

Antibody and cytokine responses to hydatid in experimentally infected Kazakh sheep with hydatidosis resistance haplotype

Ren-Yan Li · Qiang Peng · Bin Jia · Guo-Qing Shi ·
Zong-Sheng Zhao · Hong Shen · Hong-Tao Li

Received: 26 September 2010 / Accepted: 3 November 2010
© Springer-Verlag 2010

Abstract Different MHC haplotype of Kazakh sheep has different resistance and susceptibility of hydatidosis. Notably, the *Mvalbc-SacIIab-HinIIab* haplotype of MHC-*DRB1* exon two was associated with resistance hydatidosis. In order to analyze the antibody and cytokine responses to hydatidosis in Kazakh sheep with hydatidosis resistance haplotype, eight Kazakh sheep with the haplotype of *Mvalbc-SacIIab-HinIIab* were chosen as the test group, and other eight, which were not associated with hydatidosis resistance or susceptibility, were taken as control. After experimentally infected with hydatid orally, the blood was collected on 0, 7, 14, 30, 45, 60, 75, 90, 105, and 120 days. Serum and mRNA level of the cytokines IL-2, IFN- γ , TNF- α , IL-4, and IL-10 were evaluated by ELISA and fluorescence quantitative real-time polymerase chain reaction, respectively. The total white blood cells and leukomonocytes were determined by automation cytoanalyze. The level of IgE, IgG, and IgM were evaluated by ELISA. The results showed that the total white blood cells and leukomonocytes in test group were significantly higher than in control on 7, 45, 90, and 105 days post-infection (p.i.). The serum level of IL-2 in test group was significantly higher than in control on 45 days p.i., while the difference of IL-2 mRNA expression between test and control group was not significant. The serum level of TNF- α in test group was significantly higher than in control at 90 and 105 days p.i.,

and the TNF- α mRNA in test group was also significantly higher than in control on 90 days p.i. The level of IgE, IgG, and IgM in test group was higher than in control, but none was significant. The results suggested that the test group, which was predominant of Th1, could induce the protective immunity, while the control, which was predominant of Th2, could induce the susceptibility to infection of hydatidosis.

Introduction

Hydatidosis (*Echinococcus granulosus*) is recognized as one of the world's major zoonosis and is distributed all over the world (Rausch 1995; Andersen et al. 1997; Dalimi et al. 2002; Eckert and Deplazes 2004; Jenkins et al. 2005). Xinjiang Autonomous Region of China is one of the prevalent areas of hydatidosis. In sheep, the overall prevalence rate was 38.89~61.25% (Li et al. 2005) for hydatidosis. Hazakh sheep is a native sheep in XinJiang and can greatly utilize roughage, meanwhile has strong disease resistance and good performance (Adeli et al. 2009). Cystic echinococcosis in farm animals causes considerable economic problems due to the loss of edible liver and meat, as well as, the value of the fleece from infected sheep may also occur.

Helper T cell (Th) could regulate cellular and humoral immunity. According to the present concept, Th1 cell activation leads to protective immunity against hydatidosis, while Th2-dominated immune response is associated with increased susceptibility to disease (Dixon and Jenkins 1995; Gottstein and Hemphill 1997). Emery et al. (1997) reported that A/J mouse produced higher serum level of IFN- γ and IL-2, while BALB/c mouse had higher level of IL-4 after infected with alveolar echinococcosis (AE). It suggested that the production of cytokine was associated

Ren-Yan Li and Qiang Peng contributed equally to this article.

