Inhibition of COX-2 Ameliorates Murine Liver Schistosomiasis Japonica Through Splenic Cellular Immunoregulation

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Research Article

Keywords: COX-2, NS398, granulomatous inflammation, myeloid-derived suppressor cells, T cell subsets, germinal center B cells, Tfh, Tfr, Schistosoma japonicum

Posted Date: November 23rd, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-1094662/v1>

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Abstract

We have reported the positive association of cyclooxygenase 2 (COX-2)/prostaglandin E2 (PGE2) axis with liver fibrosis induced by *Schistosoma japonicum* (*Sj*) infection, and TLR4 signaling controlled this axis. However, how COX-2 regulated immune response during *Sj* infection is still unclear. Here we further studied the effect of COX-2 specific inhibitor-NS398 on liver granulomatous inflammation, fibrosis and explored the mechanisms via evaluating different immune cell during *Sj* infection. The results showed that NS398 significantly reduced the size of liver granuloma, spleen and mesenteric lymph node (MLN) and alleviated chronic granulomatous inflammation. Mechanically, it might be via decreasing the numbers of *Sj*-induced T helper type 1 (Th1), Th2, T follicular helper (Tfh), T follicular regulatory cells (Tfr) and germinal center B (GCB) cells to alleviate the liver inflammation and fibrosis. In addition, there were no difference of the numbers of macrophages, neutrophils, MDSCs, Th17 and total B cells in the spleen of the mice with or without NS-398 treatment. Our above data suggests that COX-2/PGE2 inhibition may represent a potential therapeutic approach to schistosomiasis japonica.

Introduction

Schistosomiasis is a chronic helminthic disease affecting over 250 million people in over 78 countries [(WHO 2018, <https://www.who.int/news-room/fact-sheets/detail/schistosomiasis>). The three major schistosomes infecting humans are *Schistosoma mansoni* (*Sm*), *Schistosoma haematobium* (*Sh*), and *Schistosoma japonicum* (*Sj*). *Sj* causes a hepato-intestinal form of the disease and is endemic in China, the Philippines, Indonesia, and the Mekong Delta. *Sj* resides in the mesenteric veins and hepatic portal vein, where they release eggs that induce a dramatic immune response in the intestines and liver, and then granuloma formation which characterized as eggs encapsulation within layers of immune cells embedded in extracellular matrix (ECM). Schistosomiasis japonica is divided into acute and chronic phases. The acute phase in murine occurs in the first 6 weeks after exposure, which manifested mainly as early liver granuloma stage[1]. In the chronic phase, the hepatointestinal or the hepatosplenic disease may occur[2]. Splenomegaly is a consequence and an important clinical indicator of portal hypertension[3]. Spleen is composed of three areas-white pulp, red pulp, and a transitional zone, and plays a role as a filter of the blood and as one of the major peripheral immune organs. In its red pulp area, reticuloendothelial cells such as macrophages will clear away abnormal red blood cells or pathogens.

Many innate and adaptive immune cells can exert specific effects on hepatic pathology through immunoregulation. Macrophages are the most abundant in the granuloma liver of mice with *Sm* infection. Myeloid derived suppressor cells (MDSCs) as potent suppressors of immune responses, emerge in the blood, bone marrow or spleen during cancer, infections or inflammation. Yang Q et al found that the soluble egg Ag (SEA) and soluble worm Ag (SWA) of *Sj* enhanced the accumulation of MDSCs into bone marrow, spleen, and mesenteric lymph nodes (MSN)[4]. The recruitment of neutrophils to the liver by IL-17A have been associated with the development of fibrosis in many chronic liver diseases including schistosomiasis japonica [5]. T lymphocytes are classified into CD4⁺ T helper (Th) cells and CD8⁺ cytotoxic T lymphocytes (CTLs). The roles of Th cells in the immune-pathogenesis of

schistosomiasis have been intensely reviewed[6]. Moderate Th1 responses are included in the acute schistosomiasis and early granuloma formation, while excessive Th1 response will develop easily severe acute cachexia followed by death and is detrimental to the host. Th2 immunity acts as a double-edged sword: on the one side, it exerts anti-inflammatory effects and suppress Th1-mediated immunopathology, but on the other side, it drives liver immunopathological damage especially liver fibrosis. Therefore, Th1/Th2 balance maintaining is important to control the excessive pathology of schistosomiasis. Th17/IL-17 exacerbate the egg-induced liver immunopathology in schistosomiasis. Follicular helper T cells (Tfh) are mainly located in the periphery of B cell follicles in secondary lymphoid organs, which regulate antigen-specific B cells to become professional antibody producers, and help the formation of germinal centers (GC), affinity maturation of antibodies, somatic hypermutation and for the production of memory B cells. T follicular regulatory cells (Tfr) also exist in GCs, where they play a inhibitory role in GC reactions[7][8]. Tfh promoted liver granulomas and fibrogenesis in *Sj*-infected mice[9, 10, 11]. B cell lymphoma 6 (Bcl6)&programmed death-1 (PD-1) positive Tfh in the GC of murine spleen correlate with progression of liver fibrosis [10]. Tfh and Tfr increased in patients with schistosomiasis japonica [12].

Humoral immunity requires cross-talk between Tfh, Tfr and B cells. Several studies have demonstrated that the B-cell number in the lymph nodes and spleen significantly increase during the schistosome infection[13]. *Sj* recombinant fusion proteinSjGST-32combined with tacrolimus (FK506) immunization augmented the induction of Tfh cells, and the expression of IL-21R on GCB cells and memory B cells increased in these immunized mice[14].

