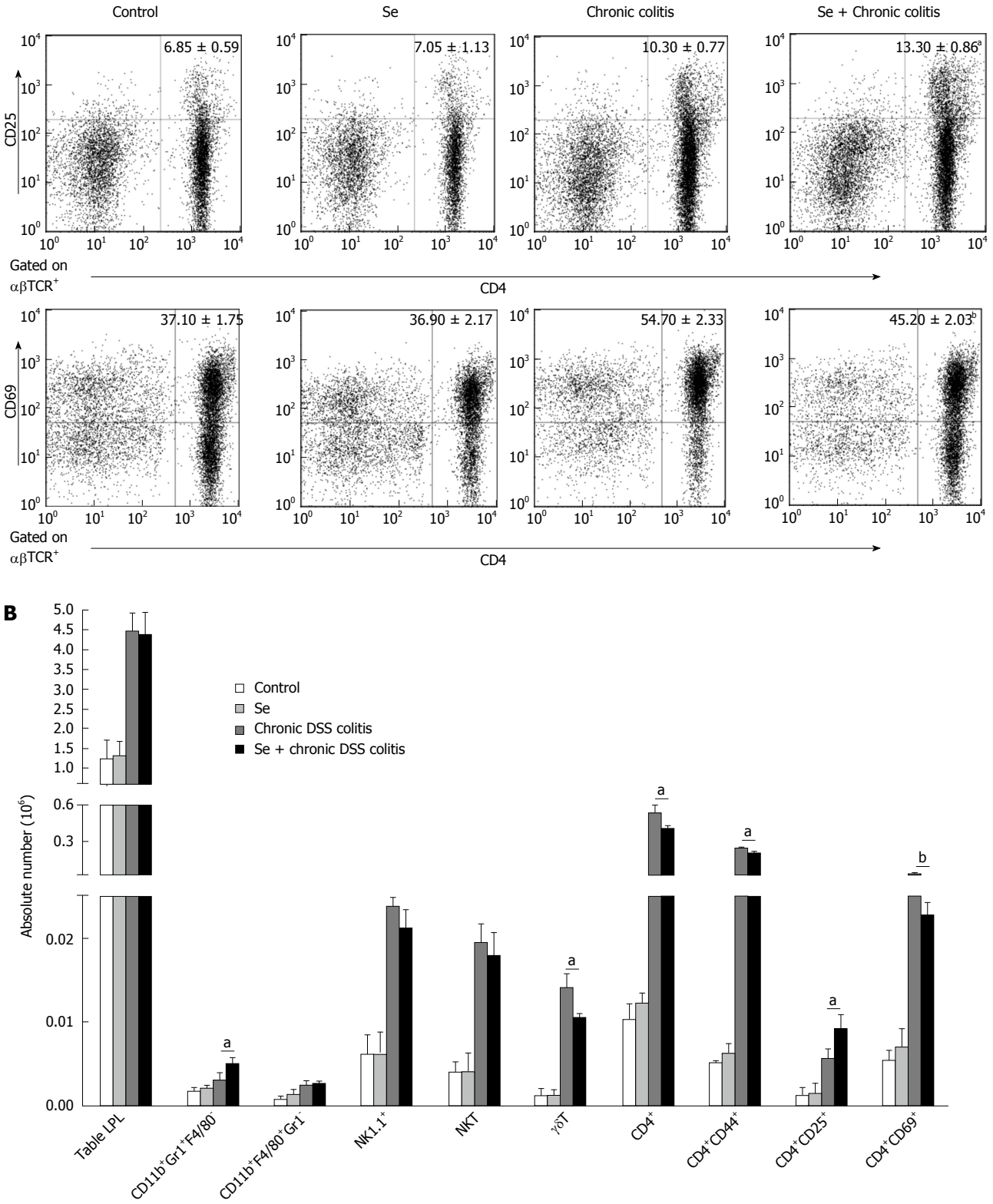


Figure 4 Flow cytometry of the populations of lamina propria lymphocytes in the colon in each group. A: The frequencies of neutrophils ($CD11b^+Gr1^+F4/80^-$), macrophages ($CD11b^+Gr1^+F4/80^+$), γδT cells (γδTCR), NK cells ($NK1.1^+$), NKT cells ($NK1.1^+\alpha\beta TCR^+$), $CD4^+$, $CD4^+CD44^+$ (effector T cells), $CD4^+CD25^+$ (regulatory T cells), $CD4^+CD69^+$ (activated T cells) T cells in LPL of the colon in each group; B: The absolute cell numbers of all kinds of cells in each group. Data indicate mean ± SD of six mice of obtained from a representative of three independent experiments ($^aP < 0.05$, $^bP < 0.01$).