R.-Y. Li · Q. Peng · B. Jia (✉) · Z.-S. Zhao · H. Shen · H.-T. Li
College of Animal Science and Technology, Shihezi University,
Shihezi, Sinkiang 832003, People's Republic of China
e-mail: 39473030@qq.com

G.-Q. Shi
The Key Sheep Breeding and Reproduction Biotechnology
Laboratory, Xinjiang Production and Construction Group,
Shihezi, Sinkiang 832003, China

with genetic factor. There is significant evidence showing that infiltration of neutrophils and macrophages occurs during the early phases of hydatidosis infection followed by leukocytosis, resulting in an increased number of myeloid cells such as eosinophils, lymphocytes, and macrophages (Fotiadis et al. 1999; Zhang et al. 2003). In the chronic phase of CE, elevated levels of Ab, particularly IgG, IgM, and IgE, occur in humans (Khabiri et al. 2006; Daeki et al. 2000; Pinon et al. 1987; Craig 1986; Dessaint et al. 1975). Hashemi Tabar and Borji (2010) tested the serum antibodies by ELISA after injection protoscolices by intraperitoneally; their study showed that the production of antibody is not able to completely to protect against hydatid. Very little is known of cytokine production in the early phases of a primary (oral challenge with eggs) *E. granulosus* infection (Zhang et al. 2008).

Hydatidosis-resistant haplotype, *MvaIbc-SacIIab-Hin1Iab*, was screened out and verified by our previous research (Li et al. 2010). The aim of this study was to analyze the level of Th1 (IL-2, IFN- γ , TNF- α)/Th2 (IL-4, IL-10) and IgG, IgM, and IgE after experimental infection of hydatidosis in Kazakh sheep with hydatidosis-resistant haplotype and those were not associated with hydatidosis resistance or susceptibility.

Materials and methods

Animals sampling and experimentally infected with hydatid

Sixteen 2-year-old Hazakh sheep, which were healthy detected by hydatidosis ELISA kit, were chosen. Among them, eight with the haplotype of *MvaIbc-SacIIab-Hin1Iab* were taken as the test group, and other eight with the haplotypes of *SacIIab-Hin1Iab* or *MvaIbc-Hin1Iab* or *MvaIbc-SacIIab*, which were not associated with hydatidosis resistance or susceptibility, were taken as the control. Each sheep were fed 20 adult cestodes with fertilized egg proglottis by mouth, and these 16 sheep were raised under the same condition.

Quantitative real-time polymerase chain reaction analysis of cytokine

The blood was collected by vacuum tube with heparin sodium on 0, 7, 14, 30, 45, 60, 75, 90, 105, and 120 days p.i. Peripheral blood lymphocyte was isolated by lymphocyte-separating medium, and using Trizol reagent (Promega, China), extracted the RNA from lymphocytes. RNA was quantified by UV absorption at 260 nm. The RNA (2 μ g) was reverse-transcribed into complementary DNA with superscript reverse transcriptase (RT; Promega, China), following the manufacturer's instructions. A mixture of 2 μ g RNA, 0.5 mmol/l dNTP, and 5 μ mol random

primers was heated to 75°C for 5 min and then quickly chilled on ice. Then 4 μ l 5 $\times$ RT buffer, 20U RNase inhibitor, and 10U AMV RTase were added at 42°C for 60 min and 5 min at 95°C. The mRNA level of IL-2, IFN- γ , TNF- α , IL-4, IL-10, and β -actin was determined by quantitative real-time polymerase chain reaction (QRT-PCR; Stratagene MX3000P, USA), and the primers sequences used to amplify were listed in table (Table 1 and Fig. 1).

PCR was performed in a 25- μ l reaction containing 12.5 μ l SYBR[®] Premix Ex Taq[™] (2 $\times$), 5 μ mol oligonucleotide primers, 2 μ l RT products, and sterile distilled water to 25 μ l. After an initial denaturation step (30 s at 95°C), followed by 40 cycles of 95°C for 5 s, anneal for 15 s, and 72°C for 15 s. Each sample was tested three times to improve the *accuratissime*. The plasmid, which was cloned with target fragment, was regarded as standardize substance. It was diluted ten times with gradient and then used to make standard curve.

Serum cytokine assay

The blood was collected at 0, 7, 14, 30, 45, 60, 75, 90, 105, and 120 days p.i. Serum samples were obtained by centrifugation at 3,000 rpm for 10 min and stored at -20°C until used. Cytokines (IL-2, IFN- γ , TNF- α , IL-4, and IL-10) and antibody (IgG, IgM, and IgE) were directly assayed in serum using commercial ELISA kits (ADL, America) following the manufacturer's instructions.