Cyclooxygenase (COX)-1 and COX-2 catalyze the first step in prostanoid biosynthesis. COX-1 is constitutively expressed whereas COX-2 is induced by some stimuli. High COX-2 expression has been detected in several liver pathologies, while the implication of COX-2inmany liver diseases is a matter of controversy[15]. Soluble egg antigen (SEA) from *Sm* drove potent Th2 responses through triggering dendritic cells to produce COX-1, COX-2 and then prostaglandin E2 (PGE2) [16]. We have reported that COX-2 inhibitor-NS398 protected mice from hepatic fibrosis induced by *Sj* infection[17].However, there is a lack of studies on the effects of hepatic COX-2 on immune cells during *Sj*-induced liver fibrosis.

Herein, we will demonstrate the effect of NS398 as the COX-2 & PGE2 synthesis inhibitoron macrophages, neutrophils, MDSCs and Th cell subsets, B cells and in the spleen during *Sj* infection.

Materials And Methods

Reagents

NS398, the inhibitor of COX-2 activity was purchased from Med Chem Express (HY-13913, New Jersey, USA).Fluoresce in-conjugated anti-mouse Abs (F4/80-FITC, CD11b-PE, Gr-1 – APC, Dead-APC-A750, CD45-PB450, CD45-KO525; CD4-FITC, CD3-PC5.5, IL-17A-PC7, IFN- γ -APC, IL-4-PB450; CD3-FITC, Foxp3-PC5.5, PD-1-PC7, CXCR5-APC, FVD-PB450, CD4-KO525; FAS-PE, CD19-PC5.5, B220-PC7, GL7-APC, CD138-APC-A750, FVD-PB450) and their corresponding isotype controls were obtained from eBioscience (San Diego, CA). Ly6G-PC7 and Ly6G-allophycocyanin were obtained from BD Biosciences (San Jose, CA).

Mice, parasite infection and NS398 treatment

6-8-week-old female C57BL/6 mice were obtained from the SPF Biotechnology Co. Ltd (Beijing) and were maintained according to institutional guidelines. All mice experiments were approved to be humane by the Institutional Animal Care and Use Committee at South China Agricultural University (2019-1013). Mice were infected by 20 ± 3 *Sj* cercariae of the Chinese mainland strain through abdomen skin penetration. NS398 (3mg/kg body weight) in 2%DMSO was medicated into mice by intraperitoneal injection 3 times a week from week 5 to week 7 with *Sj* infection (n=8), while the infection control group only received 2%DMSO (n=7). Two non-infected control mice groups were treated with NS398 (n=5) and 2%DMSO (n=9) respectively. Mice were sacrificed at week 8 (8wks) after *Sj* infection. Spleens, MSN and liver tissues were collected for further analysis.

HE staining

Fresh hepatic tissues were fixed in 4% paraformaldehyde for 24 h, and then were embedded with paraffin. The 4µm liver sections were prepared and stained with hematoxylin & eosin (HE) staining for the granuloma size and the extent of liver granulomatous inflammation analysis. The severity of liver granulomatous inflammation was evaluated according to calibrated criteria (Table 1), Fig. 1B and reference [1].

Table 1
Schistosoma japonicum-induced liver granuloma stages and inflammation

I. The pre-granulomatous stage—mini size, early inflammation (stage a)
<ul style="list-style-type: none"> a. Minimal disorganized aggregation of immune cells b. No fibroblasts and extracellular matrix, no collagen fibers c. Few eggs deposition
II. The granulomatous stages
1) Early stage—small size, acute inflammation (stage b)
<ul style="list-style-type: none"> a. Infiltration of organized immune cells including monocytes, eosinophils ect. around eggs b. No fibroblasts and collagen fibers, with few or without extracellular matrix. c. Many eggs deposition <p>Note: Stage b1: Several organized immune cells infiltration; stage b2: Many organized immune cells infiltration, without or with few extracellular matrix</p>
2) Mature stage—big size, chronic and fibrotic inflammation (stage c)
<ul style="list-style-type: none"> a. Clear outer granuloma rim which is composed of a dense population of immune cells b. Many recruited fibroblasts and their product including apparent extracellular matrix and collagen fibers inside the granuloma c. Live or dead miracidium within the deposited eggs
3) Late stage—small size, recovery (stage d)
<ul style="list-style-type: none"> Some pigmented macrophage infiltration Disintegrated granuloma structure Dead and calcified eggs

Preparation of single cell suspensions of mice spleen

Mice were anaesthetized, and sterile normal saline was injected into left ventricle to remove blood from organs. Then, the spleens were used to harvest cell suspensions by pressing these tissue pieces through a 100-mm cell strainer (BD Falcon) and then suspending in Hanks' balanced salt solution (HBSS). Red blood cells were lysed with ammonium chloride (NH₄Cl) for 10 min. Cell suspensions were incubated with LIVE/DEAD Zombie NIR™ Fixable Viability Kit (Biolegend) for 20 min, and then resuspended at 2-3×10⁶ cells/ml in complete RPMI 1640 medium with 10% FBS.

Cell surface staining and then flow cytometry analysis

Cell suspensions were preblocked with mouse Fc block antibody (BD, clone 2.4G2). The following antibodies were used for cell surface markers' staining: anti-CD45-BV510 (clone 30-F11), anti-CD11b-

PE(clone M1/70), anti-Ly6G-PE-cy7 (clone 1A8), anti-F4/80-FITC (clone BM8), anti-B220-PE-cy7 (clone RA3-6B2), anti-CD19-PerCP-Cy5.5 (clone eBio1D3), anti-PD-1-APC (clone 29F.1A12), anti-CD3e-PerCP-Cy5.5(clone 145-2C11),anti-CD4-FITC(clone RM4-5),anti-CXCR5-Biotin (clone 2G8),anti-CD95-PE (clone 15A7) and anti-GL7-Alexa Fluor® 647 (clone GL7).Flow cytometry (FACS) analysis was conducted in CytoFLEX (BECKMAN COULTER) and analyzed with FlowJo software (FlowJo LLC, Ashland, OR).

