

The Protective Effect of the Recombinant 53-kDa Protein of *Trichinella spiralis* on Experimental Colitis in Mice

Linlin Du · Hao Tang · Zhongfu Ma · Jia Xu ·
Wenchao Gao · Jiajia Chen · Wenjia Gan · Zhaoping Zhang ·
Xinbing Yu · Xingwang Zhou · Xuchu Hu

Received: 29 November 2010 / Accepted: 21 March 2011 / Published online: 8 April 2011
© Springer Science+Business Media, LLC 2011

Abstract

Background Helminth infection has been proven to reduce the severity of experimental inflammatory bowel disease (IBD). The excretory-secretory proteins of helminths play an important role in the process of immunomodulation.

Aims In the present study, we aimed to investigate the protective potential of recombinant *Trichinella spiralis* (TS) 53-kDa protein (rTsP53), a component of excretory-secretory proteins, on experimental colitis in mice.

Methods BALB/c mice were treated subcutaneously with 50 µg rTsP53 three times at an interval of 5 days. Colitis was induced by intrarectal administration of 5 mg trinitrobenzene sulfonic acid (TNBS). Disease activities and macroscopic and microscopic scores were evaluated. To determine immune response provoked by rTsP53, we measured specific IgG1 and IgG2a values against rTsP53 in sera of mice. We also detected cytokine profiles as well as the markers of alternatively activated macrophages (M2) in mice.

Results RTsP53 ameliorated significantly the disease activity index (DAI) as well as the macroscopic and microscopic scores. IgG1 but not IgG2a was the predominant specific antibody detected in the sera of immunized mice, indicating the potential of stimulating T-helper (Th) 2 bias response by rTsP3. Pre-treatment with rTsP53 decreased serum Th1 cytokines (TNF- α , IFN- γ) and elevated serum levels of serum Th2 cytokines (IL-4, IL-13); it also decreased colonic Th1 cytokines (TNF- α , IL-6) and colonic regulatory cytokines (IL-10, TGF- β 1). RTsP53 increased colonic M2 markers, arginase-1 (Arg-1), and found in inflammatory zone 1 (FIZZ1), compared to mice without rTsP53 pretreatment.

Conclusions RTsP53 is a potential protective agent for IBD.

Keywords Inflammatory bowel disease · 53-kDa protein · *Trichinella spiralis* · Alternatively activated macrophages · Th2

L. Du · H. Tang · Z. Ma · J. Xu
Department of General Internal Medicine, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, Guangdong, China

W. Gao · J. Chen · W. Gan · Z. Zhang · X. Yu · X. Hu
Department of Parasitology, Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou, Guangdong, China

X. Zhou
Department of Biochemistry, Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou, Guangdong, China

X. Hu (✉)
74 Zhongshan 2nd Road, Guangzhou 510080, China
e-mail: huxuchu@mail.sysu.edu.cn

Introduction

Inflammatory bowel diseases (IBD), including ulcerative colitis (UC) and Crohn disease (CD), are chronically relapsing disorders of the intestine [1]. IBD are diseases of different geographical distribution, with high prevalence in the north and developed countries while low in the south and developing countries. “IBD hygiene hypothesis” is proposed to explain these diseases of wealth, stating that children in extremely hygienic environments predispose to immunological diseases, such as IBD, later in life [2]. The absence of exposure to intestinal helminths in developed regions of good sanitary conditions is an important factor contributing to IBD [3].

In recent years, experimental and clinical evidence has been accumulated to demonstrate that infection with parasitic worms could protect hosts from IBD. For example, the intestinal helminth *Trichinella spiralis* (Ts), *Heligmosomoides polygyrus*, *Schistosoma* eggs, *Trichinella muris* or *Hymenolepis diminuta* (*H. diminuta*) protected rodents, rats or mice from 2, 4, 6-trinitrobenzene sulphonic acid (TNBS)-induced colitis [3–6]. Infecting IBD patients with porcine whipworm or *Trichuris suis* resulted in clinical amelioration of both CD and UC [7]. The mechanisms of the therapeutic effects of helminths are still not clearly understood. Studies showed that the proteins released from helminths preferentially activated Th2 response and suppressed Th1-prone gastrointestinal inflammation [8]. Macrophages, another important group of cells in innate immunity residing in intestinal mucosa, may also play an important role in IBD. Peripheral blood mononuclear macrophage can be differentiated into two functionally contradictory phenotypes in tissues: classically activated macrophages (M1) and alternatively activated macrophages (M2). The former are activated by Th1 cytokines interleukin (IL)-12 and IFN- γ , releasing pro-inflammatory cytokines to enhance immune responses and injured host tissues; whereas the latter are activated by Th2 cytokines IL-4 and IL-13, releasing IL-10 and TGF- β 1 to suppress immune responses and restore the injured tissues [9]. Schistosome infections prevented colitis through a novel mechanism dependent on macrophages and not simply through modulation of Th2 responses [10]. Other experiments have also demonstrated that declining M2 leads to imbalance in innate and acquired immunity in IBD [11].