Total white blood cells and lymphocytes test

The blood was collected by vacuum tube of heparin sodium on 0, 7, 14, 30, 45, 60, 75, 90, 105, and 120 days p.i. The total white blood cells and lymphocytes were tested by PE-6800Vet automatic cytoanalyze.

Statistical analysis

Using β -actin as internal standard and double standard curve analyzed the quantitation results. All the data were analyzed by SPSS 13.0, and all of them were mean $\pm$ standard error; the significant difference of results were checked by one-way ANOVA (LSD).

Results

Total white blood cells and lymphocytes

The tendency of total white blood cells and lymphocytes in test group was similar with control, but on 7, 45, 90, and 105 days p.i., the total white blood cells and

Table 1 Conditions of PCR and parameters of oligonucleotide primer pair

Target gene	GenBank no.	Products (bp)	Sequence of primer (5'→3')	Annealing temperature
IFN- γ	X52640	268	F: TTTTAATGCAAGTAACCCAG R: CAGATCATCCACCGGAAT	57°C
TNF- α	X56756	392	F: TTGTAGCCAACATCAGCG R: CTCGGCATAGTCCAGGTA	61°C
IL-2	NM_001009806	80	F: TGTCTTGCAATGCACTAACTC R: TTCTTTCATTGTGTTCCCCGT	56°C
IL-4	M96845	171	F: AACGCCGAACATCCTCACAT R: AGTCCGCCAGGAATTTGTT	60°C
IL-10	U11421	193	F: GAAAAACAAGAGCAAGGCGGTG R: TCGGGGAGTCTAGTCGAGTCA	55°C
β -actin	U39357	167	F: GCAGGTCATCACCATCGGCAATG R: TTGGCGTAGAGGTCTTTGCGGATGT	59°C

leukomonocytes in test group were significantly higher than in control.

QRT-PCR analysis of cytokine

The level of IL-2 mRNA in test group was higher than in control, but none was significant. IFN- γ mRNA in test group was significantly higher than in control on 120 days p.i. TNF- α mRNA in test group was significantly higher than in control on 90 days p.i. IL-10 and IL-4 mRNA in control was significantly higher than in test group on 120 days p.i.

Serum cytokine assay

The serum level of IL-2 in test group was significantly higher than in control on 45 days p.i. TNF- α in test group was significantly higher than in control on 90 and 105 days p.i. IFN- γ in test group was higher than in control, but none was significant. IL-10 and IL-4 in control was significantly higher than in test group, but there was no significant. The level of IgE, IgG, and IgM in test group was higher than in control, but there was also no significant.

The result of experimentally infected sheep with hydatid

Protoscoleces could develop into cysts within 20 days after infection (Dematteis et al., 2003). These 16 sheep were slaughtered on the fourth month after hydatidosis infection. Hydatid cyst on liver and lung surfaces was checked by visual inspection. The results showed that there were only two sheep infected by hydatidosis in treatment group, whereas seven sheep infected in control, and the infection rate in treatment group was significantly lower than that in control ($P < 0.05$).

Discussion

The genetic background of host had been considered as the determinative factor for anti-infection (Meyer et al. 1994; Hill et al. 1991). It was reported that the severity of disease was associated with genetic marker of MHC, and the earliest study showed that the development of hydatidosis was correlative with HLA-DR13 (Gottstein and Bettens 1994). Eiermann et al. (1998) found that MHC-DR11 was associated with a reduced risk for hydatidosis development. Godot et al. (2000) reported that haplotype of HLA B8, DR3, and DQ2 was significantly associated with hydatidosis infection. Abdul-Basit et al. (2010) identified that HLA-DR1, 8 and DR52 associate with resistance and HLA-DR16 associates with susceptibility to *E. granulosus* infection in Yemeni patients. The present study detected the difference between test groups that haplotype, *Mvalbc-SacIIab-HinIIab*, was associated with hydatidosis resistance and control in cellular and humor immunity. The results of ELISA kit testing showed the test group was predominant of Th1, while the control was predominant of Th2 after infection of hydatidosis.

