

Youfang GU, Bingyun WANG, Hongying ZHANG, Xinzhi MAO, Yonglin SHEN

Cytokines level changes in goats infected with *Fasciola hepatica*

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Abstract To study the changes of IL-2 and TNF- α in serum and the IL-2 secreted by peripheral blood lymphocytes of goats infected with *Fasciola hepatica*, thirty six clinically healthy white goats were randomly divided into groups coded as I, II, III, every 12 goats. Goats in group I and II were inoculated with a single oral dose of 200 and 500 encysted *Fasciola hepatica* metacercaria, respectively, while those in group III were untreated controls. Blood samples were collected from the jugular vein once a week for 11 weeks. Changes in the serum levels of IL-2 and TNF- α and the ability of peripheral blood lymphocytes to secrete IL-2 were detected. Serum IL-2 levels decreased in group I, but increased in group II. The levels of TNF- α and secreted IL-2 were elevated by varying degrees in both groups I and II. Lymphocytes displayed reactivity during the first week after infection with a significantly increased response to both specific and non-specific challenges. TNF- α may be involved in the hepatic injury process caused by *Fasciola hepatica*. The infective dose of *Fasciola hepatica* greatly influenced IL-2 levels in the host playing a vital role in the initiation of host defense.

Keywords *Fasciola hepatica*, goat, cytokine, radio-immune method, MTT method

1 Introduction

The liver fluke *Fasciola hepatica* is a multi-host parasite that brings enormous economic loss by causing severe

illnesses in ruminants. Goat farming is far more severely affected by *F. hepatica* infection than sheep or bovine stocks. Fascioliasis greatly affects the development of goat farming worldwide. It has been reported that the infection rate in goats is 3%–72% (Martinez et al., 1996; Wang et al., 1987). The epidemiology of Fascioliasis shows an explosion of acute cases and a high death rate, as well as a lingering chronic infection in survivors (Leathers et al., 1982). Economic losses are primarily caused by death, decreased production capacity due to chronic cases and liver injury induced by larval migration. In this study, the changes of cytokine in infected goats were measured to investigate etio-pathogenesis of Fascioliasis.

2 Materials and methods

2.1 Animals and groups

Thirty six clinically healthy white goats, weighing 20–30 kg, were purchased from the Experimental Animal Center of the Nanjing Medical University. The feces of all goats were monitored for one week using a dot-ELISA method (Leathers, 1982; Rickard, 1995) to confirm the absence of *Fasciola hepatica* infection. The anti-parasite drug ivermectin (0.2 mg·kg⁻¹) was administered subcutaneously, once for each goat. After observation for two weeks, the feces were again tested for one week to confirm a negative infection status. The goats were randomly assigned to one of three groups for every 12 goats. Goats in group I and II were inoculated with a single oral dose of 200 and 500 encysted metacercaria (kindly provided by Leeds University, UK), respectively. Animals in group III served as uninfected controls. Blood samples were collected from the jugular vein before infection (week 0) and once a week thereafter for 11 weeks. Sera and peripheral blood lymphocytes were isolated as described previously (Mizia-Stec et al., 2003; Zhang et al., 2005).

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Youfang GU (✉), Bingyun WANG, Hongying ZHANG, Xinzhi MAO, Yonglin SHEN

Animal Science College, Anhui Science and Technology University, Fengyang 233100, China

Department of Animal Medicine, Foshan Science and Technology College, Foshan 528231, China

College of Veterinary Medicine, Nanjing Agricultural University, Nanjing 210095, China

E-mail: youfanggu@163.com

2.2 Serum IL-2 and TNF- α assay

Serum IL-2 and TNF- α levels were detected using a commercial radio-immune method kit (Shanghai Institute of Nuclear Research, Chinese Academy of Sciences) according to manufacture's instructions.

2.3 IL-2 secretion assay for peripheral blood lymphocytes

2.3.1 Excretory-secretory antigen (ES-Ag) preparation

Parasites were harvested and washed 3–4 times with PBS (pH 7.2, 0.01 mol·L⁻¹) at 37°C, incubated in PBS for 1 h, and then incubated in PBS supplemented with 0.5 mmol·L⁻¹ PMSF, 100 U·mL⁻¹ penicillin and 100 U·mL⁻¹ streptomycin at 37°C for 24 h. Parasites were then centrifuged (11000 r·min⁻¹) at 4°C for 30 min and the supernatant was precipitated twice using 90% ammonium sulfate and dialyzed overnight (changing the PBS 2–3 times). Antigen was purified using a Sephadex G-25 column. The first peak was harvested and then concentrated using polyethylene glycol-10000. The concentration of the resulting excretory-secretory antigen (ESAg) was determined to be 3.458 mg·mL⁻¹ by spectrophotometry. Aliquots (100 μ L) of ESAg were stored at -20°C until use.