Cell surface staining and then flow cytometry analysis

Foxp3 intracellular staining were conducted using eBioscience™ Foxp3/transcription factor fixation/permeabilization concentrate and diluent. Cells were washed using permeabilization buffer (10×) and intracellular staining with anti-Foxp3-PerCP-Cy5.5 (clone FJK-1bs) for 40 min. For intracellular staining of cytokines, cell suspensions were stimulated with phorbol 12-myristate 13-acetate (20 ng/ml; Sigma-Aldrich), ionomycin (1 ug/ml; Sigma-Aldrich), and BFA (10µg/ml; Sigma-Aldrich) for 4 hours at 37°C. Then, cells were fixed, permeabilized, and stained for anti-IL4-BV421(clone 11B11), anti-interferon gama (IFN-γ)-APC (clone XMG1.2), anti-IL-17A-PE-cy7 (clone EBI017B7). FACS was conducted and analyzed.

Statistical analysis

The results are presented as the standard deviation (±SD) of the indicated number of replicates/experiments. Data from each group were analyzed using SPSS (v11.0). Statistical evaluation of the difference between means was performed by one or two-tailed, paired or unpaired, Student's t test. Ap-value of ≤0.05 was considered statistically significant.

Results

COX-2 inhibition by NS398 treatment reduced *Sj* infection-driven hepatomegaly, the size of granuloma, and the infiltration of immune cells around granuloma in the liver of mice

We have reported that COX-2/PGE2 axis was involved in the formation of liver fibrosis induced by *Sj* infection under the controlling of TLR4 pathway [17]. Chronic liver inflammation was supposed to develop into fibrosis. The size of granuloma induced by *Sj* egg deposition especially at mature and late stage marked as the extent of fibrosis and the amount of early stage granuloma manifested as the extent of inflammation. Herein, we found that COX-2 inhibition by NS398 treatment significantly attenuated hepatomegaly (Fig. 1A)(t-test: *Sj*/DMSO vs *Sj*/NS398: $t_{(5)}=78.17$, $P=0.046$) and the average granuloma size in the liver which including the size of mature and late stage granulomas induced by single egg deposition (Fig. 1C, 1E) (t-test: *Sj*/DMSO vs *Sj*/NS398: $t_{(39)}=24703.35$, $P=0.0486$). According to H&E staining of the liver sections, *Sj* infection-induced liver granulomas were classified into different stages (Table 1, Fig. 1B) [1]. COX-2 inhibition decreased the percentage of early stage granuloma significantly (Fig. 1D, 1E)(t-test: *Sj*/DMSO vs *Sj*/NS398: $t_{(5)}=25.74$, $P=0.047$). These supported that NS398 alleviated the extent of both inflammation and fibrosis in the *Sj*-infected mice liver.

NS398 decreased *Sj* infection-induced enlargement of mesenteric lymph nodes and splenomegaly of mice

Immune cells in the MLN and spleen tended to migrate or recycle into the liver and promoted hepatic inflammation & fibrosis induced by *Sj* infection [2]. The diameter of spleen thickness represents the severity of fibrosis. Herein, we showed that the size of MLN and spleen were significantly attenuated (Fig. 2) (t-test: *Sj*(-)/DMSO vs *Sj*(+) /DMSO: MSN size: $t_{(5)}=11.18$, $P=0.0001$; Spleen size: $t_{(5)}=18.77$, $P<0.0001$. *Sj*(+)/DMSO vs *Sj*(+)/NS398: MSN size: $t_{(5)}=7.13$, $P=0.0025$; Spleen size: $t_{(5)}=13.23$, $P=0.0008$), which suggested that NS398 lowered their contribution to liver pathogenesis during *Sj* infection.

NS398 didn't affect the percentage of macrophages, neutrophils, and MDSCs in the spleen of *Sj*-infected mice

To explore the existence of macrophages, neutrophils, and MDSCs in *Sj*-infected mice spleen at 8wk of *Sj* infection with or without NS398 treatment, mononuclear cells were isolated from mouse spleen, and the percentage of CD45, CD11b and F4/80 co-expressed macrophage (M ϕ), CD11b and Gr-1 co-expressed MDSCs, CD11b and Ly6G co-expressed neutrophils were detected by FACS (Fig. 3A). *Sj*-infection didn't change significantly the quantities of macrophages in the murine spleen, but significantly increased the percentage of neutrophils (t-test: *Sj*(-)/DMSO vs *Sj*(+) /DMSO: Neu: $t_{(5)}=11.80$, $P=0.0063$) and MDSCs (t-test: *Sj*(-)/DMSO vs *Sj*(+) /DMSO: $t_{(5)}=40.74$, $P=0.0083$), especially polymorphonuclear leucocyte (PMN)-MDSCs (t-test: *Sj*(-)/DMSO vs *Sj*(+) /DMSO: $t_{(5)}=8.86$, $P=0.018$) (Fig. 2B-E). However, NS398 treatment didn't significantly change the percentage of all of these cells distributing in the murine spleen (Fig. 3B-E).

