Parasitology

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Glutathione-S-transferase of *Trichinella spiralis* regulates maturation and function of dendritic cells

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Abstract

Immunomodulation by molecules from *Trichinella spiralis* (*T. spiralis*) has been widely reported. Glutathione-S-transferase (GST) is a major immune-modulator of the family of detoxification enzymes. Dendritic cells (DCs) are an important target for the regulation of the immune response by *T. spiralis*. In this study, the recombinant GST of *T. spiralis* (r*Ts*-GST) was expressed and purified. r*Ts*-GST induced low CD40 expression and moderate CD80, CD86 and MHC-II expressions and inhibited the increase of CD40, CD80 and CD86 on DCs induced by LPS. We showed that r*Ts*-GST decreased the LPS-induced elevated level of pro-inflammatory cytokines of DCs and enhanced the level of regulatory cytokines IL-10 and TGF-β. Furthermore, co-culture of DCs and $CD4^+$ T cells demonstrated that r*Ts*-GST-treated DCs suppressed the proliferation of OVA-specific $CD4^+$ T cells and increased the population of regulatory T cells (Tregs). r*Ts*-GST-treated DCs induced a higher level of IL-4, IL-10 and TGF-β, but inhibited the level of IFN-γ. This indicates that r*Ts*-GST-pulsed DCs induce both Th2-type responses and Tregs. These findings contribute to the current understanding of the immunomodulation of *Ts*-GST on cellular response and immunomodulation of *T. spiralis*.

Research Article

*These authors contributed equally to this study

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Introduction

Helminth parasite infections have been highly prevalent throughout history and are associated with immunomodulatory mechanisms that modify almost every facet of the host immune system. *Trichinella spiralis* (*T. spiralis*) both regulates and evades the immune system to maintain chronic infections in the host. Increasing evidence suggesting that cells of the innate and adaptive immune system could be regulated by helminths (Maizels and McSorley, 2016). However, the precise mechanisms underlying the cellular responses regulated by *T. spiralis* has remained undetermined.

Functional proteins of parasite play an important role in the invasion and evasion of the host immune response. Immune modulation associated with cellular responses to *T. spiralis* molecules has also been widely reported (Bai *et al.*, 2012; Sun *et al.*, 2015; Chen *et al.*, 2016; Ding *et al.*, 2016; Della Bella *et al.*, 2017; Xu *et al.*, 2017). Glutathione-S-transferase (GST), which belongs to a family of detoxification enzymes, is classically considered to be part of the cell defence against harmful compounds (Mannervik *et al.*, 1985). The GST gene is expressed during all developmental stages of *T. spiralis* and the *T. spiralis*-GST gene is up-regulated in intestinal infective larvae compared to muscle larvae (Ren *et al.*, 2013). The recombinant GST of *T. spiralis* (r*Ts*-GST) has been reported to have enzymatic activity (Cui *et al.*, 2015), and appears to be a central protein for the development and survivals of the parasite in the host. However, it is not fully known whether *Ts*-GST exerts immune modulation on immune cells, and the underlying mechanisms have not been reported.

Dendritic cells (DCs) are the most potent antigen-presenting cells (APCs) and have the ability to regulate naïve T cells responses (Zhu *et al.*, 2010). Molecules of helminths can regulate DC phenotypes *via* modulating the expression of several types of pattern recognition receptors such as toll-like receptors (TLR). In response, a diverse range of DC phenotypes are induced, which is characterized by increased expression of surface co-stimulatory molecules and secretion of cytokines (Ilic *et al.*, 2018). However, immature or mature DCs induce distinct differentiation, activation and proliferation of T cells (Kapsenberg, 2003). DCs treated with molecules of *Heligmosomoides polygyrus* showed significantly less MHC-II expression and were less effective for activating $CD4^+$ T cells (Sun *et al.*, 2013). Stimulation of DCs with excretory-secretory products (ESP) and components (53 kDa protein) of ESP resulted in partially matured DCs which induced the Th2 response (Cvetkovic *et al.*, 2016). A recent study reported that *T. spiralis* ESP activated DCs to semi-mature status and subsequently induced expansion of regulatory T cells (Tregs) *via* both TLR 2 and 4 (Ilic *et al.*, 2018).

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GST of *Fasciola hepatica* induced partial activation of DCs (Dowling *et al.*, 2010). These results indicate that different molecules induced distinct DC phenotypes associated with specific T cell responses.

This study first investigated the phenotypes of DCs exposed to r*Ts*-GST. Subsequently, the regulation of r*Ts*-GST-treated DCs on T cell responses was explored *in vitro*. Semi-mature DCs inhibited the activation and proliferation of OVA-specific $CD4^+$ T cells derived from OT-II mice, indicating that DCs are a key target for the immunomodulation of *Ts*-GST. In addition, r*Ts*-GST-treated DCs induced Th2-type responses and expanded the population of Tregs. These findings contribute to the explanation of the immunomodulatory effect of r*Ts*-GST on cellular responses, which may be associated with chronic infections and the survival of *T. spiralis* in the host.

