文章编号:1000-7423(2011)-05-0339-09

【论著】

Evaluation of Recombinant SjLAP and SjFBPA in Detecting Antibodies to Schistosoma japonicum

Faustina Halm-Lai[1,2], LUO Qing-li[1], ZHONG Zheng-rong[1], SONG Xiao-rong[1], CHEN Zhao-wu[1], WANG Lin[1], HU Mei-mei[1], CHU De-yong[1], SHEN Ji-long[1*]

【Abstract】 Objective To investigate the early response of immunoglobulin G (IgG) antibody responses to Schistosoma japonicum infection in mice by using the recombinant proteins, S. japonicum leucine aminopeptidase (rSjLAP) and S. japonicum fructose-1, 6-bisphosphate aldolase (rSjFBPA), and evaluate the potential of rSjLAP and rSjFBPA in diagnosis as well as in assessment of therapeutic efficacy in human schistosomiasis. Methods rSjLAP or rSjFBPA was induced from Escherichia coli BL21 strain transfected with the expression vectors, pET-28a-rSjFBPA/BL21 or pET-28a-rSjLAP/BL21 using isopropyl-β-D-thiogalactoside (IPTG), and purified by Ni-NTA His Bind resin. 88 BALB/c female mice, inbred and 6 to 8 weeks old, were randomly divided into 4 groups. Groups A, B and C each made up of 21 mice and group D comprised 25 mice. Groups A, B and C were infected with 5, 15 and 25 S. japonicum cercariae respectively. As control, mice in group D were left uninfected. 3 mice from each of groups A, B and C were sacrificed and sera collected on days 3, 7, 10, 14, 20, 30, and 60 post infection. All the 25 mice in group D were sacrificed on the first day of the experiment for serum collection. rSjLAP and rSjFBPA were screened and used in ELISA to test the antibody response of the serum samples. Also, sera of 38 acute patients, 96 chronic patients with schistosomiasis japonica, 90 healthy donors and patients with other parasite infections including Clonorchis sinensis (33 cases), Paragonimus westermani (40) and hookworms (37) were tested using the recombinant protein-based ELISA. In addition, 36 sera each from the acute and chronic patients 12 months after treatment with praziquantel and 64 of the chronic patients in more than 2 years post-treatment of praziquantel were tested. The dosage of praziquantel for both acute and chronic patients was 60 mg/kg, 2 times/d×2 d. Results IgG antibody response was first detected at day 10 post infection by rSjLAP, rSjFBPA or the combined antigen assay. The mean absorbance (A_{450}) on this day were 0.535±0.053, 0.595±0.033, 0.696±0.104 for group B; 0.548±0.060, 0.608±0.063, 0.621±0.090 for group C; and 0.415±0.038, 0.455±0.056, 0.498±0.077 for group A for rSjLAP, rSjFBPA and the combined assay respectively ($P<0.05$). Early antibody level to both antigens was significantly higher in mice infected with 15 or 25 cercariae than those with 5 cercariae ($P<0.05$). However, ELISA results in patients with confirmed schistosomiasis revealed positive rates of 97.4% (37/38) and 87.5% (84/96) for acute and chronic schistosomiasis with rSjLAP , 94.7% (36/38) and 88.5% (85/96) for acute and chronic schistosomiasis with rSjFBPA and 94.7% (36/38) and 85.4% (82/96) with both rSjLAP and rSjFBPA respectively. Statistical analysis showed no significant difference in the positive rate ($P>0.05$). Also, rSjLAP and combined antigens showed a specificity of 96.7% (87/90) while that of rSjFBPA was 97.8% (88/90). There was a general decrease in the antibody titer of the patients after treatment. In 12 months after treatment it was 0.236±0.212 with rSjLAP, 0.287±0.191 with rSjFBPA, and 0.235±0.120 with both antigens respectively for acute cases; For chronic patients, it was 0.266±0.124, 0.261±0.143 and 0.265±0.140 in 12 months post-treatment, and 0.204±0.074, 0.176±0.074, and 0.176±0.073 in 2 years, respectively. For healthy control, it was 0.188±0.056, 0.173±0.45, and 0.184±0.051, respectively. No significant difference on antibody titer was found between treated patients and control ($P>0.05$). The cross reaction with C. sinensis was 15.2% (5/33) for rSjLAP, 12.1% (4/33) for rSjFBPAand 9.2% (3/33) for combined antigens. With P. westermani, it was 15.0% (6/40), 12.5% (5/40) and 15.0% (6/40), respectively, and 8.1% (3/37) with hookworm infection. Conclusion The study showed a satisfactory sensitivity and specificity of rSjLAP and rSjFBPA by ELISA which is promising for the immunological diagnosis of schistosomiasis.