However, persistent parasite infection in the human could cause pathology [12], so, living helminth worm infections or oral eggs cannot be accepted easily by patients. Therefore, the new strategy aims at helminth antigens that are viewed as a safe substitute for living parasite infection. Treatment with proteins of *S. mansoni* ameliorated TNBS-induced colitis [13], and Ts excretory–secretory antigens (ESA) reduced the severity of TNBS-induced colitis significantly [14]. However, the scanty source of antigens obtained from worms limits its practical application. If the component contributing to protection can be identified and produced abundantly by genetic engineering, the problem can be solved.

A Ts-specific 53-kDa glycoprotein (TsP53) has been identified as a major component of its ESA and is present in much greater amounts from Ts larva [15], so it is used as an immunodiagnostic antigen [16], however, its immunomodulatory function has not yet been investigated. In our preliminary study, mice immunized with recombinant TsP53 (rTsP53) produced high levels of specific IgG1, but not of IgG2a, either with complete

Freund's adjuvant (CFA) or without any adjuvant, suggesting it is a potent antigen to stimulate Th2-prone response (data shown in the result 5). Therefore, the aim of this study was to evaluate the protective effect of pretreatment with rTsP53 in the TNBS-induced colitis mice model and investigate the immunological mechanisms of rTsP53 modulating Th1 cytokines and Th2 cytokines to activate M2 macrophages.

Materials and Methods

Cloning, Expression, Purification and Identification of rTsP53

Total RNA was extracted from the Ts larvae using Trizol (Invitrogen). Total mRNA was reversely transcribed to cDNA using SuperScript III reverse transcriptase kit according to the manufacturer's instructions (Invitrogen). The cDNA was used as a template to amplify the complete coding sequence of TsP53 by polymerase chain reaction (PCR). The primers were designed according to the nucleotide sequence of TsP53 in GenBank (Access No. AAA97512): sense (CCCCATATGTCCTACAGACAATGAGAATG) and antisense (ATTCTCGAGGAA CAACAACTGTAGTTC) with *Nde*I and *Xho*I restriction enzyme sites (underlined), respectively. PCR product was inserted into the pET-28a (+) expression vector. The recombinant plasmid was transformed into an *E. coli* BL21, and rTsP53 expression was induced by 1.0 mM IPTG at 28°C. The recombinant protein was solubilized completely with 6 M urea in 20 mM Tris–HCl buffer (pH 8.0) and then purified with a Ni–NTA His* Bind Purification Kit (Novagen). For the refolding of recombinant proteins, the urea was removed from the sample protein solutions by stepwise dialysis against 20 mM Tris–HCl buffer (pH 8.0) before loading to in a His trap chelating column. rTsP53 were eluted with 200 mM imidazole, and then imidazole was removed from the sample with a PD-10 column (GE Healthcare Bio-Sciences). The rTsP53 was dialyzed against PBS repeatedly. Endotoxin was removed by ToxinEraser™ endotoxin removal resin (Genmed) resulting in levels < 0.1 endotoxin units/ml as indicated by the Limulus Amebocyte Lysate (LAL) assay (Genmed) following the manufacturer's protocol. The concentration of rTsP53 was determined by Bradford assay. Before use the protein solution was sterilized by ultrafiltration with the help of 0.2- μ m pore microfilter (Sarstedt, Numbrecht, Germany). The expression and purification of rTsP53 were analyzed by 12.0% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and identified by Western blotting using the serum of Ts-infected mice.

Experimental Animals

Male BALB/c mice aged 6–8 weeks were purchased from the experimental animal center in Sun Yat-sen University. Experiments followed a protocol drafted by the Animal Ethics Committee of the University. Mice were housed in ventilated cages with wire-net floors in a controlled room and were fed a normal laboratory diet. Mice were deprived of food for 24 h prior to the induction of colitis, but were allowed free access to tap water throughout. Mice were treated with rTsP53 (50 µg in 200 µl PBS) or PBS by subcutaneous injection three times at an interval of 5 days before the induction of colitis by TNBS. Experimental mice were assigned to two groups: the rTsP53 +TNBS group and the TNBS group (with PBS).

Induction of Colitis

At the fifth day after the last injection, mice were slightly anesthetized with 10% chloral hydrate following a 24 h abrosia. A medical-grade polyurethane cannula for enteral feeding (external diameter 2 mm) was inserted into the anus and the tip was advanced to 4 cm proximal to the anus verge. TNBS (Sigma-Chemical Co, St Louis, MO, USA) was instilled into the colon (5 mg per mouse) to induce acute colitis (a stock solution of TNBS was made by dissolving 50 mg of DNBS per ml of 50% ethanol). The animals were then maintained in a head-down position for a few minutes (2–3 min) to prevent leakage of the intracolonic instillate. Mice were bled to collect sera and then sacrificed for pathological investigation on the fifth day after TNBS administration.