tory cytokines and downregulates NO and ROS production[40]. Elimination of local macrophages in the intestine was shown to prevent chronic colitis in IL-10-deficient mice[41]. Macrophages may also ameliorate colitis by secreting immunosuppressive factors[42], suggesting that macrophages may play a dual role in different stages of IBD. Our study found that Se did not alter the number of macrophages, but alterations

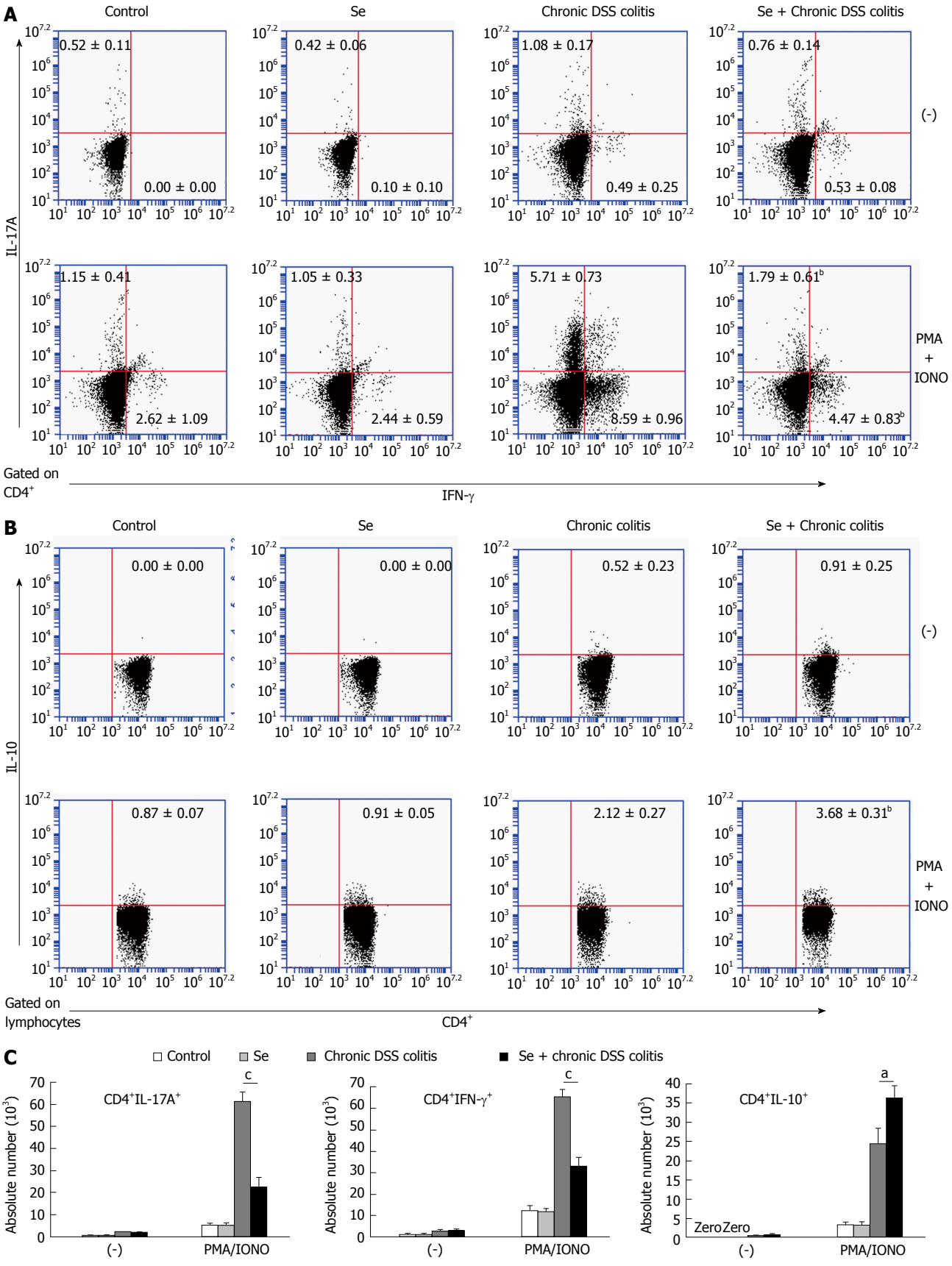


Figure 5 Cytokine-producing T cells in the lamina propria lymphocytes of the colon in each group. A: The frequencies of $CD4^+IL\text{-}17A^+$ and $CD4^+IFN\text{-}\gamma^+$ T LPL. B: The frequency of $CD4^+IL\text{-}10^+$T LPL. C: The absolute numbers of cytokine producing $CD4^+IL\text{-}17A^+$, $CD4^+IFN\text{-}\gamma^+$ T LPL and $CD4^+IL\text{-}10^+$T LPL with and without stimulation. Data indicate mean ± SD of six mice of obtained from a representative of three independent experiments ($^aP < 0.05$, $^bP < 0.01$, $^cP < 0.001$).

in function were not determined. Selenoproteins in macrophages protect mice from DSS-colitis by enhancing 15-hydroxy-prostaglandin dehydrogenase (15-PGDH)-dependent oxidation of prostaglandin E2 (PGE2) to alleviate inflammation[43]. The study showed that Se through its incorporation into selenoproteins suppressed inflammation and decreased the production of TNF-α and PGE2[44]. We speculate that macrophages in selenoproteins may affect the differentiation of T cells. Therefore, further research is needed.

Mouse intestinal LP T lymphocytes include αβ and γδT cells. γδT cells are a minor T-cell subset present in the LP, but are active in inflammatory processes[45], for example, in patients with IBD[46,47]. Changes in the repertoire and function of human mucosal γδT cells have been associated with the disease process in IBD[46]. γδT cells play a protective role in acute