Supported by the National High Technology Research and Development Program of China (863 Program, No. 2006AA02A251), the National Natural Science Foundation of China (No. 81071376; No. 30872209) and the Doctoral Fund of Ministry of Education of China (No. 200803660003)

1 Anhui Provincial Laboratory of Microbiology & Parasitology; Anhui Key Laboratory of Zoonoses, Department of Microbiology and Parasitology, Anhui Medical University, Hefei 230022, China; 2 Department of Microbiology, University of Cape Coast, Ghana

* Corresponding author, E-mail: shenjilong53@126.com

【Key words】 Schistosomiasis japonica; Immunodiagnosis; Leucine aminopeptidase; Fructose-1, 6-bisphosphate aldolase

CLC No: R383.24 Document code: A

重组果糖二磷酸醛缩酶 SjLAP 和亮氨酸氨基肽酶 SjFBPA 用于日本血吸虫病的诊断和疗效考核的评价

Faustina Halm-Lai[1,2], 罗庆礼[1], 钟政荣[1], 宋晓蓉[1], 陈兆武[1], 王林[1], 胡梅梅[1], 储德勇[1], 沈继龙[1*]

(1 安徽病原生物学省级实验室, 安徽省人兽共患病重点实验室, 安徽医科大学病原生物学教研室, 合肥 230022, 中国; 2 Cape Coast 大学微生物学教研室, 加纳)