COX-2 inhibition reduced *Sj* infection-driven Th1, Th2 but not Th17 cells in the spleen

To investigate whether T helper cell subsets in the spleen were involved in the alleviation of mice liver pathogenesis induced by *Sj* infection during NS398 treatment, mononuclear cells from infected mouse spleen with or without NS398 treatment were stained by fluorescence-labeled anti-CD3, CD4, IFN- γ , IL-4 and IL-17A, and then were detected by FACS (Fig. 4A). *Sj*-infection significantly increased the percentage of Th1 (t-test: *Sj*(-)/DMSO vs *Sj*(+) /DMSO: $t_{(5)}=15.76$, $P=0.022$) and Th2 (t-test: *Sj*(-)/DMSO vs *Sj*(+) /DMSO: $t_{(5)}=8.94$, $P=0.0032$) in the spleen, and these increasing were significantly lowered by NS398 treatment (t-test: *Sj*/DMSO vs *Sj*/NS398: Th1: $t_{(5)}=8.84$, $P=0.0095$; Th2: $t_{(5)}=3.03$, $P=0.0021$) (Fig. 4B, 4C). However, *Sj*-infection didn't increase the percentage of Th17 and NS398 didn't affect this Th subpopulation in the mice spleen (Fig. 4D). The effect of NS398 on decreasing Th1 and Th2 differentiation explained its implication on alleviating the extent of liver granulomatous inflammation and fibrosis.

NS398 decreased *Sj* infection-triggered Tfh and Tfr cell generation

Profoundly impaired CD4⁺ T cell responses are associated with *Sj* infection. Tfh and Tfr increased in patients with *Sj* infection [12]. However, the function of Tfr-mediated immune responses to *Sj* infection

and the implication of NS398 to this cell subset is unclear. Flow cytometry was performed to analyze Tfh and Tfr populations within spleen mononuclear cell preparations, as shown in Fig. 5A. As expected, the frequency of Foxp3⁻CD4⁺ T cells in CD4⁺CD3⁺ T cells was increased in *Sj* infected mice spleen (t-test: *Sj*(-)/DMSO vs *Sj*(+) /DMSO: $t_{(5)}=81.96, P=0.051$), while Foxp3⁺ CD4⁺ T cells (regulatory T cells, Treg) was significantly decreased (t-test: *Sj*(-)/DMSO vs *Sj*(+) /DMSO: $t_{(5)}=17.54, P=0.039$) (Fig. 5B). No significantly increasing of PD-1⁺CXCR5⁺ Tfh cells (t-test: *Sj*(-)/DMSO vs *Sj*(+) /DMSO: $t_{(5)}=31.5, P=0.097$) but significantly elevating level of Tfr cells (t-test: *Sj*(-)/DMSO vs *Sj*(+) /DMSO: $t_{(5)}=30.18, P=0.0032$) were shown in the *Sj*-infected murine spleen (Fig. 5C-D). NS398 treatment didn't change the amount of Foxp3⁻ and Foxp3⁺ Th cells (Fig. 5B), but it significantly decreased Tfh cells (Fig. 5C) (t-test: *Sj*/DMSO vs *Sj*/NS398: $t_{(5)}=26.56, P=0.0087$) and Tfr populations (Fig. 5D) (t-test: *Sj*/DMSO vs *Sj*/NS398: $t_{(5)}=16.08, P=0.024$). Therefore, PD-1⁺CXCR5⁺ Tfh cells and Tfr cells in the spleen might play important role in the schistosomiasis japonica and NS398 will control their role.

NS398 treatment reduced the percentage of GCB cells in the spleen

As reported, during *Sj* infection, splenic B-cell number was significantly increased [13, 18]. Here, we found that at 8wk of *Sj* infection, the number of B cells in the mice spleen and GCB cells were increased, especially GCB cells (Fig. 6). NS398 treatment showed no change in B cells, but significantly decreased the amount of GCB cells (Fig. 6B and 6C) (t-test: *Sj*(-)/DMSO vs *Sj*(+) /DMSO: $t_{(4)}=5.49, P=0.024$; *Sj*/DMSO vs *Sj*/NS398: $t_{(5)}=1.29, P=0.012$). The effect of NS398 on GCB cells was consistent to Tfh and Tfr.

Discussion

In this study, we investigated the infiltration of multiple immune cells into the mice spleen during 8wks of *Sj* infection, and the relationship between NS398 (COX-2 specific inhibitor) treatment and the amount of these infiltrating cells and the extent of schistosomiasis japonica. We found that NS398 decreased *Sj* infection-driven hepatomegaly, the size of granuloma, and the extent of liver fibrosis and granulomatous inflammation, which was consistent to our last study [17]. The involved mechanisms might include the NS398's significantly decreasing *Sj* infection-induced enlargement of mesenteric lymph nodes and splenomegaly, and Th1, Th2, Tfh, Tfr differentiation and GCB maturation in the mice spleen. We firstly identified that 8wk of *Sj* infection significantly increased the amount of MDSCs, Tfr and GCB cells in the spleen. These suggested that liver pathogenesis induced by *Sj* infection might be through the activation of COX-2/PGE2 axis in the liver and then the induction of Th1, Th2, Tfh, Tfr differentiation and GCB maturation in the spleen.

In terms of the effect of *Sj*-infection on the cellular immune regulation, Zheng L et al [5] showed that neutrophils in the spleen of C57BL/6 mice increased gradually from 6, 8 and 12 wks of 20 *cercariae* infection. The tendency between neutrophils and Th17 cells was consistent in the mice with Schistosomiasis [19][20]. Yang Q et al identified that MDSCs in the spleen of C57BL/6 mice with 5-6wks