Lymphocytosis is one of the innate immunity factors to resistance hydatidosis (Zhang et al. 2008). Our study showed that on 7, 45, 90, and 105 days p.i. the total white blood cells and leukomonocytes in test group were significantly higher than in control. It suggested that lymphocytosis contributed to host resistance hydatidosis. The level of IgE, IgG, and IgM in test group was higher than in control, even though none was significant. IgG and IgM could activate complement and IgG could mediate phagocyte for phagotrophy parasite antigen. In addition, in vitro experimentation has shown that neutrophils in association with Ab can bring about killing of *E. granulosus* oncospheres (Rogan et al. 1992), indicating a possible role for Ab-dependent, cell-mediated cytotoxicity reactions. The main function of mast

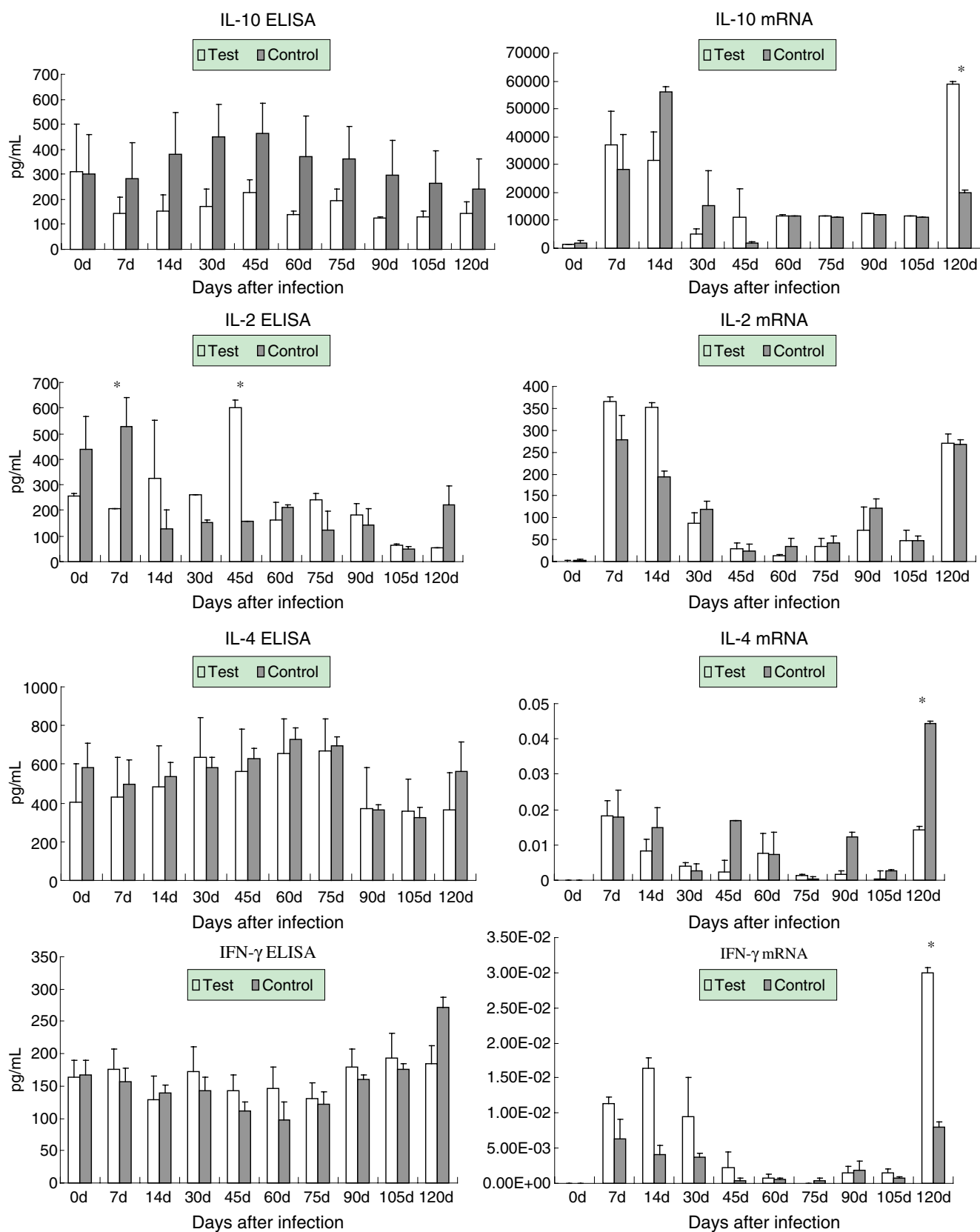


Fig. 1 The expression level of cytokines, antibody, and blood cells in test and control group