Assessment of Colon Damage

Weight loss, fecal character, fecal occult blood, and hematochezia were evaluated individually according to the previously described disease activity index (DAI) scores [17]. The colon was then removed and opened longitudinally, after which the damage was assessed macroscopically and histologically using previous criteria. Macroscopic damage was scored based on ulceration, the presence of adhesions, hyperemia, and thickening of the colonic wall [17]. Tissue samples from the distal inflamed colon of each animal were fixed in 4% buffered formaldehyde, dehydrated by increasing concentrations of ethanol, and embedded in paraffin. Sections of tissue were cut to 5 µm, mounted on clean glass slides and dried overnight at 37°C. Sections were cleared, hydrated, and stained with haematoxylin and eosin. The histology damage score was calculated on a 12-point scale: loss of architecture, 0–3; inflammatory infiltrate, 0–3; goblet cell depletion, 0 or 1; ulceration, 0 or 1; edema, 0 or 1; muscle thickening, 0–2;

and presence of crypt abscesses, 0 or 1. Additional sections were stained with periodic acid-Schiff's reagent for goblet cell enumeration per five fields of view [18]. This scoring was done in a blinded fashion.

Detection of Colon Tissue Cytokine Levels

Distal colon tissues were removed, washed in phosphate buffered saline (PBS), and frozen in liquid nitrogen. Total RNA was extracted using Trizol reagent according to the manufacturer's protocol (Invitrogen). The cDNA of each sample was synthesized from 1 µg total RNA using SuperScript II according to the manufacturer's instruction (Invitrogen). Th1 cytokines (IL-6, TNF- α) and regulatory cytokines (IL-10, TGF- β 1) and M2 markers found in inflammatory zone 1 (FIZZ1) and arginase-1 (Arg-1), were all assessed with quantitative real-time PCR, which was done using a real-time PCR kit in a Bio-Rad iQ5 real-time PCR machine using SYBR Green detection protocol. Gene specific primers were used for amplifying genes. House-keeping gene β -actin was used as the endogenous control. The relative mRNA expression of each studied gene was calculated with the comparative $\Delta\Delta C_t$ method using the formula $2^{-\Delta\Delta C_t}$.

Measurement of Antibody Isotypes anti-TsP53

Sera from sacrificed mice of the two groups were used in an enzyme-linked immunosorbent assay (ELISA) to measure the levels of IgG1 and IgG2a against rTsP53. Ninety-six-well flat-bottom microtitre plates (Nunc) were coated overnight at 4°C with 100 µl of rTsP53 at a concentration of 5 µg/ml in 0.1 M carbonate bicarbonate buffer (pH 9.6) per well. Serum samples diluted 1:100 (IgG1) and 1:100 (Ig2a) in PBST (100 µl/well) were then added in duplicate.

Sera Cytokine Analysis

At the end of the experiment before sacrifice, a 0.2-ml blood sample was collected from each mouse through the caudal vein. The samples were centrifuged at 5,000 rpm at 4°C for 5 min. Blood sera were stored in -20°C. The concentrations of IL-4, IL-13, IFN- γ and TNF- α in the blood sera were measured with an ELISA kit according to its manufacturers' protocol (Bender Medsystems).

Statistical Analysis

All values in the figures and text are expressed as arithmetic means \pm standard error of the mean (SEM). Colitis scores were analyzed by Mann-Whitney *U* test, and values of $P < 0.05$ were considered significant. The statistical significance of any difference in each parameter among the

groups was evaluated by one-way analysis of variance (ANOVA) followed by Tukey test. P values of < 0.05 were considered statistically significant.

Results

Expression, Purification, and Identification of rTsP53

The gene encoding TsP53 was 1,239 bp in length and encoded a protein of 412 amino acids with a molecular weight of 53 kDa (<http://www.expasy.ch/tools/protparam.html>). The highly expressed fusion protein was purified by Ni–NTA affinity column. The purified thrombin-cleave products were analyzed with SDS–PAGE, with a single band in size as expected (Fig. 1).

Mice Administered with rTsP53 Are Resistant to TNBS-Induced Colitis

Weight Change and DAI Score

In this acute model of colitis, the animals that received intracolonic TNBS administration showed prostration, piloerection, and hypomotility. Diarrhea and bloody stool occurred in TNBS groups. The administration of rTsP53 partly attenuated these symptoms. Body weight loss in the rTsP53 + TNBS group was less than that in the TNBS group ($P < 0.01$). rTsP53 also attenuated the DAI score (Table 1).