of 40 ± 5 *Sj cercariae* infection were strikingly increased[4].Huang P et al [21] showed that Th2, Th17 and CD25⁺Foxp3⁺ CD4⁺T cells (Treg) but not Th1 significantly increased in the BALB/c mice spleen post 7wks of 30 ± 2 *Sj cercariae* infection. Tebeje BM et al[22]identified that 5wks of 34 *Sj cercariae*-infected splenic Th1 cells responded more highly to *Sj* adult worm antigen preparation (SWAP)compared to SEA. Th2 immune response to SEA was dominant at week 6, and Treg response was high in the CBA mice spleen at week 5 followed by a decline at week 6. Su C's group [23] identified the dynamics of Th1, Th2, Th17 and Treg cells and their role in 12 *Sjcercariae* infection in C57BL/6 mice and showed that all of these T cell subsets increased gradually in the infected mice spleen at week 5, 8 and 13.Elevated frequencies of Th17 cells have been shown in the *Sm*-infected C57BL/6 mice spleen at week 6, but not at week 4 or 8 [24]. In contrast to C57BL/c, CBA mice develop more severe lesions driven by Th17 cells [25].Tfh cells in the spleen of C57BL/6 mice with both 5 and 6 weeks of 40 ± 5 *Sj cercariae* infection was significantly increased, especially at week 5[11]. High frequency of Tfh and Tfr cells were significantly increased in the peripheral blood mononuclear cells (PBMCs) of patients with schistosomiasis japonica [26]. Infection with *Sj* induced TGF- β & IL-10 producing B cells while decreased CR5⁺ B1a cells [13, 18, 27]. Herein, we showed the consistent increasing of neutrophils, MDSCs, Th1, Th2, Tfh, Tfr cells in the C57BL/6 mice with 8wks of 20 ± 3 *Sj cercariae* infection. However, the amount of Th17 didn't increased as reported. Different dose of *Sjcercariae* infection and different mice strain and infecting duration might explain.

COX-2 plays an important role in the progression of liver fibrosis[28][17]. We have found that the COX-2/PGE2 axis was positively associated with the extent of liver fibrosis induced by *Sj* infection [17]. Herein, we showed that NS398, the COX-2 specific inhibitor lowered the granuloma size, and ameliorated splenomegaly and the size of MSN, which supported its effect on liver inflammation and fibrosis. Macrophages in the *Sj*-infected mice spleen were almost significantly decreased by NS398 (p=0.055). Macrophage deletion with chlodronate significantly attenuated granuloma formation in the liver of mice induced by *Sj* infection [29]. COX-2 blockage by NS398 inhibited accumulation and function of MDSCs and promoted proliferation and inhibited apoptosis of CD4⁺ T cells in the spleen and bone marrow of mice with traumatic stress [30]. Triggering the COX-2-PGE2/EP2 pathway resulted on the induction of Th2 immune response [31]. Septic rats given NS398 showed amelioration of IL-6, tumor necrosis factor alpha (TNF- α)and CD4⁺/CD8⁺ T cells' imbalance in the liver and decreased liver injury [32]. NS398 significantly increased IL-4 secretion while decreased IFN- γ secretion by splenocytes after ovalbumin stimulation in mice with allergic skin inflammation [33]. NS398 stimulated Th1 and inhibited Th2 type cytokines which were produced by PBMCs co-culturing with supernatant of A549 cell line [34]. However, *Staphylococcus aureus* Cowan I-induced IFN- γ production was increased markedly in spleen cells from BALB/c mice with NS398 treatment[35]. Therefore, the effect of NS398 on MDSCs, Th1 and Th2 relied on the disease model. Overexpression of COX-2 enhanced survival of chronic lymphocytic leukemia B cells. NS398 significantly reduced the generation of CD38⁺ IgM⁺ and CD38⁺ IgG⁺ antibody-secreting cells [36]. However, no study showed the role of NS398 in Tfh, Tfr differentiation and GCB maturation. Since both Tfh and Tfr were dominant populations in the *Sj*-infected mice spleen and NS398 significantly decreased them. The role and mechanism of them in *Sj* infection-induced liver fibrosis worth deep investigation.

Conclusions

Our study firstly outlined the reciprocal relationships between the COX-2/PGE2 axis and the size of liver, spleen, MSN and liver granuloma, and multiplex immune cell infiltration in the spleen. We provided the evidence of COX-2 inhibition ameliorated liver inflammation and fibrosis induced by *Sj* infection through suppressing Th1, Th2, Tfh, Tfr and GCB cells accumulation in the spleen. COX-2/PGE2 inhibition may represent a potential therapeutic approach to schistosomiasis japonica.

Abbreviations

cyclooxygenase 2 (COX-2); prostaglandin E2 (PGE2); *Schistosoma japonicum* (*Sj*); myeloid derived suppressor cells (MDSCs); mesenteric lymph nodes (MLN); T helper type 1 (Th1); T follicular helper (Tfh), T follicular regulatory cells (Tfr); germinal center B (GCB); *Schistosoma mansoni* (*Sm*); *Schistosoma haematobium* (*Sh*); extracellular matrix (ECM); soluble egg Ag (SEA); soluble worm Ag (SWA); CD8⁺ cytotoxic T lymphocytes (CTLs); B cell lymphoma 6 (Bcl6); programmed death-1 (PD-1); week 8 (8 wks); hematoxylin & eosin (HE); ammonium chloride (NH₄Cl); Flow cytometry (FACS); interferon gamma (IFN-γ); standard deviation (SD); macrophage (Mφ); polymorphonuclear leucocyte (PMN); regulatory T cells (Treg); *Sj* adult worm antigen preparation (SWAP); peripheral blood mononuclear cells (PBMCs); tumor necrosis factor alpha (TNF-α)

Declarations

Acknowledgements

We acknowledge the expert technical help of Guiying Lin, Xiaomin Chang and Yaotang Wu.

Authors' contributions

Zhang Q, Chen L, Ji XF, Fu C and Huang T performed the research. Shen EX, Tang JJ and Li Z designed the research. Li Z, Shen EX, Zhang Q and Chen Lan analyzed the data. Li Z and Chen L wrote the paper. Li Z and Shen EX revised the paper. All authors read and approved the final manuscript.