Materials and methods

Animals and parasites

C57BL/6 mice wild type (WT, female, 6–8 weeks old) were purchased from the Shanghai SLAC Company. OT-II ovalbumin (OVA)-specific T-cell receptor (TCR) transgenic mice (on C57BL/6 background) were purchased from the Nanjing University Model Animal Research Centre (Nanjing, China). Female Wistar rats were purchased from the Experimental Animal Centre of College of Basic Medical Sciences, Jilin University (Changchun, China). All animal experiments were performed according to regulations of the Administration of Affairs Concerning Experimental Animals in China. The protocol was approved by the Institutional Animal Care and Use Committee of Jilin University (20170318).

T. spiralis (ISS534) was prepared as previously described (Tang *et al.*, 2015). Briefly, Wistar rats were orally infected with 3000 infective larvae and *T. spiralis* muscle larvae were recovered at 35 days post-infection (dpi) *via* artificial digestion with pepsin-HCl (1% pepsin and 1% HCl at 37 °C for 2 h). All parasites were washed three times in saline solution.

Preparation and identification of r*Ts*-GST

The *Ts*-GST gene (GenBank accession no. XM 003371707.1) was amplified by PCR using specific primers with *Nde* I and *Xho* I restriction enzyme sites (5′-CGC*CATATG*GCTCCTCTATACAA GC-3′; 5′-CCG*CTCGAG*TTAATTTAATCGAATTTTCTTG-3′). The purified PCR product was cloned into the pCold I vector (Takara, Dalian, China). The recombinant plasmid carrying the *Ts*-GST gene was transformed into *Escherichia coli* BL21 (DE3) (Novagen) and expressed under 0.1 mM IPTG induction. The r*Ts*-GST was purified using Ni-NTA-Sefinose resin (Sangon Biotech, China), according to the manufacturer's instruction. The concentration of the purified r*Ts*-GST was determined with Microplate BCA Protein Assay Kit-Reducing Agent Compatible (Thermo Fisher Scientific, Waltham, USA). Purity of the recombinant protein was identified by 12% SDS-PAGE analysis and the gel was stained with 0.25% Coomassie brilliant blue R-250 (AMRESCO). The contaminated endotoxin was removed by ToxOut High Capacity Endotoxin Removal Kit (Biovision, USA). The endotoxin was 0.1812 EU mg^{-1} in the purified r*Ts*-GST, approximately equivalent to 20 pg mg^{-1} endotoxin in r*Ts*-GST, which is lower than the minimal amount that could stimulate TLR2/4 based on the standard LPS O55: B5 (Invivogen, USA) (Schwarz *et al.*, 2014).

Generation of bone marrow-dendritic cells and $CD4^+$ T cells

Bone marrow-dendritic cells (BMDCs) were generated from bone marrow cells of mice as previously described (Lutz *et al.*, 1999). Briefly, bone marrow cells were obtained from C57BL/6 mice and cultured for at 37 °C, 5% CO_2 in RPMI 1640 medium containing the growth factors recombinant GM-CSF (20 ng mL^{-1}) and IL-4 (20 ng mL^{-1}; Sigma–Aldrich) and 10% FBS. Immature DCs were harvested on day 7 for further experiments.

$CD4^+$ T cells of OT-II mice were purified by magnetic sorting, using anti-CD4 magnetic beads (Miltenyi Biotec, Auburn, CA, USA) from spleen cells. The purified $CD4^+$ T cells had >90% purity.

In vitro BMDCs stimulation assay

To analyse the effects of r*Ts*-GST on DC maturation, immature DCs were isolated. DCs from mice were stimulated with sterile phosphate-buffered saline (PBS), r*Ts*-GST (10 μg mL^{-1}) and LPS (100 ng mL^{-1}, Sigma–Aldrich) alone or in combination, *in vitro* for 48 h. Cell culture supernatants were collected and stored at −80 °C. The cytokine in the supernatants was measured by enzyme-linked immunosorbent assay (ELISA) kits (Huamei, Wuhan, China) according to the manufacturer's instructions. Stimulated DCs were stained with FITC-conjugated monoclonal antibody (mAb) to CD11c and PE-conjugated mAbs to MHC-II, CD40, CD80 or CD86 respectively (Biolegend, USA). Appropriately labelled isotype-matched antibodies were used as controls. Cells were analysed using a BD FACS Calibur Flow Cytometer and FlowJo software (Tree star Inc, Ashland, OR). Results are shown as mean ± S.D. ($n = 3$) of three individual different experiments.