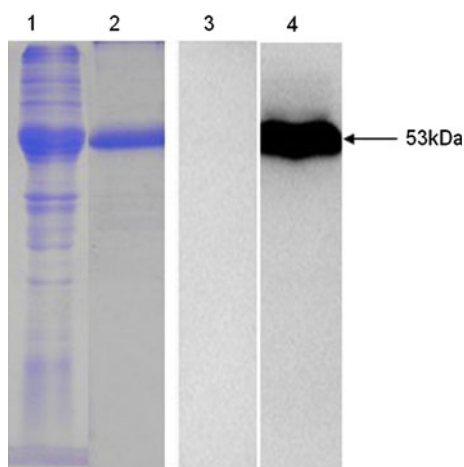


Fig. 1 Column 1 Sediments of lysate of *E. coli* BL21 containing pET-28a (+)-TsP53 with IPTG induction. Column 2 the purified product of pET-28a (+)-TsP53. Column 3 Western blot analysis of the purified rTsP53 using serum of naive mice. Column 4 Western blot analysis of the purified rTsP53 using serum of Ts infection mice

Table 1 Parameters quantified after the administration of rTsP53 in mice with acute colitis induced by trinitrobenzene sulfonic acid (TNBS) intracolonic instillation

Group	<i>n</i>	Body weight change (g)	DAI score
TNBS	10	-2.40 ± 0.87	8.15 ± 1.69
TsP53 + TNBS	10	$-0.93 \pm 1.44^*$	$4.6 \pm 2.17^*$

Data are presented as means \pm standard error of the mean (SEM)

* $P < 0.05$ vs. TNBS group

Macroscopic Colonic Damage

Macroscopic damage of the colon on the fifth day after administration of TNBS showed ulceration, thickening of the colonic wall, hyperemia and severe adhesions between the colon and other organs. Less mucosal damage, less thickening of the colonic wall, and fewer adhesions were present in the rTsP53 group. Pretreatment with rTsP53 protected mice from TNBS-induced damage in the colon. Macroscopic scores are presented in Fig. 2 (4.37 ± 1.30 vs. 2.4 ± 1.17 , $P < 0.05$).

Microscopic Colonic Damage

Histological damage of the colons from mice in the TNBS group showed large areas with mucosal erosion, necrosis of epithelium, focal ulceration of the mucosa and diffuse infiltration of inflammatory cells in the mucosa and submucosa, as well as mucosal and submucosal edema (Fig. 3B-a). Administration of rTsP53 presented less mucosal damage and ameliorated mucosal structure and epithelium. rTsP53 also reduced the amount of inflammatory cells in the mucosa and submucosa (Fig. 3B-b). Microscopic scores in the rTsP53 + TNBS group and TNBS

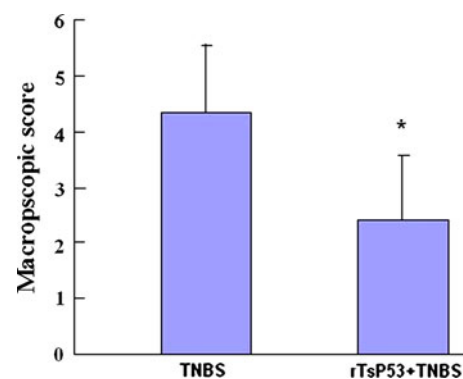


Fig. 2 Effects of administration with rTsP53 on macroscopic score. Macroscopic damage was evaluated on day 5 post-induction of colitis. Data are expressed as the means \pm standard error of the mean (SEM). * $P < 0.05$ vs. trinitrobenzene sulfonic acid (TNBS) group

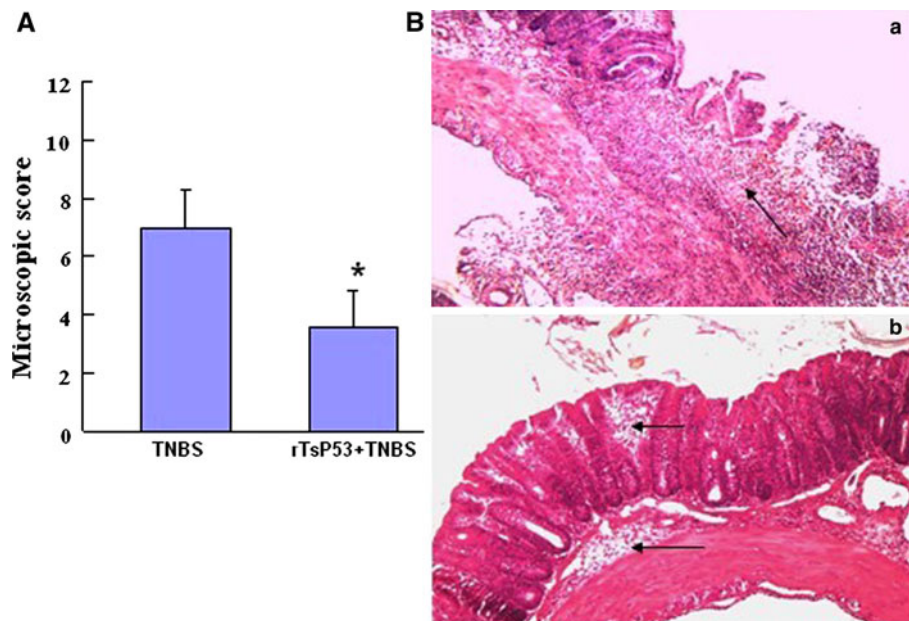


Fig. 3 Effects of administration with rTsP53 on microscopic score. Microscopic damage was evaluated on day 5 post-induction of colitis. Data are expressed as the means \pm SEM. **a** Microscopic score. * $P < 0.05$ vs. trinitrobenzene sulfonic acid (TNBS) group. **b** Representative histological findings in mice with TNBS-induced colitis treated with rTsP53. Sections of the distal colon were collected at