DSS colitis[45], but are involved in the exacerbation of chronic colitis[48], suggesting that γδT cells may represent a promising target in the treatment of human IBD. Our study showed that the absolute numbers and percentages of γδT cells in T-LPL were significantly lower in the Se + chronic colitis group than in the chronic colitis group. Se has been found to upregulate CD4(+)CD25(+) Treg cells in iodine-induced autoimmune thyroiditis in NOD.H-2(h4) mice[49]. γδT cells positively regulate contact sensitivity reactions by modulating INF-γ, IL-12, and TNF-α production[50]. Treg cells can inhibit the production of IFN-γ by antigen-specific memory γδT cells[51]. In addition, IL-10, TGF-β, and the transcription factor Foxp3 mediate immunoregulation[52]. The Treg cells restrain immune responses that are dependent upon expression of the IL-10 and the transcription factor Foxp3. Treg cell-derived IL-10 limits inflammation at environmental interfaces[53]. IL-10 may therefore play an important role in the suppressive function of Treg cells[54]. Other mechanisms, however, cannot be excluded, including direct cell-surface contact. These findings indicate a potential new mechanism by which CD4(+)CD25(+) Tregs can specifically suppress γδT cells and highlight the strategy of combining Treg inhibition with subsequent γδT-cell activation to enhance γδT-cell-mediated immunotherapy[55]. Tregs can inhibit γδT-cell proliferation *in vitro via* a cell-cell contact-independent mechanism[55]. Our data showed that IL-10 expression, as well as the absolute numbers and percentage of CD4(+)CD25(+) Treg cells in T-LPL, were significantly higher in the Se + chronic colitis group than in the chronic colitis group. Further understanding of the molecular mechanisms underlying γδT-cell-mediated exacerbation of chronic colitis may suggest better therapeutic strategies for human IBD.