Co-culture of BMDC with $CD4^+$ T cells

$CD11c^+$ DCs were typically of >90% purity as determined by flow cytometry. The purified $CD4^+$ T cells had >90% purity. To determine the roles of r*Ts*-GST-pulsed DC in $CD4^+$ T-cell proliferation, the DCs (2×10^5 per well) were firstly treated with PBS or r*Ts*-GST (10 μg mL^{-1}) for 8 h (using PBS as the control group). After washing three times with sterile PBS, DCs were stimulated either with or without OVA (1 mg mL^{-1}; Calbiochem) for 2 h. The DCs were then treated with 50 μg mL^{-1} mitomycin (Sigma–Aldrich) for 30 min and washed with medium. $CD4^+$ T cells (2×10^6 per well) stained with 5-and 6-carboxyfluorescein diacetate succinimidyl ester (CFSE) (eBioscience, San Diego, CA, USA) were co-cultured with DC for 72 h. Then, cell samples were analysed using a BD FACS Calibur Flow Cytometer and FlowJo software (Tree star Inc). Results are shown as means ± standard deviation (S.D.) ($n = 3$) of three individual different experiments.

In addition, the Treg populations induced by r*Ts*-GST-treated DCs were measured. $CD4^+$ T cells were co-cultured with DC treated with PBS, OVA (1 mg mL^{-1}) alone or OVA + r*Ts*-GST for 72 h, respectively. Co-cultured cells were collected and firstly pre-incubated with Fc Blocker (Anti-Mouse CD16/CD32, BD Biosciences, USA) for 15 min to decrease non-specific binding of labelled antibodies. Cells were then stained with APC-labeled anti-CD25, fixed and permeabilized using the Foxp3 Transcription Factor staining buffer kit (eBioscience) according to the manufacturer's instructions. Cells were then blocked in 5% rat serum prior to intracellular staining with Percp Cy5.5-labelled anti-Foxp3. Appropriately labelled isotype-matched antibodies were used as controls. Samples were analysed using BD FACS Calibur Flow Cytometer and FlowJo software (Tree star Inc, Ashland, OR). Results are shown as means ±

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standard deviation (S.D.) ($n = 3$) of three individual different experiments.

To determine cytokine production, $CD4^+$ T cells were co-cultured with DC treated with PBS, OVA (1 mg mL^{-1}) alone or OVA + r*Ts*-GST for 48 h respectively, and the DC and $CD4^+$ T-cell co-culture was stimulated by Concanavalin-A (Con-A) (2.5 μg mL^{-1}) (Sigma–Aldrich) for the final 18 h of co-culture. The supernatants in DCs and $CD4^+$ T-cells co-culture were harvested for cytokine analysis by ELISA kits (Huamei, Wuhan, China). Results are shown as means ± standard deviation (S.D.) ($n = 3$) of three individual different experiments.

Statistical analysis

All results were expressed as mean ± S.D. Statistical analysis was performed using the GraphPad Prism 5 for Windows. One-way and two-way analysis of variance (ANOVA) were used to compare statistical differences at different conditions. P values are expressed as $^*P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.

Results

Expression and identification of rTs-GST

The *Ts*-GST gene was cloned into the pCold I vector and r*Ts*-GST was successfully expressed in *Escherichia coli* BL21 (DE3) under IPTG induction. After purification, soluble r*Ts*-GST was obtained, with an approximate molecular weight of 26 kDa (Fig. 1).

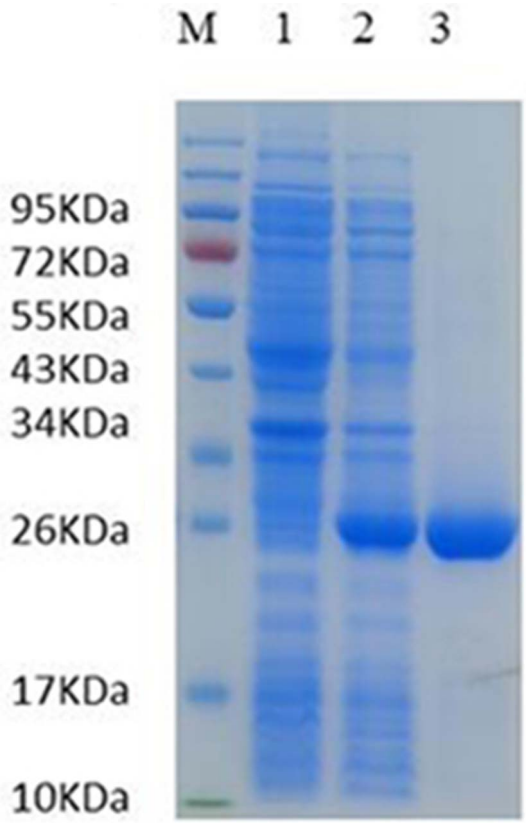

Fig. 1. Identification of r*Ts*-GST. r*Ts*-GST was purified using Ni-NTA-Sefinose resin, according to the manufacturer's instruction. Purity of the recombinant protein was identified by 12% SDS-PAGE analysis and the gel was stained with 0.25% Coomassie brilliant blue R-250. SDS-PAGE analysis of r*Ts*-GST. M: protein molecular weight marker; 1: lysis of recombinant bacteria; 2: lysis of induced recombinant bacteria harbouring pCold I vector *Ts*-GST after ultrasonication and 3: purified r*Ts*-GST.

rTs-GST induced semi-matured DCs and impair TLR ligand-induced DC maturation in vitro

To investigate the effect of r*Ts*-GST on the maturation of DCs, surface co-stimulatory molecules on $CD11c^+$ DCs (>90% $CD11c^+$, Fig. 2A) were analysed. Expression of surface molecules CD40, CD80, CD86 and MHC-II on LPS stimulated $CD11c^+$ DCs were significantly upregulated compared to PBS or r*Ts*-GST alone. In contrast, r*Ts*-GST inhibited upregulation of CD40, CD80 and CD86 on LPS stimulated DCs (Fig. 2B and C). However, r*Ts*-GST was not able to down-regulate the expression of MHC-II. Moreover, the expression of CD40 on $CD11c^+$ DCs was similar to PBS and expressions of CD80, CD86 and MHC-II significantly increased by r*Ts*-GST compared to the PBS group.