necropsy (on day 5) and stained with H&E. **a** TNBS-induced colitis, showing severe musocal damage with bowel-wall marked inflammatory infiltration (*arrow*). **b** RTsP53 protected the musocal structure and improved inflammation in mucosa (*up arrow*) and submucosa (*down arrow*) (original magnification, $\times 100$)

group are shown as Fig. 3A (7.0 ± 1.25 vs. 3.6 ± 1.26 , $P < 0.05$). RTsP53 reduced microscopic score significantly.

The mRNA Level of Cytokines and Arg-1 and FIZZ1 Expressed in the Colon Tissues

The expression level of IL-6 and TNF- α mRNA in the rTsP53 + TNBS group is significantly lower than that in the TNBS group ($P < 0.05$) (Fig. 4a), whereas the levels of IL-10 and TGF- $\beta 1$ in the rTsP53 + TNBS group were much higher than that in the TNBS group (Fig. 4b). Next, we investigated two M2 markers, Arg-1 and FIZZ1 mRNA, and confirmed that there were both higher than the values in the TNBS group ($P < 0.05$) (Fig. 4c).

IgG1 and IgG2a Antibody Responses to TsP53 in Mice

In our preliminary study, naive normal mice immunized three times with rTsP53 produced high levels of specific IgG1, but not IgG2a, with or without CFA (Fig. 5a). In the present study, the titres of antibody isotypes IgG1 and IgG2a in the sera from the two groups against rTsP53 without any adjuvant were compared in terms of OD value (450 nm). The rTsP53 + TNBS group produced statistically significant levels of TsP53-specific IgG1 (0.625 ± 0.181) compared with the TNBS group (0.13 ± 0.035) ($P < 0.05$). The

IgG2a against rTsP53 in the two groups were negligible (Fig. 5b).

Serum Levels of TNF- α , IFN- γ , IL-4 and IL-13

The levels of serum TNF- α and IFN- γ levels in the TNBS group were significantly downregulated by rTsP53 ($P < 0.05$). The levels of serum IL-4 and IL-13 were increased simultaneously in the rTsP53 + TNBS group compared with those in the TNBS group ($P < 0.05$) (Fig. 6).

Discussion

We investigated the rTsP53, a major component of ESA of *Ts*, and validated that it could be a protective agent for IBD. All inflammatory parameters, including DAI score, macroscopic, and microscopic scores in the rTsP53 + TNBS group were significantly lower than those in the TNBS group. RTsP53 could effectively attenuate the degree of damage and colonic inflammation caused by TNBS, showing its ability to prevent colitis.

More than 20 years ago, Stewart et al. [19] suggested that the ESA of *Ts* are likely involved in immunosuppression, but no further studies have been reported. The