Funding

This work was funded by Provincial Natural Science Foundation of Guangdong (no. 2019A1515012171), the general project program of state key laboratory of respiratory disease (no. SKLRD-MS-201904) and the 111 Project (no. D18010).

Availability of data and materials

The datasets supporting the conclusions of this article are included within the article.

Ethics approval and consent to participate

Six to eight-week-old female C57BL/6 wild-type mice were obtained from the specific-pathogen-free (SPF) Biotechnology Co. Ltd (Beijing). All mice were approved to be appropriate and humane by the Institutional Animal Care and Use Committee at South China Agricultural University (authorization no: 2019c013).

Consent for publication

Not applicable.

Competing interests

The authors declared that they have no competing interests.

References

1. Chuah C, Jones MK, Burke ML, McManus DP, Gobert GN. Cellular and chemokine-mediated regulation in schistosome-induced hepatic pathology. *Trends in parasitology*.2014;30 3:141–50; doi: 10.1016/j.pt.2013.12.009.
2. Masi B, Perles-Barbacaru TA, Bernard M, Viola A. Clinical and Preclinical Imaging of Hepatosplenic Schistosomiasis. *Trends in parasitology*.2020;36 2:206–26; doi: 10.1016/j.pt.2019.11.007.
3. Khanna R, Sarin SK. Noncirrhotic Portal Hypertension: Current and Emerging Perspectives. *Clinics in liver disease*.2019;23 4:781–807; doi: 10.1016/j.cld.2019.07.006.
4. Yang Q, Qiu H, Xie H, Qi Y, Cha H, Qu J, et al. A *Schistosoma japonicum* Infection Promotes the Expansion of Myeloid-Derived Suppressor Cells by Activating the JAK/STAT3 Pathway. *Journal of immunology (Baltimore, Md: 1950)*.2017;198 12:4716-27; doi: 10.4049/jimmunol.1601860.
5. Zheng L, Hu Y, Wang Y, Huang X, Xu Y, Shen Y, et al. Recruitment of Neutrophils Mediated by V γ 2 $\gamma\delta$ T Cells Deteriorates Liver Fibrosis Induced by *Schistosoma japonicum* Infection in C57BL/6 Mice. *Infection and immunity*.2017;85 8; doi: 10.1128/iai.01020-16.
6. Zheng B, Zhang J, Chen H, Nie H, Miller H, Gong Q, et al. T Lymphocyte-Mediated Liver Immunopathology of Schistosomiasis. *Frontiers in immunology*.2020;11:61; doi: 10.3389/fimmu.2020.00061.
7. Schaerli P, Willmann K, Lang AB, Lipp M, Loetscher P, Moser B. CXC chemokine receptor 5 expression defines follicular homing T cells with B cell helper function. *The Journal of experimental medicine*.2000;192 11:1553–62; doi: 10.1084/jem.192.11.1553.
8. Biram A, Shulman Z. T cell help to B cells: Cognate and atypical interactions in peripheral and intestinal lymphoid tissues. *Immunol Rev*.2020;296 1:36–47; doi: 10.1111/imr.12890.
9. Chen X, Yang X, Li Y, Zhu J, Zhou S, Xu Z, et al. Follicular helper T cells promote liver pathology in mice during *Schistosoma japonicum* infection. *PLoS pathogens*.2014;10 5:e1004097; doi: 10.1371/journal.ppat.1004097.

10. Wang Y, Lin C, Cao Y, Duan Z, Guan Z, Xu J, et al. Up-regulation of Interleukin-21 Contributes to Liver Pathology of Schistosomiasis by Driving GC Immune Responses and Activating HSCs in Mice. *Scientific reports*.2017;7 1:16682; doi: 10.1038/s41598-017-16783-7.
11. Yang Q, Qu J, Jin C, Feng Y, Xie S, Zhu J, et al. *Schistosoma japonicum* Infection Promotes the Response of Tfh Cells Through Down-Regulation of Caspase-3-Mediating Apoptosis. *Frontiers in immunology*.2019;10:2154; doi: 10.3389/fimmu.2019.02154.
12. Fairfax KC, Everts B, Amiel E, Smith AM, Schramm G, Haas H, et al. IL-4-secreting secondary T follicular helper (Tfh) cells arise from memory T cells, not persisting Tfh cells, through a B cell-dependent mechanism. *Journal of immunology (Baltimore, Md: 1950)*.2015;194 7:2999-3010; doi: 10.4049/jimmunol.1401225.
13. Xiao J, Guan F, Sun L, Zhang Y, Zhang X, Lu S, et al. B cells induced by *Schistosoma japonicum* infection display diverse regulatory phenotypes and modulate CD4(+) T cell response. *Parasites & vectors*.2020;13 1:147; doi: 10.1186/s13071-020-04015-3.
14. Zhang J, Gao W, Guo Q, Huang B, Wang B, Xia G, et al. Helminth protein vaccine induced follicular T helper cell for enhancement of humoral immunity against *Schistosoma japonicum*. *Biomed Res Int*.2013;2013:798164; doi: 10.1155/2013/798164.
15. Motiño O, Francés DE, Casanova N, Fuertes-Agudo M, Cucarella C, Flores JM, et al. Protective Role of Hepatocyte Cyclooxygenase-2 Expression Against Liver Ischemia-Reperfusion Injury in Mice. *Hepatology (Baltimore, Md)*.2019;70 2:650–65; doi: 10.1002/hep.30241.
16. Kaisar MMM, Ritter M, Del Fresno C, Jónasdóttir HS, van der Ham AJ, Pelgrom LR, et al. Dectin-1/2-induced autocrine PGE2 signaling licenses dendritic cells to prime Th2 responses. *PLoS biology*.2018;16 4:e2005504; doi: 10.1371/journal.pbio.2005504.
17. Chen L, Ji X, Wang M, Liao X, Liang C, Tang J, et al. Involvement of TLR4 signaling regulated-COX-2/PGE2 axis in liver fibrosis induced by *Schistosoma japonicum* infection. *Parasites & vectors*.2021;14 1:279; doi: 10.1186/s13071-021-04790-7.
18. Kong H, He J, Guo S, Song Q, Xiang D, Tao R, et al. Endothelin receptors promote schistosomiasis-induced hepatic fibrosis via splenic B cells. *PLoS pathogens*.2020;16 10:e1008947; doi: 10.1371/journal.ppat.1008947.
19. Zhang Y, Chen L, Gao W, Hou X, Gu Y, Gui L, et al. IL-17 neutralization significantly ameliorates hepatic granulomatous inflammation and liver damage in *Schistosoma japonicum* infected mice. *European journal of immunology*.2012;42 6:1523–35; doi: 10.1002/eji.201141933.
20. Zhou W, Yang Y, Mei C, Dong P, Mu S, Wu H, et al. Inhibition of Rho-Kinase Downregulates Th17 Cells and Ameliorates Hepatic Fibrosis by *Schistosoma japonicum* Infection. *Cells*.2019;8 10; doi: 10.3390/cells8101262.
21. Huang P, Zhou M, Cheng S, Hu Y, Gao M, Ma Y, et al. Myricetin Possesses Anthelmintic Activity and Attenuates Hepatic Fibrosis via Modulating TGFβ1 and Akt Signaling and Shifting Th1/Th2 Balance in *Schistosoma japonicum*-Infected Mice. *Frontiers in immunology*.2020;11:593; doi: 10.3389/fimmu.2020.00593.