Then, the production of inflammatory cytokines of DCs was examined. Stimulation of LPS resulted in increased cytokine production including pro-inflammatory cytokines and anti-inflammatory cytokines. Stimulation of r*Ts*-GST alone increased the levels of IL-10 and TGF-β, which are regulatory cytokines. The levels of IL-10 and TGF-β in r*Ts*-GST-treated LPS-matured DCs were not significantly different, compared to LPS-matured DCs (Fig. 3A). In addition, r*Ts*-GST decreased the production of TNF-α, IL-1β and IL-12 induced by LPS (Fig. 3B). In summary, these results indicate that r*Ts*-GST inhibited the LPS-induced proinflammatory response on DCs.

DCs treated with rTs-GST regulated the proliferation of OVA-specific $CD4^+$ T cells

The immunoregulatory activity of $CD11c^+$ DCs on T cells proliferation was determined. OVA-specific $CD4^+$ T cells were labelled with CFSE and co-cultured with DCs that had been treated with or without r*Ts*-GST in the presence of OVA. FACS results showed that proliferation of OVA-specific $CD4^+$ T cells was significantly boosted by DCs treated with OVA alone compared to PBS (Fig. 4B). This effect was diminished when cells were treated with r*Ts*-GST, suggesting that DCs treated with r*Ts*-GST impaired OVA-induced T cell proliferation.

DCs treated with rTs-GST expanded the Treg cells population

To determine the effect of DCs treated by r*Ts*-GST on the generation of Tregs, r*Ts*-GST-treated DCs were cultured with T cells isolated from spleens of OT-II (C57BL/6 background) for 48 h. The FACS results demonstrated that the population of $CD4^+CD25^+Foxp3^+$ T cells significantly increased when OVA-specific $CD4^+$ T cells were co-cultivated with r*Ts*-GST-treated $CD11c^+$ DCs in the presence of OVA, compared to PBS or the OVA alone treatment group (Fig. 5).

DCs treated with rTs-GST regulated production of inflammatory cytokines of $CD4^+$ T cells

The culture supernatants from co-culture were collected, and the secretion levels of the cytokines associated with each type of T cell were determined by ELISA. Production of cytokines significantly increased by OVA compared to the group without OVA. Then, treatments with or without *rTs*-GST in the presence of OVA were compared. Compared to the PBS-treated DCs group, r*Ts*-GST-treated DCs not only induced OVA-specific $CD4^+$ T cells to secrete cytokine IL-4 (Th2) ($P < 0.001$), but also induced higher levels of IL-10 and TGF-β (IL-10, $P < 0.01$; TGF-β, $P < 0.01$), which is consistent with the increase of the Tregs population observed by FACS. Furthermore, the levels of IFN-γ (Th1)