2.3.2 Preparation of recombinant bovine IL-2

Recombinant bovine IL-2 was prepared following the method described by Jin et al. (1998). IL-2 was diluted in 7 mol·L⁻¹ guanidine hydrochloride solution and stored at -20°C until use.

2.3.3 Lymphocyte preparation

Lymphocytes were isolated by Ficoll-Paque (Amersham Pharmacia Biotech, Piscataway, NJ) gradient centrifugation as described previously (Zhang et al., 2005) and diluted to 5 \times 10⁶ cells per mL.

2.3.4 Cellular response and IL-2 detection

The IL-2 containing supernatant and responder cells were prepared following the method described by Yu et al. (1991). Cells were stimulated with ConA (Sigma) at a final concentration of 12.5 μ g·mL⁻¹ and ES-Ag at 10 μ g·mL⁻¹. The responder cells were adjusted to (2.5–5.0) \times 10⁶ cells per mL and maintained in RPMI 1640 medium.

2.3.5 MTT assay

Responder cells (100 μ L of 1 \times 10⁶–2 \times 10⁶ cells per mL) containing 1-methyl-D-mannopyranoside (10 mg·mL⁻¹) were plated in a 96-well plate and treated with 100 μ L of recombinant bovine IL-2 (positive control) serum for testing or RPMI 1640 medium (blank, control) at 37°C for

36–48 h. Ten microlitre (μ L) of MTT (5 mg·mL⁻¹) was added to each well at 2.5 h before the end of the incubation and 100 μ L SDS-HCL was added to stop the reaction. The OD value of each well at 570 nm was measured using a microculture plate reader. Conversion percentage was expressed as the result of the following equation: Conversion percentage = (ConA or Ag-OD value / control OD value) \times 100%.

2.4 Statistics

The statistical package used was Statistica 6.0 (StatSoft Inc., Tulsa, Nebraska, USA). All values were expressed as the mean \pm SD. The means between two groups were compared with the Student's *t*-test at the statistically significant level of *P* < 0.05.

3 Results and discussion

As shown in Table 1, the IL-2 levels in sera from group I goats, which were orally inoculated with 200 encysted metacercariae, were elevated early in infection compared to the control group. The levels in the group II (oral inoculation with 500 metacercariae) sera were lower than that in controls. Thus, the different infective doses may have caused different immunologic responses in the hosts. We

Table 1 Changes of serum IL-2 levels in goats infected with *F. hepatica* (μ g·L⁻¹)

week	group I	group II	group III
0	3.25 \pm 0.05	3.02 \pm 0.33	3.14 \pm 0.05
1	2.52 \pm 0.54	3.15 \pm 0.49	3.14 \pm 0.07
2	2.87 \pm 0.18	5.11 \pm 2.46	3.03 \pm 0.10
3	3.07 \pm 0.37	7.88 \pm 3.66	2.93 \pm 0.15
4	2.68 \pm 0.13	6.05 \pm 1.80	2.33 \pm 0.36
5	1.95 \pm 0.03	5.38 \pm 1.68	2.44 \pm 0.13
6	2.83 \pm 0.07	5.32 \pm 1.76	3.86 \pm 0.05
7	3.00 \pm 0.01	6.45 \pm 1.12	3.41 \pm 0.19
8	2.79 \pm 0.11	5.89 \pm 1.56	3.44 \pm 0.72
9	2.16 \pm 0.36	5.64 \pm 2.53	2.68 \pm 0.30
10	2.35 \pm 0.25	5.74 \pm 1.19	2.60 \pm 0.14
11	2.13 \pm 0.15	3.11 \pm 1.02	2.32 \pm 0.41

Table 2 Changes of serum TNF- α levels in goats infected with *Fasciola hepatica* (μ g·L⁻¹)

week	group I	group II	group III
0	0.75 \pm 0.23	0.72 \pm 0.11	0.72 \pm 0.14
1	0.65 \pm 0.10	0.53 \pm 0.12	0.65 \pm 0.04
3	0.84 \pm 0.17	0.73 \pm 0.09	0.87 \pm 0.05
5	0.71 \pm 0.08	0.81 \pm 0.10	0.60 \pm 0.07
7	1.02 \pm 0.21	0.98 \pm 0.17	0.86 \pm 0.07
9	1.42 \pm 0.20*	1.28 \pm 0.24	0.72 \pm 0.03
11	1.94 \pm 0.10*	1.61 \pm 0.62*	0.85 \pm 0.04

Note: * indicates significantly different at *P* < 0.05 compared with control group.