22. Tebeje BM, Harvie M, You H, Rivera V, McManus DP. T cell-mediated immunity in CBA mice during *Schistosoma japonicum* infection. *Experimental parasitology*.2019;204:107725; doi: 10.1016/j.exppara.2019.107725.
23. Wen X, He L, Chi Y, Zhou S, Hoellwarth J, Zhang C, et al. Dynamics of Th17 cells and their role in *Schistosoma japonicum* infection in C57BL/6 mice. *PLoS neglected tropical diseases*.2011;5 11:e1399; doi: 10.1371/journal.pntd.0001399.
24. Soloviova K, Fox EC, Dalton JP, Caffrey CR, Davies SJ. A secreted schistosome cathepsin B1 cysteine protease and acute schistosome infection induce a transient T helper 17 response. *PLoS neglected tropical diseases*.2019;13 1:e0007070; doi: 10.1371/journal.pntd.0007070.
25. Kalantari P, Bunnell SC, Stadercker MJ. The C-type Lectin Receptor-Driven, Th17 Cell-Mediated Severe Pathology in Schistosomiasis: Not All Immune Responses to Helminth Parasites Are Th2 Dominated. *Frontiers in immunology*.2019;10:26; doi: 10.3389/fimmu.2019.00026.
26. Chen X, Li W, Zhang Y, Song X, Xu L, Xu Z, et al. Distribution of Peripheral Memory T Follicular Helper Cells in Patients with Schistosomiasis Japonica. *PLoS neglected tropical diseases*.2015;9 8:e0004015; doi: 10.1371/journal.pntd.0004015.
27. Tian F, Hu X, Xian K, Zong D, Liu H, Wei H, et al. B10 cells induced by *Schistosoma japonicum* soluble egg antigens modulated regulatory T cells and cytokine production of T cells. *Parasitology research*.2015;114 10:3827–34; doi: 10.1007/s00436-015-4613-x.
28. Yang H, Xuefeng Y, Shandong W, Jianhua X. COX-2 in liver fibrosis. *Clinica chimica acta; international journal of clinical chemistry*.2020;506:196–203; doi: 10.1016/j.cca.2020.03.024.
29. Peng H, Zhang Q, Li X, Liu Z, Shen J, Sun R, et al. IL-33 Contributes to *Schistosoma japonicum*-induced Hepatic Pathology through Induction of M2 Macrophages. *Scientific reports*.2016;6:29844; doi: 10.1038/srep29844.
30. Li RJ, Liu L, Gao W, Song XZ, Bai XJ, Li ZF. Cyclooxygenase-2 blockade inhibits accumulation and function of myeloid-derived suppressor cells and restores T cell response after traumatic stress. *Journal of Huazhong University of Science and Technology Medical sciences = Hua zhong ke ji da xue xue bao Yi xue Ying De wen ban = Huazhong keji daxue xuebao Yixue Yingdewen ban*.2014;34 2:234–40; doi: 10.1007/s11596-014-1264-6.
31. Harizi H, Limem I, Gualde N. CD40 engagement on dendritic cells induces cyclooxygenase-2 and EP2 receptor via p38 and ERK MAPKs. *Immunology and cell biology*.2011;89 2:275–82; doi: 10.1038/icb.2010.94.
32. Li B, Li YM, Li X, Shi B, He MY, Zhu XL, et al. COX-2 inhibition improves immune system homeostasis and decreases liver damage in septic rats. *The Journal of surgical research*.2009;157 1:43–7; doi: 10.1016/j.jss.2008.12.020.
33. Laouini D, Elkhail A, Yalcindag A, Kawamoto S, Oettgen H, Geha RS. COX-2 inhibition enhances the TH2 immune response to epicutaneous sensitization. *The Journal of allergy and clinical immunology*.2005;116 2:390–6; doi: 10.1016/j.jaci.2005.03.042.