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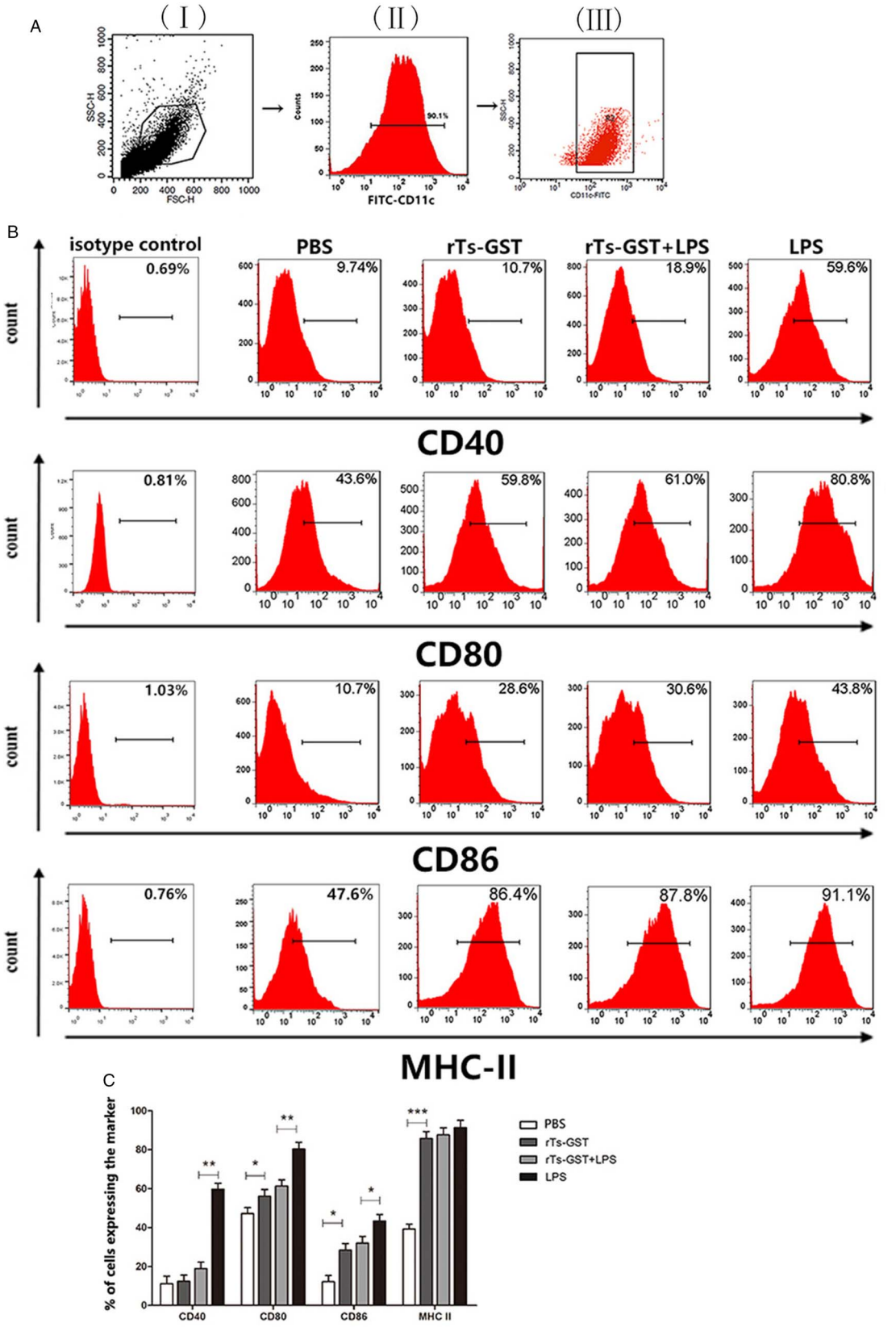

Fig. 2. Expression of surface co-stimulatory molecules on DCs induced by LPS, r*Ts*-GST or PBS. DCs were stimulated with sterile phosphate-buffered saline (PBS), r*Ts*-GST (10 μg mL^{-1}), LPS (100 ng mL^{-1}) alone or r*Ts*-GST+LPS, *in vitro* for 48 h. (A) Expression of CD11c^{+} DCs was measured by FACS. Representative dot plots for the gating strategy: (I) gating on viable cells, (II) expression of CD11c^{+} and (III) gating on CD11c^{+} cells. (B) Expression of CD40, CD80, CD86 and MHC-II measured by FACS. (C) Data represent means ± standard deviations ($n = 3$) of the results of three individual experiments. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ *vs* control groups.

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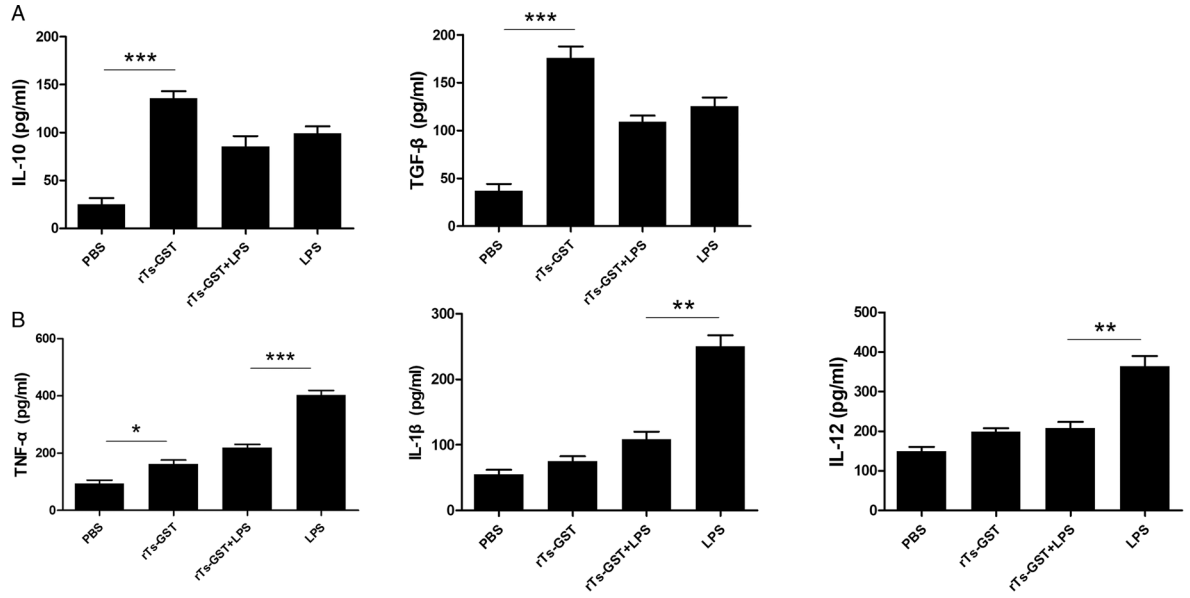

