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Polyinosinic–polycytidylic acid attenuates hepatic fibrosis in C57BL/6 mice with *Schistosoma japonicum* infection

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ABSTRACT

The development of hepatic fibrosis is the principal cause of morbidity and mortality in human beings infected with schistosoma. In this study, we investigated the effect of polyinosinic–polycytidylic acid (poly I:C) on *Schistosoma japonicum* (*S. japonicum*) egg-induced liver fibrosis. *S. japonicum* cercariae infected mice were injected with poly I:C at the onset of egg granuloma formation (early phase poly I:C treatment) or after the formation of liver fibrosis (late phase poly I:C treatment). Our results showed that both early and late phase poly I:C treatment significantly reduced collagen deposition and hepatic stellate cell activation in the liver. Poly I:C is one of the most effective adjuvants for Th1 type responses, and its protective effect on liver fibrosis was accompanied by increased IFN-α, IFN-β, IFN-γ, IL-12, TNF-α, and IL-10 mRNA expression, and decreased IL-4 and IL-5 mRNA expression. Moreover, poly I:C injection also enhanced the mRNA expression of natural killer group 2 member D (NKG2D) and tumor necrosis factor related apoptosis-inducing ligand (TRAIL). Therefore, it is indicated that poly I:C can significantly attenuate *S. japonicum* egg-induced hepatic fibrosis, which may be partly dependent on the increased Th1 response and decreased Th2 response.

1. Introduction

Schistosoma parasites infect an estimated 207 million people worldwide (Steinmann et al., 2006), and the global burden of disease due to schistosomiasis is thought to be largely underestimated (King, 2008). Egg-induced fibrosis is less severe in the earliest phase of egg deposition when Th1-associated cytokines are present, while a few weeks later the Th2 cytokines IL-4, IL-5, and IL-13 dominate and liver fibrosis increases dramatically. Animal model researches have proved that Th1 cytokines reduce fibrotic pathology, whereas Th2 cytokines lead to the development of hepatic fibrosis and portal hypertension (Chevillard et al., 2003; Chiaramonte et al., 1999; Pearce and MacDonald, 2002; Wynn et al., 1995). Therefore, during the fibrotic progression, the replacement of the Th2-dominated pattern of cytokine expression characteristic of schistosoma infection with one dominated by Th1 cytokines might play a key role in slowing down or reversing the fibrosis.

Polyinosinic–polycytidylic acid (poly I:C) is one of the most effective adjuvants for Th1 CD4+ T cell responses via inducing dendritic cell (DC) maturation, and it can maintain long-lasting T cell immunity (Longhi et al., 2009; Stahl-Hennig et al., 2009; Trumpfheller et al., 2008). Poly I:C is recognized by Toll like receptor 3 in the endosomal compartment of specialized cells (Alexopoulou et al., 2001). Poly I:C also binds the cytoplasmic RNA helicase MDA5 that is expressed in the cytosol of all somatic cells (Kalali et al., 2008; Kato et al., 2006). The binding of poly I:C to its receptors leads to strong induction of IFNs (Alexopoulou et al., 2001). Besides DCs and macrophages, many nonhematopoietic cell types can produce type I IFNs upon poly I:C stimulation (Gitlin et al., 2006). At the presence of DCs or macrophages, poly I:C stimulates NK cells to secrete IFN-γ (Hou et al., 2009; Tu et al., 2008). IFN-γ has been shown to have potent antifibrogenic effect (Hoffmann et al., 1998; Jeong and Gao, 2008; Mukai et al., 2006). Over the past several years, it has been demonstrated that poly I:C is essential for promoting protective immune responses against infectious organisms (Gibbert et al., 2010; Herbst-Kralovetz and Pyles, 2006), as well as assisting in immune-mediated antitumor and antimetastatic activities (Chin et al., 2010; Jiang et al., 2008; Stone et al., 2009). Thus, the studies described above suggest that poly I:C may be valuable in the immunotherapeutic or immunoprophylactic treatments of clinical diverse diseases.

In this paper, we assessed the antifibrotic effect of poly I:C on *Schistosoma japonicum* (*S. japonicum*)-infected mice. We found that

Abbreviations: Poly I:C, polyinosinic–polycytidylic acid; *S. japonicum*, *Schistosoma japonicum*; α-SMA, alpha-smooth muscle actin; DC, dendritic cell; ALT, alanine aminotransferase; AST, aspartate aminotransferase; p.i., post-infection; HSC, hepatic stellate cells; CCl_4, carbon tetrachloride; NKG2D, natural killer group 2 member D; TRAIL, tumor necrosis factor related apoptosis-inducing ligand; SOCS1, suppressor of cytokine signaling 1.

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both early phase poly I:C treatment (injection since 5 weeks postinfection (p.i.)) and late phase poly I:C treatment (injection since 8 weeks p.i.) increased Th1 cytokine mRNA levels while decreasing Th2 cytokine mRNA levels, thereby ameliorating egg-induced liver fibrosis. These results suggest that poly I:C-induced immunomodulation may have major potential as a strategy for reducing fibrosis in schistosomiasis.

2. Materials and methods

2.1. Animals

Six-week old female C57BL/6 mice were purchased from Experimental Animal Center, Chinese Science Academy (Shanghai, China). All mice were maintained in a specific pathogen-free microenvironment, and received care in compliance with the guidelines outlined in the *Guide for the Care and Use of Laboratory Animals*.