【摘要】 **目的** 评价重组亮氨酸氨基肽酶(rSjLAP)和重组果糖二磷酸醛缩酶(rSjFBPA)抗原用于诊断人血吸虫感染以及疗效考核的价值。 **方法** 异丙基-β-D-硫代半乳糖苷(IPTG)诱导 pET-28a-rSjLAP/BL21 和 pET-28a-rSjFBPA/BL21 表达目的蛋白, 组氨酸标签亲和纯化柱纯化 rSjLAP 和 rSjFBPA 蛋白。88 只 BALB/c 雌性小鼠随机分为 4 组, A、B 和 C 组(各 21 只小鼠), D组(25 只小鼠)。A、B 和 C 组分别感染 5、15 和 25 条日本血吸虫尾蚴。D 组为不感染对照组, 在实验的第 1 天全部处死。A、B 和 C 组在感染后第 3、7、10、14、20、30 和 60 天, 分别处死小鼠 3 只, 采眼球血制备血清, 检测其抗体水平。采用单独或联合 rSjLAP 和 rSjFBPA 为抗原, ELISA 法检测小鼠血清、急性血吸虫病(38 份)和慢性血吸虫病患者(96 份)血清中的抗体, 以健康人(90 份)血清为对照, 同时检测华支睾吸虫病(33 份)、卫氏并殖吸虫病(40 份)和钩虫病患者(37 份)血清, 并检测急性血吸虫病患者吡喹酮治疗(60 mg/kg, 2 次/d×2 d)后 1 年的血清(36 份)、慢性血吸虫病患者吡喹酮治疗(剂量, 疗程同前)后 1 年(36 份)和 2 年(64 份)的血清。 **结果** BALB/c 小鼠在感染后第 10 天, rSjLAP 和 rSjFBPA 单独或联合使用均可检测到小鼠血清中的 IgG 抗体; B 组 (0.535±0.053, 0.595±0.033, 0.696±0.104)和 C 组(0.548±0.060, 0.608±0.063, 0.621±0.090)早期抗体水平明显高于 A 组(0.415±0.038, 0.455±0.056, 0.498±0.077)(P<0.05)。用 rSjLAP 为抗原可检测急性血吸虫病和慢性血吸虫病患者血清, 阳性率分别为 97.4% (37/38) 和 87.5% (84/96) (P>0.05); 用 rSjFBPA 为抗原检测, 其阳性率分别为 94.7% (36/38)和 88.5% (85/96) (P>0.05); 用 rSjLAP 和 rSjFBPA 联合为抗原检测, 则其阳性率分别为 94.7% (36/38)和 85.4% (82/96)(P>0.05)。rSjLAP 或联合抗原的特异性均为 96.7% (87/90), 而 rSjFBPA 的特异性为 97.8% (88/90)。给予吡喹酮治疗后, rSjLAP 和 rSjFBPA 单独或联合使用检测急性血吸虫病血清(0.236±0.212, 0.287±0.191, 0.235±0.120)和慢性血吸虫病患者(0.266±0.124, 0.261±0.143, 0.265±0.140; 0.204±0.074, 0.176±0.074, 0.176±0.073), 抗体滴度普遍下降, 与对照组 (0.188±0.056, 0.173±0.045, 0.184±0.051)相比, 差异无统计学意义(P>0.05)。rSjLAP 和 rSjFBPA 单独为抗原检测华支睾吸虫病患者血清, 交叉反应率为 15.2% (5/33)和 12.1% (4/33), 两者联合则为 9.2% (3/33)。rSjLAP 检测卫氏并殖吸虫的交叉反应率为 15.0% (6/40), rSjFBPA 为 12.5% (5/40), 两种抗原联合检测为 15.0% (6/40)。上述抗原单独或联合检测钩虫的交叉反应率均为 8.1% (3/37)。上述各组阳性率与健康人群之间差异有统计学意义 (P<0.05), 显示该重组抗原在其他蠕虫检测中存在一定的交叉反应。 **结论** 用 rSjFBPA 和 rSjLAP 作为抗原的 ELISA 法诊断血吸虫病具有良好的敏感性和特异性。

【关键词】 **日本血吸虫病; 免疫诊断; 亮氨酸氨基肽酶; 果糖二磷酸醛缩酶**

基金项目: 国家重点基础研究发展计划(863 项目) (No. 2006AA02A251); 国家自然科学基金(No. 81071376, 30872209); 教育部博士基金(No. 200803660003)

* 通讯作者, E-mail: shenjilong53@126.com

Schistosomiasis is still ranked the second most socio-economically devastating parasitic disease following malaria worldwide [1]. This disease is most prevalent in areas characterized by low socioeconomic conditions [2]. The tropical and subtropical parts of many developing countries, especially in Africa account for 85% of people infected globally[3]. The disease is also prevalent in Middle East, Far East, and South America among others[4-7]. It is estimated that at least 207 million people are infected globally with an estimated 700 million people at risk of infection in 74 endemic countries[3,8]. Thus, schistosomiasis has caused significant morbidity and mortality, hence is a major public health problem.

Diagnosis of this disease is crucial as it forms the basis for the assessment of morbidity, development of new drugs, vaccine, community treatment and evaluation of successful eradication or control measures [9]. Previous studies have shown that immunoserological diagnosis, antigens of recombinant peptides including rSjFBPA and rSjLAP have high sensitivity and specificity and are convenient for large-scale operations[10-13]. But, so far no immnunodiagnostic assay is considered best in the detection of early infection.

The principal objective of the present work was to determine the early days at which immunoglobulin G (IgG) antibody responses to infection could be detected. Also, we evaluated the potentials of rSjLAP and rSjFBPA in diagnoses of infection as well as in assessment of therapeutic efficacy in human schistosomiasis. The information obtained could form the basis for assessing the efficacy of these recombinant protein-based assays for detecting S. japonicum infection.