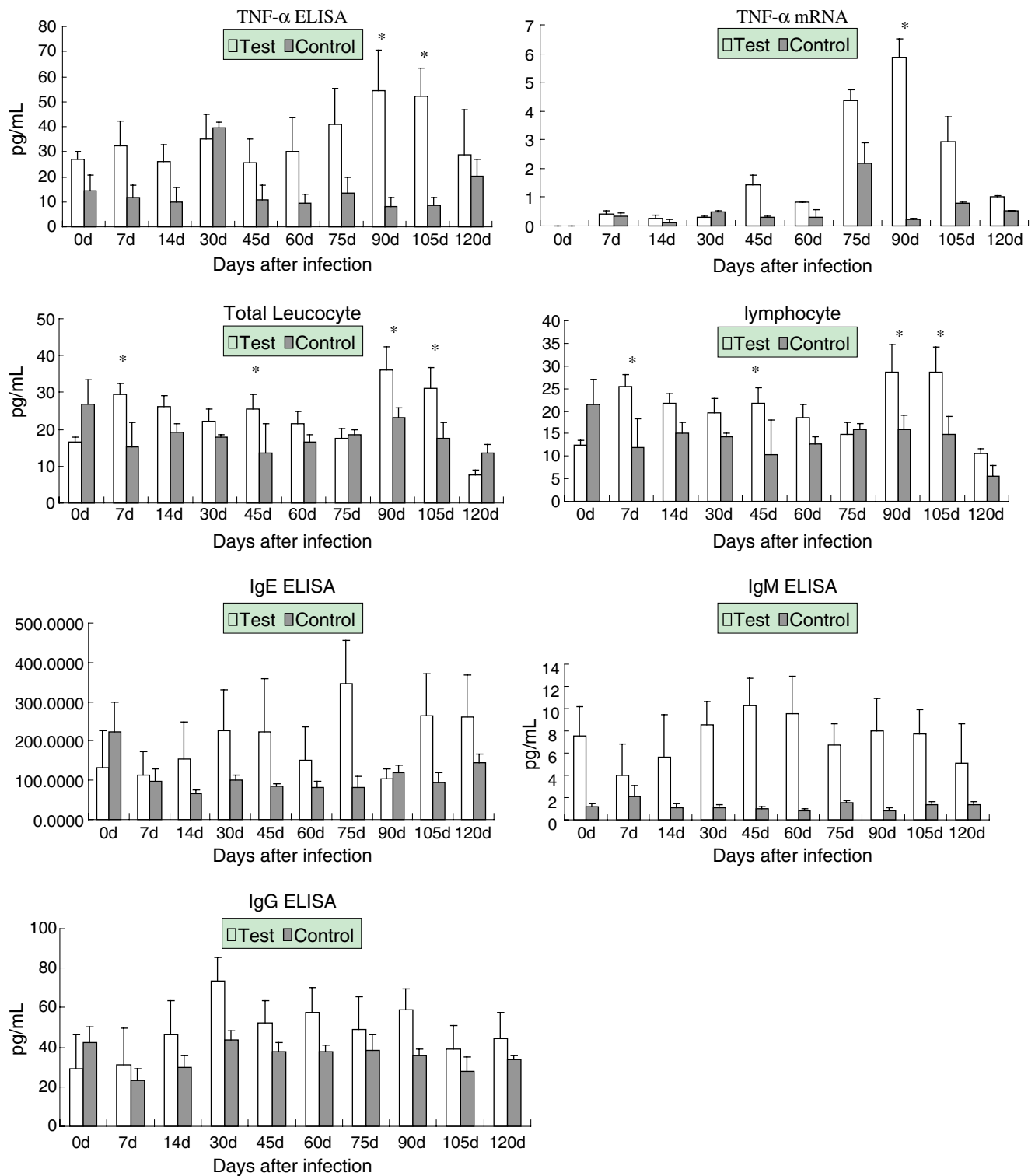


Fig. 1 (continued)

cells, which rely on IgE, is to enhance the ability of eosinophils to kill the parasites (Sterla et al. 1999). This may also help host resistance hydatidosis.

After experimentally infecting the sheep with hydatid, it mainly produced Th1 cytokines, including IL-2, IFN- γ , and

TNF- α in test group, while in control, mainly generated Th2 cytokines, including IL-4 and IL-10. IL-4 could promote the precursor cell differentiation into Th2 cell (Zhang et al. 2003; Rigano et al. 2007) and inhibit differentiation into Th1 cell (Bresson-Hadni et al. 1990;

Touil-Boukoffa et al. 1997). It was reported that the Th2 cytokines are responsible for inhibition of parasite killing because of the anti-inflammatory action of IL-10 (Bauder et al. 1999; Vuitton 2003). The principal function of IL-10 appears to be to limit and ultimately, terminate inflammatory responses (Moore et al. 2001). It also promoted the growth of parasites (Emery et al. 1996). Perhaps, this was the reason why the control group, which was dominant by Th2 cytokines, was susceptible to hydatidosis.

The cytokines of Th1 including IL-2 and IFN- γ could retard the growth of parasites (Emery et al. 1996). IL-2 could promote the activity of cytotoxic lymphocyte, NK cells, and lympholine-activated killer cell (Fang et al. 1999). Wei et al. (2004) confirmed IL-2 inhibited the growth of AE. During the defense parasites infection, IFN- γ could inhibit the secretion of Th2 cytokines by enhancement