NK cells are a subset of innate lymphocytes that contribute to host resistance and provide immune surveillance. The roles of NK cells in DSS-induced colitis, however, are less clear. NK cells produce cytokines, including TNF-α and IFN-γ[56], as well as protect mice from DSS-induced colitis by regulating neutrophil function *via* the NKG2A receptor[57]. Several proinflammatory cytokines, including IL-15, IL-21, and IL-23, may potently activate NK cells, inducing the secretion of high levels of the proinflammatory cytokines IFN-γ and TNF-α[58]. IL-21 enhances IBD NK cell cytotoxic responses, triggers T cells to produce proinflammatory cytokines, and induces IBD CD4(+) T cells to differentiate into Th17 cells, suggesting that IL-21 is involved in the pathogenesis of IBD and that blocking IL-21R signaling may have a therapeutic benefit in IBD[59]. NK T cells, a subset of T lymphocytes that express the TCR, play an important role in the pathogenesis of intestinal inflammation[60,61]. NK T cells elicit effector function by producing large amounts of IFN-γ, IL-4, and IL-10[62]. Activation of NK T cells has been shown to protect mice from DSS-induced colitis[60]. We found that the percentages and absolute numbers of NK and NKT cells in T-LPL did not differ significantly in the chronic colitis and Se + chronic colitis groups.

The Th17 pathway was shown to be very important in the pathogenesis of human IBD[63]. Th1 cell subsets are considered major factors in DSS-induced colitis[64]. The orphan nuclear receptor RORγt and T-bet direct Th17 and Th1 differentiation, respectively[65,66]. The amounts of Th1 and Th17 cells were increased and Treg cells were decreased in chronic colitis. In contrast, Treg cells may have protective effects in colitis. The expression of transcription factor Foxp3 has been shown to direct Treg differentiation[67]. However, Th1 and Th17 promote colitis whereas Tregs have protective effects[68,69]. The numbers of Th1 and Th17 cells were increased by the high expression of cytokines supporting Th17 cell differentiation, thus exacerbating the immunopathogenesis of IBD. These cytokines include IL-1, TGFβ, IL-6, IL-21, and IL-23. Our results showed that the percentages and absolute numbers of Th1 and Th17 cells in T-LPL were significantly lower in the Se + chronic colitis than in the chronic colitis group. In addition, IL-6, IFN-γ, IL-17A, IL-21, T-bet, and RORγt expression levels were lower. IL-10 is a negative regulator of inflammation and counters the activity of many proinflammatory cytokines. IL-10 has been shown to suppress intestinal inflammation. Foxp3 functionally inhibits RORγt[70]. Our results showed that IL-10 and Foxp3 expression levels were higher in the Se + chronic colitis group than in the chronic colitis group.Th17 cells are a subset of $CD4^+$ T cells that produce IL-17A. IL-21 induces and maintains T-cell-dependent inflammatory responses, including both Th1 and Th17 responses[71]. IL-21 promotes Th17 cell differentiation by suppressing Foxp3 expression, with inhibition of IL-21 signaling reducing IL-17A and IFN-γ expression[72-74]. T-cell subsets are regarded as unstable and may convert into other subsets of T cells under certain circumstances[75].

In summary, our results suggest that sodium selenite has a protective role in DSS-induced chronic

colitis. Se may alleviate colitis by inducing Tregs to suppress the secretion of proinflammatory cytokines and populations of Th1, Th17, and γδT cells. As sodium selenite has been widely used to treat human autoimmune diseases, it may be useful in treating IBD.

COMMENTS

Background

Inflammatory bowel disease (IBD) is a group of diseases responsible for chronic inflammation of the intestine. In a mouse model of dextran sulfate sodium (DSS)-induced colitis, Th17 cells promote, whereas regulatory T (Treg) cells protect against, intestinal inflammation. Selenium (Se) is an important natural antioxidant, with serum Se concentrations inversely correlated with IBD.

Research frontiers

$CD4^+$ T cells have been divided into subsets, based on the cytokines they produce; these subsets include Th1, Th2, Th17, and Treg cells. Th1 and Th17 cells are involved in the pathogenesis of IBD, whereas Treg cells ameliorate intestinal inflammation by suppressing Th17 cells.

Innovations and breakthroughs

These results suggest that Se protects against DSS-induced chronic colitis perhaps by increasing the number of CD4(+)CD25(+) Tregs that suppress the secretion of proinflammatory cytokines and populations of Th1, Th17, and γδT cells.

Applications

The results suggest that sodium selenite has a protective role in DSS-induced chronic colitis. Se may alleviate colitis by inducing Tregs to suppress the secretion of proinflammatory cytokines and populations of Th1, Th17, and γδT cells. As sodium selenite has been widely used to treat human autoimmune diseases, it may be useful in treating IBD.

Peer-review

This is a well-designed and well-presented study for examining the antiinflammatory potential of sodium selenite in DSS colitis in mice.

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