Fig. 3. Cytokine production by DCs treated with LPS or r*Ts*-GST. DCs were cultured for 48 h and supernatants were measured by ELISA. (A) Regulatory cytokine (IL-10 and TGF-β) production was measured. (B) The production of pro-inflammatory cytokines (TNF-α, IL-1β and IL-12) was measured. Results are presented as mean ± standard deviation ($n = 3$) from three individual experiments. *$P < 0.05$; **$P < 0.01$, ***$P < 0.001$ (one-way ANOVA with Tukey's post-test).

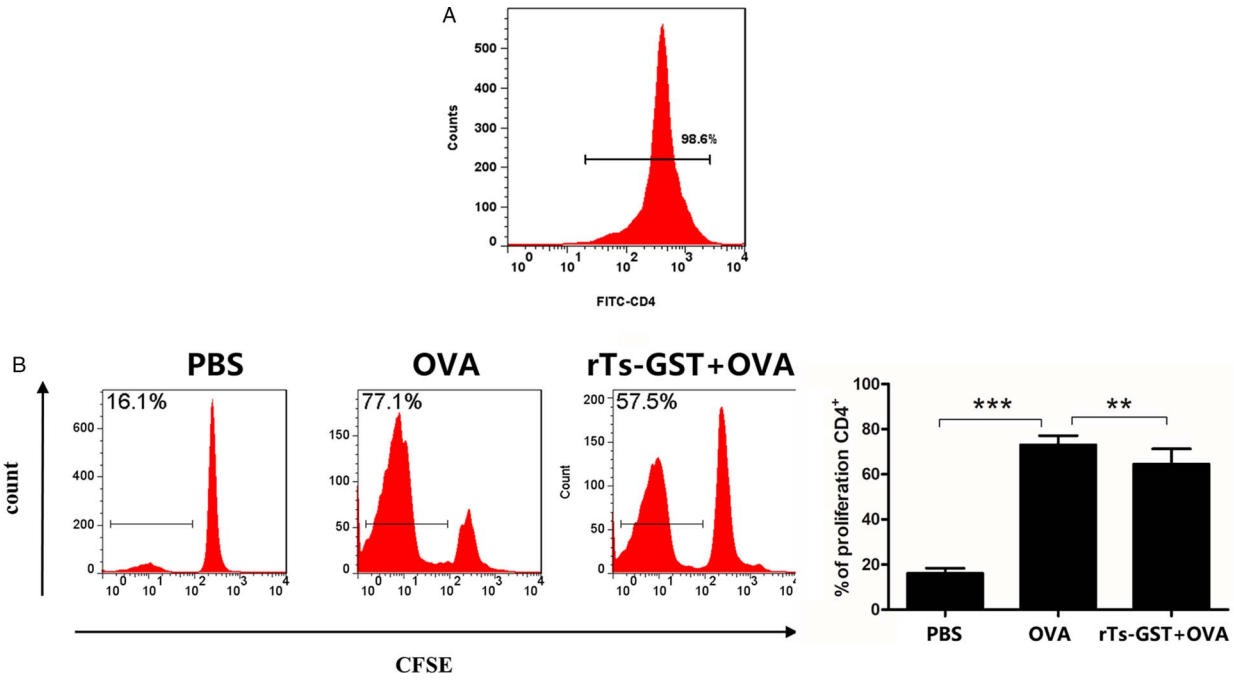

Fig. 4. Proliferation of T cells by r*Ts*-GST-pulsed CD11c$^+$ DCs. (A) The purity of CD4$^+$ T cells was analysis by FACS after magnetic sorting using anti-CD4 magnetic beads. (B) To assess the effect of r*Ts*-GST-treated DCs for the proliferation of OVA-specific CD4$^+$ T cells, CD4$^+$ T cells were stained with CFSE prior to co-culture with DCs. Proliferation of CD4$^+$ T Cells was determined by CFSE labelling and flow cytometry. Results are shown as means ± standard deviation (S.D.) ($n = 3$) of three individual different experiments. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (one-way ANOVA with Tukey's post-test).

were significantly inhibited ($P < 0.01$) by r*Ts*-GST-pulsed DCs, compared to the OVA group (Fig. 6).