the phagocytosis of macrophage and limit the proliferation of cytozoon (Wei et al. 2004). TNF- α , which was an immunological reaction medium and secreted by Th1, participated in inflammatory and immunological response of several infection diseases and synergism with IFN- γ (Jiang 2001). Therefore, the test group, which was dominant of Th1 cytokines, could be resistance hydatidosis. Khaled et al. (2008) found a significant high protection rate against secondary hydatidosis in BALB/c mice, which were accompanied with the induction of Th1 response.

The result of establishment model of infection of hydatidosis showed that taking protoscolex intraperitoneally was less accurate than orally. The reason was that majority of protoscolex will be killed in 2 weeks through intraperitoneal infection (Rogan 1987). Therefore, in our study, the sheep was orally infected with hydatid, and it will be more accurate to simulate the process of infection of hydatidosis by nature. That will provide reliable proof for clinical cure.

The study confirmed that Th1 induce protective immunity and Th2 increase the susceptibility of hydatidosis. It also suggested that the hydatidosis resistance haplotype, *MvaIbc-SacIIab-HinIIab*, of Kazakh sheep could contribute to the immunoloregulation reaction for protection from hydatidosis infection.

Acknowledgments We thank all the people who helped us for the present study. This study was supported by a grant from the Natural Science Foundation of China (30660124) to Prof. Bin Jia.