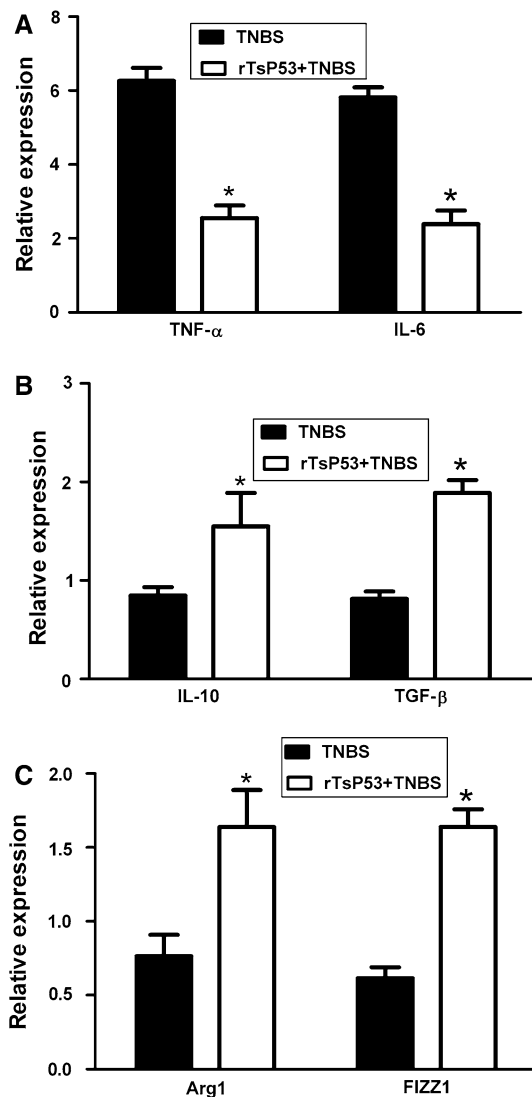


Fig. 4 Relative expression of each gene transcript (IL-10, TGF-β, IL-6, TNF-α, Arg-1, and FIZZ1) was obtained from the real time-PCR. Results are expressed as means ± standard error of the mean (SEM). **a** The relative mRNA expression of IL-6 or TNF-α in colon tissue. **P* < 0.05 vs. trinitrobenzene sulfonic acid (TNBS) group. **b** The relative mRNA expression of IL-10 or TGF-β1 in colon tissue.**P* < 0.05 vs. TNBS group. **c** The relative mRNA expression of Arg-1 or FIZZ1 in colon tissue. **P* < 0.05 vs. TNBS group

consideration of using rTsP53 as an immunomodulatory agent has been derived from its striking antigenicity of stimulating strong IgG1, with no specific IgG2a production in mice immunized with CFA and without any adjuvant in our previous and present studies. Antibody production and class switch depends on T-cells and their cytokines. IL-4 and IL-13 preferentially switch activated B cells to the IgG1 isotype (Th2 type); IFN-γ and IL-12 enhance IgG2a (Th1 type) [20, 21]. Because of stimulating strong IgG1, rTsP53 can be considered as a Th2-prone immunomodulator. TNBS-induced colitis is a Th1-driven inflammation

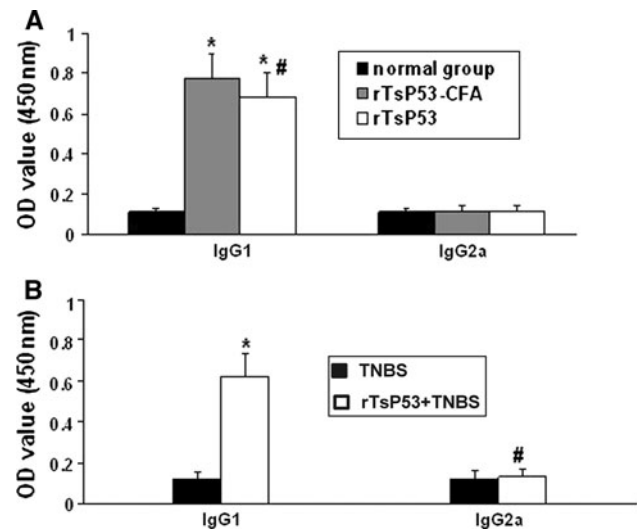


Fig. 5 Isotype profiles to rTsP53. Results are expressed as means ± standard error of the mean (SEM). **a** In our previous results, **P* < 0.05 vs. normal group and #*P* < 0.05 vs. rTsP53-CFA group. The levels of IgG2a in the three groups were not significant. **b** In the present study, **P* < 0.05 vs. trinitrobenzene sulfonic acid (TNBS) group. # Not significant compared to TNBS group (*P* > 0.05)

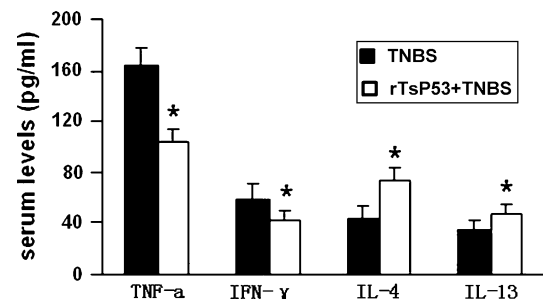


Fig. 6 Levels of serum Th1 cytokines (TNF-α and IFN-γ) and Th2 cytokines (IL-4 and IL-13) in two groups. Data are expressed as the means ± standard error of the mean (SEM). **P* < 0.05 vs. trinitrobenzene sulfonic acid (TNBS) group

process in which TNF-α, IFN-γ, and IL-6 play a fundamental role by triggering leukocyte activation and tissue accumulation in IBD [22]. In this study, rTsP53 significantly downregulated Th1 cytokines (TNF-α, IFN-γ) and inversely upregulated Th2 cytokines (IL-4, IL-13) in the sera of a TNBS-induced mice model. This Th2-prone immune status also influenced local immunity in mucosa. In the rTsP53 + TNBS group, the mRNA of TNF-α and IL-6 in the local colon tissue have been significantly decreased compared to the TNBS group. In addition, significant upregulation of IL-10 and TGF-β1 mRNA have been detected from colonic tissues in rTsP53 + TNBS treatment mice. IL-10 is an important protective factor to colitis. IL-10 neutralized or deficient mice spontaneously developed colitis [18, 23]. So, rTsP53 has a protective

ability against colitis by upregulation of Th2 cytokines and regulatory factors.