Table 3 Results of lymphocytes in goats infected with *Fasciola hepatica* (%)

week	proliferation rate stimulated by ConA			proliferation rate stimulated by antigen		
	group I	group II	group III	group I	group II	group III
0	124.67 ± 23.42	123.49 ± 5.85	115.37 ± 3.23	108.65 ± 15.42	105.52 ± 0.35	105.23 ± 2.36
1	113.04 ± 18.47	116.71 ± 0.57	112.59 ± 2.56	108.12 ± 27.05	116.25 ± 14.79	106.69 ± 2.40
2	126.72 ± 1.06*	124.34 ± 6.70	119.10 ± 0.92	106.98 ± 9.69	139.22 ± 3.93*	105.89 ± 0.16
3	133.78 ± 16.23	139.83 ± 11.91	114.00 ± 2.62	163.14 ± 44.25	153.31 ± 8.46*	107.25 ± 4.19
4	126.05 ± 1.73*	135.75 ± 0.8046**	116.92 ± 0.24	162.71 ± 7.34*	180.98 ± 39.59	105.72 ± 5.72
5	135.14 ± 14.87	139.23 ± 5.53	115.56 ± 4.12	154.63 ± 4.65*	172.86 ± 7.89*	107.29 ± 2.74
6	119.12 ± 12.39	138.98 ± 11.06	110.96 ± 3.37	127.78 ± 27.78	145.79 ± 36.98	105.17 ± 0.88
7	135.58 ± 2.25	133.80 ± 5.88	114.56 ± 5.47	135.72 ± 8.72	137.59 ± 3.80*	104.39 ± 1.35
8	125.17 ± 21.12	138.34 ± 3.05*	111.84 ± 1.81	176.03 ± 66.59	155.17 ± 44.89	105.94 ± 1.65
9	107.87 ± 25.46	128.29 ± 6.23	138.25 ± 0.39	171.60 ± 43.23	186.95 ± 0.18**	104.97 ± 6.47
10	194.45 ± 94.45	121.39 ± 9.66	112.46 ± 1.87	223.76 ± 52.18	223.66 ± 7.79	104.39 ± 1.35
11	141.15 ± 38.46	154.93 ± 12.32	113.64 ± 0.15	223.12 ± 37.99	207.32 ± 0.15**	105.89 ± 0.16

Note: * and ** indicate significantly different at $P < 0.05$ and $P < 0.01$ levels respectively, compared with control group.

have therefore come to the conclusion that the host cellular immune response may undergo intense changes during *F. hepatica* growth or migration in the liver parenchyma while this effect is less evident during the parasitism of biliary ducts after maturation. That is to say, the effects of *Fasciola hepatica* on the host cellular immune response are related to parasite metabolism and secretory products at different developmental stages.

As shown in Table 2, TNF- α levels were elevated to different degrees in goat sera after infection with different doses of *F. hepatica* indicating that TNF- α is involved in the liver injury process caused by *F. hepatica*. TNF- α may cause liver injury either directly, as an inflammatory mediator, or indirectly, by inducing the release of nitric oxide (NO) and other free radicals (Harbrecht et al., 1994). TNF- α has also been reported to induce cell death in worms and to enhance the macrophage killing of *Plasmodium yoelii* (Zhang et al., 1997). As such, TNF- α is associated with both the *F. hepatica*-inducing liver injury process and the mechanism of host resistance, but these functions require further study to draw a firm conclusion.

In this study, we also indirectly assessed the *in vitro* proliferation index of lymphocytes by measuring production of IL-2 in response to mitogen activation. We found that secreted IL-2 levels increased at the first week post-infection *F. hepatica* and were significantly elevated to both specific and non-specific stimulation (Table 3). The IL-2 levels in serum of goats in group I displayed a significant difference in the 2nd and 4th week after infection when stimulated with ConA ($P < 0.05$). In group II, this effect was evident in the 8th week ($P < 0.05$) and enhanced during the 4th week ($P < 0.01$). Compared with the control group, the IL-2 levels in the serum of goats in group I show a significant difference in the 4th and 5th week after stimulation with Ag ($P < 0.05$). This also occurred in group II in the 2nd, 3rd, 5th, 7th and 10th week ($P < 0.05$) and to a greater extent in the 9th and 11th week ($P < 0.01$). Therefore, IL-2 was found to play a vital role in host immune defense.

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