Discussion

GSTs form a family of detoxification enzymes and are major immune-modulatory molecules. GSTs from helminths, such as *T. spiralis* (Cui *et al.*, 2015), *Schistosoma* (Grezel *et al.*, 1993), *Fasciola* (Morrison *et al.*, 1996), *Setaria cervi* (Gupta *et al.*, 2005), *Ancylostoma caninum* (Zhan *et al.*, 2005) and *Necator americanus* (Zhan *et al.*, 2010) indicated immunoprotective potential. The GST is a *T. spiralis* functional protein, and the GST gene of *T. spiralis* is expressed during all developmental stages of *T. spiralis*. *T. spiralis*-GST gene is up-regulated in intestinal infective larvae compared to muscle larvae (Ren *et al.*, 2013). And the recombinant GST of *T. spiralis* (r*Ts*-GST) has been reported to exert enzymatic activity (Cui *et al.*, 2015). It has been reported that *Ts*-GST expressed in the stichosome which are secretory cells in *Trichinella* (Rojas *et al.*, 1997; Li *et al.*, 2015). The presence of anti-*Ts*-GST antibodies in infected animals agrees with its localization on the secretory organ of the parasite. Furthermore, variable levels of GST are observed in the

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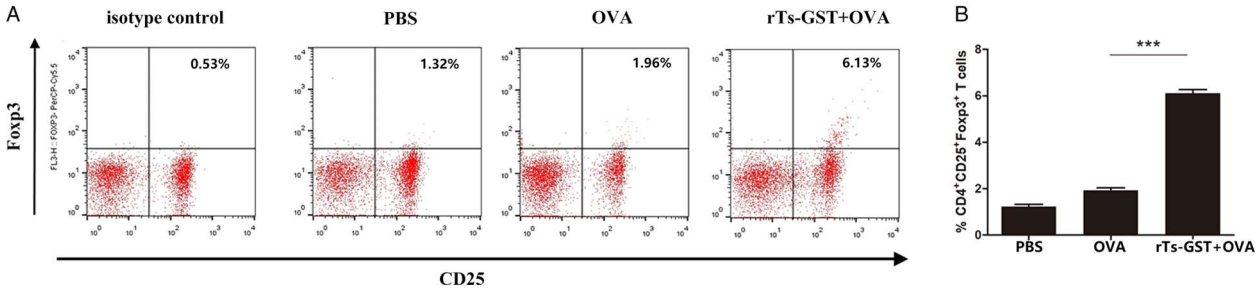

Fig. 5. Population of Tregs induced by r*Ts*-GST-treated CD11c$^+$ DCs. Treg populations induced by r*Ts*-GST-treated DCs. CD4$^+$CD25$^+$Foxp3$^+$ T cells were determined by flow cytometry. (A) Representative analysis of Tregs are shown and (B) the summarized results are presented as means ± standard deviation (S.D.) ($n = 3$) of three individual different experiments. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (one-way ANOVA with Tukey's post-test).

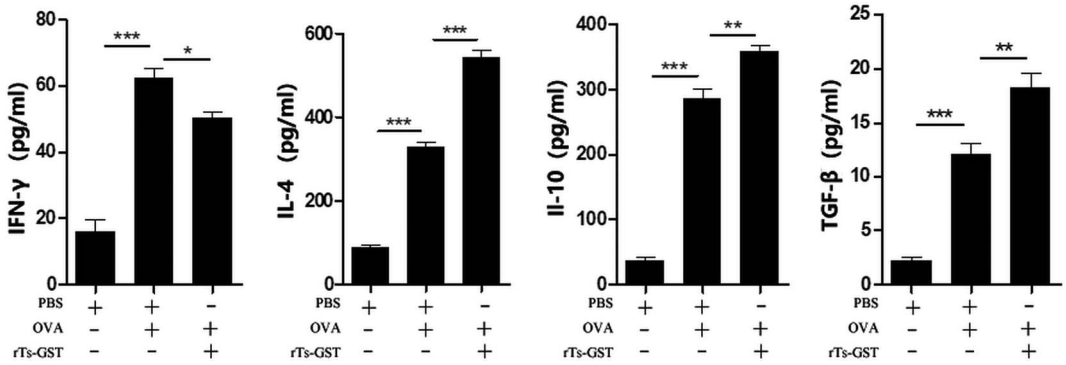

Fig. 6. Cytokine production of T cells induced by r*Ts*-GST-treated CD11c$^+$ DCs. DCs (2×10^5 per well) were treated with PBS or r*Ts*-GST (10 μg mL^{-1}) for 8 h. DCs were washed three times with sterile PBS and resuspended with RPMI 1640 medium. Then DCs and CD4$^+$ T cells (2×10^6 per well) were co-cultured with or without OVA (1 mg mL^{-1}) for 48 h. During the last 18 h, 2.5 μg mL^{-1} Concanavalin-A (Con-A) was added. The supernatants in DCs and CD4$^+$ T-cells co-culture were harvested for cytokine analysis by ELISA. Results are shown as means ± standard deviation (S.D.) ($n = 3$) of three individual different experiments. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (one-way ANOVA with Tukey's post-test).

blood from infected animals at different stages and the first peak corresponds to the presence of adults during the intestinal phase, whereas larvae have already settled when the higher levels are reached (Rojas *et al.*, 1997). And the presence of *Ts*-GST in sera from infected animals was confirmed after its isolation by GSH affinity chromatography. These results indicated that the *Ts*-GST directly interact with host immune system and could play an important role in regulating immune response. It has also been also previously reported that the GST of *F. hepatica* showed immunomodulatory activity for cell response (Dowling *et al.*, 2010). However, whether *Ts*-GST is immune-modulatory for immune cells remains unknown.