Upregulation of serum IL-4, IL-13, and colonic IL-10 might indicate the activation of M2 macrophages that have been confirmed as anti-inflammatory regulator factors in colitis [24]. Herbert et al. [25] showed that alternative macrophages were essential during schistosomiasis for protection against organ injury through down-regulation of egg-induced inflammation. Infection with *H. diminuta* increased intestine expression of Arg-1 and FIZZ1, markers of M2 [26]. Depletion of intestinal macrophages using clodronate-liposomes prevented the anti-colitic effect of infection with *H. diminuta*. Meanwhile, the injection of M2 significantly reduced the severity of DNBS-induced colitis [27]. Attenuation of TNBS-induced inflammation in colon by rTsP53 might be linked to the activation of M2 in intestinal mucosa. In this work, we detected mRNA of Arg-1 and FIZZ1, two markers for M2 macrophages, in the colon tissue of TNBS-induced colitis mice. Arg-1 is the counter-regulatory enzyme to inducible NO synthase (iNOS) and can thus act to suppress NO production. Arg-1 also has well documented roles in tissue repair and has recently been implicated as an anti-nematode effector molecule [28, 29]. The functions of FIZZ1 are not yet fully understood but, like Arg-1, they have been strongly implicated in the response to injury [30]. The mRNA levels of Arg-1 and FIZZ1 were higher in rTsP53 + TNBS treatment than in the TNBS group. This demonstrates that rTsP53 evoked a M2-bias innate immunity in the colitis model. Does rTsP53 act on macrophages directly or indirectly? ESA-62 from filarial nematodes modulates cytokine production (IL-12, TNF- α) in DCs and macrophages through the phosphorylcholine which it contains to bind the toll like receptor (TLR)-4 [31, 32]. Therefore, further investigation is required in order to investigate whether the M2 macrophages in the intestinal mucosa induced by subcutaneously inoculated rTsP53 is executed through direct interaction between rTsP53 and macrophages or through other immune cells such as DCs.

This study demonstrated rTsP53 is an effective agent to prevent experimental colitis by stimulating Th2 responses and suppressing Th1 responses. Its great availability renders preventing IBD and even other Th1-prone acute inflammatory diseases and autoimmune disorders with helminth proteins as a feasible option, although there are many issues that must be elucidated, such as the effective dosage, potential toxicity, and inoculation times. However, there are some limitations. The data show helminths as a therapy for heterogeneous inflammatory disorders depends on the immunologic basis of the condition. *H. diminuta* infection is beneficial in other models of colitis, but causes a significant exacerbation of oxazolone-induced colitis (a Th2 model) [33]. So, rTsP53 as a Th2-prone

immunomodulator may be a benefit to TNBS-induced colitis (a Th1 model) but not suitable to a Th2 model. Furthermore, rTsP53 as a heterogeneous protein may cause possible adverse effects such as allergic reaction. So, there are many practical questions to solve.

Acknowledgments This work was supported by the China National Great Basic Research Program (the 973 program, No. 2010CB530003) and the China National Natural Science Foundation (No. 30771887).

References

- Podolsky DK. Inflammatory bowel disease. *N Engl J Med*. 2002;347:417–429.
- Wurzelmann JI, Lyles CM, Sandler RS. Childhood infections and the risk of inflammatory bowel disease. *Dig Dis Sci*. 1994;39:555–560.
- Elliott DE, Urban JF Jr, Argo CK, Weinstock JV. Does the failure to acquire helminthic parasites predispose to Crohn's disease? *FASEB J*. 2000;14:1848–1855.
- Fox JG, Beck P, Dangler CA, et al. Concurrent enteric helminth infection modulates inflammation and gastric immune responses and reduces *Helicobacter*-induced gastric atrophy. *Nat Med*. 2000;6:536–542.
- Khan WI, Blennerhasset PA, Varghese AK, et al. Intestinal nematode infection ameliorates experimental colitis in mice. *Infect Immun*. 2002;70:5931–5937.
- Elliott DE, Li J, Blum A, et al. Exposure to schistosome eggs protects mice from TNBS-induced colitis. *Am J Physiol Gastrointest Liver Physiol*. 2003;284:G385–G391.
- Summers RW, Elliott DE, Urban JF Jr, Thompson R, Weinstock JV. *Trichuris suis* therapy in Crohn's disease. *Gut*. 2005;54:87–90.
- Summers RW, Elliott DE, Urban JF Jr, Thompson R, Weinstock JV. *Trichuris suis* therapy for active ulcerative colitis: a randomized controlled trial. *Gastroenterology*. 2005;128:825–832.
- Brown BN, Valentin JE, Stewart-Akers AM, McCabe GP, Badylak SF. Macrophage phenotype and remodeling outcomes in response to biologic scaffolds with and without a cellular component. *Biomaterials*. 2009;30:1482–1491.
- Khan WI, Collins SM. Immune-mediated alteration in gut physiology and its role in host defence in nematode infection. *Parasite Immunol*. 2004;26:319–326.
- Ruysers NE, De Winter BY, De Man JG, et al. Therapeutic potential of helminth soluble proteins in TNBS-induced colitis in mice. *Inflamm Bowel Dis*. 2009;15:491–500.
- Motomura Y, Wang H, Deng Y, El-Sharkawy RT, Verdu EF, Khan WI. Helminth antigen-based strategy to ameliorate inflammation in an experimental model of colitis. *Clin Exp Immunol*. 2009;155:88–95.
- Yera H, Andiva S, Perret C, Limonne D, Boireau P, Dupouy-Camet J. Development and evaluation of a Western blot kit for diagnosis of human trichinellosis. *Clin Diagn Lab Immunol*. 2003;10:793–796.
- Smith P, Mangan NE, Walsh CM, et al. Infection with a helminth parasite prevents experimental colitis via a macrophage-mediated mechanism. *J Immunol*. 2007;178:4557–4566.
- Wendelsdorf K, Bassaganya-Riera J, Hontecillas R, Eubank S. Model of colonic inflammation: immune modulatory mechanisms in inflammatory bowel disease. *J Theor Biol*. 2010;264:1225–1239.