34. Yoon JM, Lee KH, Lee SM, Lim JJ, Yang SC, Yoo CG, et al. The immune modulation of Clara cell-10 in human peripheral monocytes and dendritic cells. *International journal of molecular medicine*.2010;26 3:415–23.
35. Kuroda E, Sugiura T, Zeki K, Yoshida Y, Yamashita U. Sensitivity difference to the suppressive effect of prostaglandin E2 among mouse strains: a possible mechanism to polarize Th2 type response in BALB/c mice. *Journal of immunology (Baltimore, Md: 1950)*.2000;164 5:2386-95; doi: 10.4049/jimmunol.164.5.2386.
36. Bernard MP, Phipps RP. Inhibition of cyclooxygenase-2 impairs the expression of essential plasma cell transcription factors and human B-lymphocyte differentiation. *Immunology*.2010;129 1:87–96; doi: 10.1111/j.1365-2567.2009.03152.x.

Figures

Figure 1

COX-2 inhibition by NS398 treatment reduced Sjinfection-driven hepatomegaly, the size of granuloma, and the infiltration of immune cells around granuloma in the liver of mice Each C57BL/6 mouse was infected with 20 ± 3 Sj cercariae. Fresh liver tissue from non-infected and infected mice with or without NS398 treatment were harvested, fixed in 4% paraformaldehyde, embedded with paraffin, and sections were prepared for H&E staining. (A) Fresh liver tissue from indicated groups were photographed and the area (liver size) was analyzed by CAD software. (B) Typical photographs of different granuloma stages in liver sections from indicated mice groups with H&E staining, (C) the size of granuloma of each stage induced by single egg deposition and (D) the percentage of each granuloma stage were shown. (E) Representing images from Sj-infected mice liver section with H&E staining without or with NS398 treatment were shown. Data in (A right panel) and (C,D) were expressed as the mean $\pm$ SD (Student's t-test, statistic p value was marked into the figures).

Figure 2

NS398 decreased Sj infection-induced enlargement of mesenteric lymph nodes and splenomegaly of mice. Fresh MSN (A) and spleen (B) from non-infected and infected mice with or without NS398 treatment were harvested, photographed, and the area (size) was analyzed by CAD software. Data in (A right panel) and (B right panel) were expressed as the mean $\pm$ SD (Student's t-test, statistic p value was marked into the figures).

Figure 3

NS398 didn't affect the amount of macrophages, neutrophils, and MDSC in the spleen of Sj-infected mice. Splens from non-infected and infected mice with or without NS398 treatment were harvested and cells were isolated. (A) Spleen cells were stained with dead-APC-A750, CD45-PB450 or KO525, CD11b-PE, F4/80-FITC, Ly6G-PC7 and Gr-1-APC antibodies. F4/80+CD11b+ cells (macrophage), Ly6G+CD11b+ cells (neutrophil or PMN) or Gr-1+CD11b+ cells (MDSCs) were analyzed and data shown are gated on CD45+ live cells. Ly6G+cells in MDSCs (PMN-MDSCs) were also analyzed. The expression of macrophage (B), neutrophil (C) in CD45+ cells in spleen was evaluated. MDSC in CD45+ cells (D) and PMN-MDSCs in MDSCs or in CD45+ cells (E) in spleen were evaluated. Data were expressed as the mean $\pm$ SD of indicated numbers of mice from different groups (Student's t-test, statistic p value was marked into the figures).

Figure 4

COX-2 inhibition lowered Sj infection-driven Th1, Th2 but not Th17 cells in the spleen (A) Isolated spleen cells were stained with dead-APC-A750, CD3-PC5.5, CD4- FITC, IFN- γ -APC, IL-4-PB450 and IL-17-PC7 antibodies. IFN- γ ⁺ CD4⁺ cells (Th1), IL-4⁺CD4⁺ cells (Th2) or IL-17⁺CD4⁺ cells (Th17) were analyzed and data shown are gated on CD3⁺CD4⁺ T cells. Numbers represent the percentage of the boxed population; (B) The expression of Th1 cells in CD3⁺CD4⁺ T cells in spleen was evaluated. (C) Th2 or (D) Th17 cells in CD3⁺CD4⁺ T cells in spleen were evaluated. Data were expressed as the mean $\pm$ SD (Student's t-test, statistic p value was marked into the figures).

Figure 5

NS398 decreased Sj infection-triggered Tfr (A) Isolated spleen cells were stained with CD3-FITC, CD4-KO525, Foxp3-PC5.5, CXCR5-APC, PD-1-PC7. Foxp3+ or Foxp3- cells were analyzed and data shown are gated on CD3+CD4+ T cells. CXCR5+ PD-1+ cells (Tfh) were analyzed and data shown are gated on Foxp3+ or Foxp3- CD4+ T cells respectively. Numbers represent the percentage of the boxed population; (B) The expression of Foxp3+ or Foxp3- cells in CD3+CD4+ T cells in spleen was evaluated. (C) CXCR5+ PD-1+ cells or (D) CXCR5+ PD-1+T cells (Tfr) in spleen were evaluated. Data were expressed as the mean $\pm$ SD (Student's t-test, statistic p value was marked into the figures).

Figure 6

NS398 reduced Sj infection-induced splenic GCB cells. (A) Isolated spleen cells were stained with CD19-PC5.5, B220-PC7, Fas-PE and GL7-APC antibodies. CD19+ B220+ cells (B cell) and GL7+Fas+ cells in B cell (GCB) were gated and analyzed. Numbers represent the percentage of the boxed population; The

expression of B cells (B) and GCB cells (C) in spleen was evaluated. Data were expressed as the mean $\pm$ SD (Student's t-test, statistic p value was marked into the figures).

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