DCs play an important role in the balance between tolerance and immunity. Protein expressed by nematode parasites can modulate differentiation and activation of DCs. Paramyosin, one of the proteins expressed by *T. spiralis*, has been shown to induce semi-maturation in DCs (Guo *et al.*, 2016). The results of the present study show that r*Ts*-GST significantly inhibited LPS-induction of DC maturation, as indicated by the attenuation of CD40, CD80 and CD86 markers by r*Ts*-GST. Moreover, r*Ts*-GST induced low CD40 expression and moderate CD80, CD86 and MHC-II expression, suggesting that r*Ts*-GST regulated DCs to a semi-maturation phenotype. Between the r*Ts*-GST and LPS group, no significant changes in the levels of MHC-II were observed, which is related to the recognition of the T cell antigen receptor, indicating that DCs treated with r*Ts*-GST retained the capacity of antigen presentation to the T cell. Secretion of cytokines related analysis indicated that r*Ts*-GST alone induced low levels of pro-inflammatory cytokines (TNF-α, IL-1β and IL-12) on DCs. r*Ts*-GST was able to inhibit the increasing pro-inflammatory cytokine levels (TNF-α, IL-1β and IL-12) induced by LPS. The observed low production of IL-12 and upregulation of IL-10 secreted by r*Ts*-GST-treated-DCs might be related to Treg expansion, as previously reported (Rutella *et al.*, 2006). Furthermore, IL-10 cytokine production in DCs has been reported to be related to Th2 differentiation (Williams *et al.*, 2013), which is also critical for the polarization of naïve T cells into Tregs (Villablanca *et al.*, 2008). In the present study, IL-10 and TGF-β, as regulatory cytokines, increased in DCs treated with r*Ts*-GST alone, compared to the PBS group. IL-10 and TGF-β were able to maintain the tolerogenic function of DCs and the ability of IL-10-producing Tregs (Torres-Aguilar *et al.*, 2010). Thus, our results indicated that DCs treated with r*Ts*-GST may be involved in both the Th2 immune response and Tregs population.

Furthermore, investigation of DC function showed that DCs treated with r*Ts*-GST not only induced OVA-specific CD4$^+$ T cells to secrete increased levels of cytokine IL-4 (Th2), IL-10 and TGF-β, but also significantly inhibited IFN-γ (Th1) compared to the OVA group, indicating that T cell immune responses switched toward Th2 and Treg by DCs treated by r*Ts*-GST. Furthermore, FACS results demonstrated that r*Ts*-GST-treated-DCs increased the percentage of Tregs, further confirming that *Ts*-GST has the ability to regulate Treg differentiation and to maintain immunologic tolerance. Several studies showed that ES of *T. spiralis* muscle larvae, or its components, possess the ability to induce semi-mature DCs, which can induce Tregs expansion *in vitro* (Guo *et al.*, 2016; Ilic *et al.*, 2018). Tregs are a fundamental mechanism of immune regulation during helminth infections, and the induction of Foxp3$^+$ Treg responses is of principal importance for the design of both prophylactic helminth treatments and therapies for autoimmunity (Shevach, 2018). Moreover, r*Ts*-GST-treated DCs were less effective in stimulating

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$CD4^+$ T cell proliferation. These results suggest that r*Ts*-GST often inhibit the T cell response *via* DCs during infection to establish chronic infections. A similar result was reported for *H. polygyrus* cysteine protease inhibitor (CPI), which was shown to induce a weaker proliferation of OVA-specific $CD4^+$ T cells (Sun *et al.*, 2013).

In conclusion, this study demonstrated that r*Ts*-GST exert immunomodulation on the phenotypes of DCs and T cells differentiation. r*Ts*-GST induced semi-maturation of DCs, by decreasing the expressions of co-stimulatory molecules on DCs surface and cytokines release. Moreover, the proliferation of $CD4^+$ T cells was reduced by DCs treated with r*Ts*-GST, resulting in modulated T cell response into Th2 and Tregs. These findings contribute to the understanding of chronic *T. spiralis* infections in the host through the immunomodulatory effect of *Ts*-GST on cellular responses. In addition, semi-matured DCs treated with parasitic antigens attenuate immune responses in autoimmune disorders (Blum *et al.*, 2012; Sofronic-Milosavljevic *et al.*, 2013; Xiao *et al.*, 2016; Matisz *et al.*, 2017). *Schistosome* derived GST protected against inflammatory bowel diseases through a Th2-type response (Driss *et al.*, 2016). Whether r*Ts*-GST is a potential therapeutic anti-inflammatory agent for the treatment of inflammatory bowel disease or other autoimmune diseases requires further study.

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Conflict of interests. The authors declare that they have no competing interests.

Ethical standards. All animal experiments were carried out in accordance with the European Communities Council Directive 86/609/EEC for animal welfare and were approved by the Institutional Animal Care and Use Committee of Jilin University.

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