2.2. Infection of mice with S. japonicum

Mice were anesthetized and percutaneously infected with 18–20 cercariae of *S. japonicum* (strain from Jiangxi Province, China) that were obtained from infected *Oncomelania hupensis* snails.

2.3. Treatment of mice with polyinosinic:polycytidylic acid

Poly I:C (Sigma, Chemical Co., St. Louis, MO) was dissolved in the pyrogen-free saline. Mice were injected intraperitoneally with poly I:C (0.5 μg/g). For early phase poly I:C treatment, mice were injected with poly I:C or saline every 3 days since week 5 p.i. For late phase poly I:C treatment, mice were given praziquantel on week 7 to eliminate the adult worms and were injected with poly I:C or saline every 3 days from week 8 to week 13.

2.4. Analysis of liver transaminase activities

Serum samples from individual mice were obtained at week 8 and week 10 p.i. Liver injury was assessed by measuring serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities using commercially available kit (Rong Sheng, Shanghai, China).

2.5. Histology and immunohistochemistry

Liver tissues were fixed in 10% buffered formalin and embedded in paraffin. Tissue sections were affixed to sides, deparaffinized, stained with Masson trichrome for collagen deposition. Immunostaining for alpha-smooth muscle actin (α-SMA) was performed using monoclonal α-SMA primary antibody (clone 1A4; Dako, Carpinteria, CA), and a horseradish peroxidase-labeled secondary antibody. Six to ten images per mouse liver were photographed using an inverted microscope (Nikon 80I, Japan) and percent positive staining for α-SMA and Masson trichrome sections was determined by computerised morphometry (Image-Pro Plus software).

2.6. Quantitative PCR

Total RNA of liver tissue was extracted by using Trizol Reagent (Invitrogen). Two micrograms of the total RNA was used for each reverse transcriptase reaction for cDNA synthesis. Quantitative PCR was performed using a sequence detector (ABI-Prism 7500; Applied Biosystems) and a SYBR Premix Ex Taq (Takara), according to the manufacturer's instructions. The primer sequences used are listed in Table 1. For analysis, all expression levels of target genes were normalized to the housekeeping gene β-actin (ΔCt). Gene expression values were then calculated based on the ΔΔCt method as mentioned previously (Hou et al., 2009). The mean of the respective cytokines in uninfected mice was used as a calibrator. Relative quantities (RQs) were determined using the equation: $RQ = 2^{-\Delta\Delta Ct}$.

2.7. Statistical analysis

The results were analyzed by Student's *t* test or analysis of variance where appropriate. All data were shown as mean ± standard error of the mean (SEM). *P* value <0.05 was considered to be statistically significant.

3. Results

3.1. Early phase poly I:C treatment prevents S. japonicum egg-induced liver fibrosis

To investigate the effect of poly I:C on *S. japonicum* egg-induced liver fibrosis, we have been treating infected mice with poly I:C since week 5 p.i. At week 8 and week 10 p.i., the formation of liver fibrosis was monitored by Masson trichrome staining for collagen deposition (blue staining) and immunohistochemical staining for hepatic stellate cell (HSC) activation (α-SMA-positive staining). As shown in Fig. 1A, collagen deposition was suppressed in poly I:C-treated mice compared with saline-treated mice (5.81 ± 0.64% versus 9.32 ± 1.81% at week 8; 7.01 ± 0.91% versus 12.12 ± 2.01% at week 10). Consistent with these findings, poly I:C-treatment markedly reduced HSC activation (α-SMA+ cells; Fig. 1B).

3.2. Early phase poly I:C treatment has no effect on worm load, egg burden, granuloma size, and liver injury

The decrease in liver fibrosis was not due to differences in the intensity of infection or egg burden because worm pairs, total worms, and total parasite eggs in the livers of poly I:C-treatment groups and saline-treatment groups were identical (Table 2). Furthermore, poly I:C treatment did not significantly affect granuloma size (Table 2), as well as ALT and AST levels (Fig. 2).

3.3. Early phase poly I:C treatment markedly increases Th1 cytokine and IL-10 mRNA levels while decreasing Th2 cytokine mRNA levels

To explore the molecular mechanism underlying the antifibrogenic effect mediated by early phase poly I:C treatment, we compared Th1- and Th2-associated cytokine mRNA profiles respectively in the livers of poly I:C-treated mice and saline-treated mice at several time points p.i. As shown in Fig. 3, early poly I:C treatment dramatically enhanced IFN-γ and IL-12 mRNA expression at all the time points and increased IFN-α and IFN-β mRNA expression at week 8 and week 10 p.i. Elevated TNF-α mRNA expression at week 6 p.i. and IL-10 mRNA expression at all the time points were also observed with early poly I:C treatment. In contrast, early poly I:C treatment markedly decreased IL-4 and IL-5 mRNA expression at week 6 and week 8 p.i., but had no effect on IL-13 mRNA expression.

Since natural killer group 2 member D (NKG2D), tumor necrosis factor related apoptosis-inducing ligand (TRAIL), and suppressor of cytokine signaling 1 (SOCS1) have been shown to play important roles in poly I:C-mediated-regulation of liver fibrosis induced by carbon tetrachloride (CCl_4) injection (Radaeva et al., 2006; Jeong et al., 2011), we also examined the expression of these genes. As shown in Fig. 3, poly I:C treatment increased NKG2D and TRAIL mRNA expression at week 10 p.i., but had little effect on SOCS1 mRNA expression.