16. Nagano I, Wu Z, Takahashi Y. Species-specific antibody responses to the recombinant 53-kilodalton excretory and secretory proteins in mice infected with *Trichinella* spp. *Clin Vaccine Immunol*. 2008;15:468–473.
17. Bobin-Dubigeon C, Collin X, Grimaud N, Robert JM, Le Baut G, Petit JY. Effects of tumour necrosis factor-alpha synthesis inhibitors on rat trinitrobenzene sulphonic acid-induced chronic colitis. *Eur J Pharmacol*. 2001;431:103–110.
18. Hunter MM, Wang A, Hirota CL, McKay DM. Neutralizing anti-IL-10 antibody blocks the protective effect of tapeworm infection in a murine model of chemically induced colitis. *J Immunol*. 2005;174:7368–7375.
19. Stewart GL, Wood BG, Boley RB. Modulation of host response by *Trichinella pseudospiralis*. *Parasite Immunol*. 1985;7:223–233.
20. Chakir H, Wang H, Lefebvre D, Webb J, Scott FW. T-bet/GATA-3 ratio as a measure of the Th1/Th2 cytokine profile in mixed cell populations: predominant role of GATA-3. *J Immunol Methods*. 2003;278:157–169.
21. Rengarajan J, Szabo S, Glimcher L. Transcriptional regulation of Th1/Th2 polarization. *Immunol Today*. 2000;21:479–483.
22. Deventer SJH. Tumor necrosis factor and Crohn's disease. *Gut*. 1997;40:443–448.
23. Elliott DE, Setiawan T, Metwali A, Blum A, Urban JF Jr, Weinstock JV. *Heligmosomoides polygyrus* inhibits established colitis in IL-10-deficient mice. *Eur J Immunol*. 2004;34:2690–2698.
24. Zurawski G, De Vries JE. Interleukin-13, an interleukin-4 like cytokine that acts on monocytes and B cells, but not on T-cells. *Immunol Today*. 1994;15:19–26.
25. Herbert DR, Hölscher C, Mohrs M, et al. Alternative macrophage activation is essential for survival during schistosomiasis and downmodulates T helper 1 responses and immunopathology. *Immunity*. 2004;20:623–635.
26. Persaud R, Wang A, Reardon C, McKay DM. Characterization of the immuno-regulatory response to the tapeworm *Hymenolepis diminuta* in the non-permissive mouse host. *Int J Parasitol*. 2007;37:393–403.
27. Hunter MM, Wang A, Parhar KS, et al. In vitro-derived alternatively activated macrophages reduce colonic inflammation in mice. *Gastroenterology*. 2010;138:1395–1405.
28. Witte MB, Barbul A. Arginine physiology and its implication for wound healing. *Wound Repair Regen*. 2003;11:419–423.
29. Anthony RM, Urban JF, Alem F, et al. Memory T(H)2 cells induce alternatively activated macrophages to mediate protection against nematode parasites. *Nat Med*. 2006;12:955–960.
30. Loke P, Gallagher I, Nair MG, et al. Alternative activation is an innate response to injury that requires CD4 + T cells to be sustained during chronic infection. *J Immunol*. 2007;179:3926–3936.
31. Goodridge HS, Marshall FA, Else KJ, et al. Immunomodulation via novel use of TLR4 by the filarial nematode phosphorylcholine-containing secreted product, ES-62. *J Immunol*. 2005;174:284–293.
32. Kane CM, Cervi L, Sun J, et al. Helminth antigens modulate TLR-initiated dendritic cell activation. *J Immunol*. 2004;173:7454–7461.
33. Hunter MM, Wang A, McKay DM. Helminth infection enhances disease in a murine TH2 model of colitis. *Gastroenterology*. 2007;132:1320–1330.