References

- Abdul-Basit A, Al-Ghoury, Eman M, El-Hamshary (2010) HLA class II alleles: susceptibility or resistance to cystic echinococcosis in Yemeni patients. *Parasitol Res* 107:355–361
- Adeli, Ayiguli, Chen WG (2009) The suggestions about resources and utilizing of Hazakah sheep. *Xinjiang animal husbandry* 1:48–49, China
- Andersen FL, Ouhelli H, Kashani M (1997) Compendium on cystic echinococcosis. Brigham Young University, Provo, UT 84602, USA
- Bauder B, Auer H, Schilcher F, Gabler C, Romig T, Bilger B, Aspöck H (1999) Experimental investigations on the B and T cell immune response in primary alveolar echinococcosis. *Parasite Immunol* 21:409–421
- Bresson-Hadni, Liance SM, Meyer JP, Houin R, Bresson JL, Vuitton DA (1990) Cellular immunity in experimental *Echinococcus multilocularis* infection. II. Sequential and comparative phenotypic study of the periparasitic mononuclear cells in resistant and sensitive mice. *Clin Exp Immunol* 82:378–383
- Craig PS (1986) Detection of specific circulating antigen, immune complexes and antibodies in human hydatidosis from Turkana (Kenya) and Great Britain, by enzyme immunoassay. *Parasite Immunol* 8:171–188
- Daeki AO, Craig PS, Shambesh MK (2000) IgG-subclass antibody responses and the natural history of hepatic cystic echinococcosis in asymptomatic patients. *Ann Trop Med Parasitol* 94:319–328
- Dalimi A, Motamedi G, Hosseini M, Mohammadian B, Malaki H, Ghamari Z, Ghaffari FF (2002) Echinococcosis/hydatidosis in western Iran. *Vet Parasitol* 105(2):161–171
- Dematteis S, Rottenberg M, Baz A (2003) Cytokine response and outcome of infection depends on the infective dose of parasites in experimental infection by *Echinococcus granulosus*. *Parasite Immunol* 25:189–219
- Dessaint JP, Bout D, Watre P, Capron A (1975) Quantitative determination of specific IgE antibodies to *Echinococcus granulosus* and IgE levels in sera from patients with hydatid disease. *Immunology* 29:813–823
- Dixon JB, Jenkin PS (1995) Immunology of mammalian metacystode infections. II. Immune recognition and effector function. *Helminthol* 64:599–613
- Eckert J, Deplazes P (2004) Biological, epidemiological, and clinical aspects of echinococcosis, a zoonosis of increasing concern. *Clin Microbiol Rev* 17(1):107–135
- Eiermann TH, Bettens F, Tiberghien P (1998) HLA and alveolar echinococcosis. *Tissue Antigens* 52(2):124–129
- Emery I, Liance M, Deriaud E, Vuitton DA, Houin R, Leclerc C (1996) Characterization of T-cell immune responses of *Echinococcus multilocularis* -infected C57BL/6 J mice. *Parasite Immunol* 18:463–472
- Emery I, Liance M, Leclerc CC (1997) Secondary *Echinococcus multilocularis* infection in A/J mice: delayed metacystode development is associated with Th1 cytokine production. *Parasite Immunol* 19:493–503
- Fang YQ, Tan Y, Yang GZ (1999) The effects of IL-2 against *Toxoplasma gondii* infection in vivo. *Immunol J* 115(1):32–34
- Fotiadis C, Sergiou C, Kirou J, Troupis TG, Tselentis J, Doussaitou P, Gorgoulis VG, Sechas MN (1999) Experimental *Echinococcus* infection in the mouse model: pericystic cellular immunity reaction and effects on the lymphoid organs of immunocompetent and thymectomized mice. *In Vivo* 13: 541–546
- Godot V, Harraga S, Beurton I (2000) Resistance/susceptibility to *Echinococcus multilocularis* infection and cytokine profile in humans. II. Influence of the HLA B8, DR3, DQ2 haplotype. *Clin Exp Immunol* 121:491–498
- Gottstein B, Hemphill A (1997) Immunopathology of echinococcosis. *Chem Immunol* 66:177–208
- Gottstein B, Bettens F (1994) Association between HLA-DR13 and susceptibility to alveolar echinococcosis. *J Infect Dis* 169:1416–1423
- Hashemi Tabar GR, Borji H (2010) Antibody responses to hydatid cyst in experimentally infected lambs. *World Appl Sci J* 8 (8):1001–1006

- Hill AVS, Allsopp CEM, Kwiatkowski D (1991) Common West African HLA antigens are associated with protection from severe malaria. *Nature* 352:595–600
- Jenkins DJ, Romig T, Thompson RCA (2005) Emergence re-emergence of *Echinococcus* spp. a global update. *Int J Parasitol* 35:1205–1219
- Jiang CP (2001) Parasite infection and cytokine. *Chin J Parasit Dis Con* 14(2):150–153
- Khabiri AR, Bagheri F, Assmar M, Siavashi MR (2006) Analysis of specific IgE and IgG subclass antibodies for diagnosis of *Echinococcus granulosus*. *Parasite Immunol* 28:357–362
- Khaled M, Al-Qaoud, Sami K, Abdel-Hafez (2008) The induction of T helper type 1 response by cytokine gene transfection protects mice against secondary hydatidosis. *Parasitol Res* 102:1151–1155
- Li Y, Yan JL, Li J (2005) Investigation and analysis of epidemic conditions of hydatidosis disease of sheep in Xinjiang. *J Shihezi University Natural Sci* 23:60–64, China
- Meyer CG, Gallin M, Erttmann KD (1994) HLA-D alleles associated with generalized disease, localized disease, and putative immunity in *Onchocerca volvulus* infection. *Proc Natl Acad Sci USA* 91:7515–7524
- Moore KW, deWaal Malefyt R, Coffman RL, O'Garra A (2001) Interleukin-10 and the interleukin-10 receptor. *Annu Rev Immunol* 19:683–765
- Rausch RL (1995) Life cycle patterns and geographic distribution of *Echinococcus* species. In: Thompson RCA, Lymbery AJ (eds) *Echinococcus* and hydatid disease CAB International. Oxon, UK
- Pinon JM, Poirriez J, Lapan H, Geers R, Penna R, Fernandez D (1987) Value of isotypic characterization of antibodies to *Echinococcus granulosus* by enzyme-linked immuno-filtration assay. *Eur J Clin Microbiol* 6:291–295
- Rogan MT (1987) *Echinococcus granulosus*. Studies on the development of the metacestode tegument. University of Salford, UK
- Rogan MT, Craig PS, Zehly E, Masinde G, Wen H, Zhou P (1992) In vitro killing of taeniid oncospheres, mediated by human sera from hydatid endemic areas. *Acta Trop* 51:291–296
- Rigano R, Buttari B, Profumo E, Ortona E, Delunardo F, Margutti P, Mattei V, Teggi A, Sorice M, Siracusano A (2007) *Echinococcus granulosus* antigen B impairs human dendritic cell differentiation and polarizes immature dendritic cell maturation towards a Th2 cell response. *Infect Immun* 75:1667–1678
- Li RY, Jia B, Zhang WJ, Zhao ZS, Shi GQ, Shen H, Peng Q (2010) Analysis of the Relationship between MHC-DRB1 gene polymorphism and hydatidosis in Kazakh sheep. *Asian Aust J Anim Sci* 23(9):1145–1151
- Sterla S, Sato H, Nieto A (1999) *Echinococcus granulosus* human infection stimulates low avidity anticarbohydrate IgG2 and high avidity antipeptide IgG4 antibodies. *Parasite Immunol* 21:27–34
- Touil-Boukoffa C, Sanceau J, Tayebi B, Wietzerbin J (1997) Relationship among circulating interferon, tumor necrosis factor- α , and interleukin-6 and serologic reaction against parasitic antigen in human hydatidosis. *J Interferon Cytokine Res* 17:211–217
- Vuitton DA (2003) The ambiguous role of immunity in echinococcosis: protection of the host or of the parasite? *Acta Trop* 85:119–132
- Zhang WB, Allen GR, Donald PM (2008) Mechanisms of immunity in hydatid development disease: implications for vaccine. *J Immunol* 181:6679–6685
- Wei XL, Ding JB, Xu Y, Wen H, Lin RY (2004) Change of cytokines in mice with *Echinococcus multilocularis* infection. *Chin J Parasitol Parasit Dis* 22(6):361–364
- Zhang W, Li J, McManus DP (2003) Concepts in immunology and diagnosis of hydatid disease. *Clin Microbiol Rev* 16:18–36