MATERIALS AND METHODS

1 Sampling and inoculation

Eighty-eight susceptible BALB/c female mice, inbred, healthy and 6 to 8 weeks old were obtained from the Center of Laboratory Animals of Anhui Medical University, approved by the Animal Welfare and Ethics Committee of Anhui Medical University. The mice were randomly divided into 4 groups. Groups A, B, C each made up of 21 mice and group D comprised 25 mice. The mice in groups A, B, and C were infected percutaneously with 5, 15, and 25 S. japonicum cercariae respectively released from the intermediate snail host Oncomelania hapensis (purchased from Jiangsu Institute for Parasitic Diseases Control, Wuxi, China). Then, they were maintained under appropriate conditions. As control, mice in group D were left uninfected and sacrificed on the first day of the experiment for serum collection. 3 mice were sacrificed from each of the groups A, B and C and sera collected on days 3, 7, 10, 14, 20, 30, and 60 post infection. Blood samples were taken from the retro-orbital sinus of the eyes.

Also, sera from 38 acute and 96 chronic patients were obtained from the schistosomiasis patients at the endemic areas of Anhui Province. Among those, 36 sera each from the acute and chronic patients within 12 months after treatment with praziquantel and 64 of the chronic patients more than 2 years post-treatment of praziquantel were obtained. The dosage of praziquantel for both acute and chronic patients was 60 mg/kg, 2 times/d×2 d. Sera from 90 healthy donors (control), 33, 40 and 37 patients with Clonorchis. sinensis, Paragonimus. westermani, and hookworm infections respectively were collected from non-endemic area of schistosomiasis, Anhui Province. All the serum samples were stored at -80℃.

2 Expression and purification of recombinant antigens, LAP and FBPA

Ni-NTA His·Bind ® Resins Kits (TB054 Rev. F0106, Novagen, USA) was used for sonication and purification. The transformed Escherichia coli BL21 strains (pET-28a-rSjLAP/BL21 and pET-28a-rSjFBPA/BL21) were kindly provided by Dr. ZHONG Zheng-rong (The Key Laboratories of Parasitology and Zoonoses, Anhui Province, China).

The strains (4 ml each) were grown separately in 400 ml sterilized Luria-Bertani (LB) medium which contained 4.0 g tryptone, 2.0 g yeast extract, 4.0 g sodium chloride, 395 ml deionized water, and 200 μl of 100 mg/ml Kanamycin. The pH of the LB medium was 7.4. The E. coli BL21 strains were induced by the addition of 1 mmol/L IPTG of final concentration and harvested by centrifugation at 4 000×g, 4 ℃ for 10 min and decanting the supernatant solution. It was ensured that samples were taken before and after the addition of IPTG at time intervals(1 or 2, 4, and 6 h) for protein visualization or immunoblotting. Then, the harvested cells were disrupted by sonication in a lysis buffer containing 6 mol/L urea and centrifuged at 4 000×g, 4 ℃ for 10 min. The supernatant extracts (rSjLAP and rSjFBPA) were sterile-filtered through 0.45-μm membrane filter. 1 500 μl resin was transferred to empty novagen column prewashed with sterilized deionised water and then allowed to settle down. The resin column was charged and equilibrated by sequential washing with 2 250 μl of sterile deionised water, 3 750 μl of 1×charge buffer and 2 250 μl of 1×binding buffer. Then, the columns were loaded with

the prepared antigens, allowed to drain and washed with 7 500 μl of 1×binding buffer followed by 4 500 μl of 1×wash buffer and the recombinant proteins were eluted in fractions with 4 500 μl of 1×elute buffer. Protein concentrations were determined using Pierce bicinchoninic acid Protein Assay Kit (23225 23227, Pierce Bio, USA).

3 Visualization and immune specificity of recombinant proteins

Unpurified and purified samples collected were subjected to 12% sodium dodecylsulfate-polyacrylamide gel electrophoresis after which the proteins were visualized by comassie brilliant blue staining solution. For immune specificity, the electrophoresed recombinant SjLAP or SjFBPA was transferred onto a nitrocellulose membrane and electrophoresed on ice at 200 mA for 2 h. The membrane was blocked in 20 ml phosphate buffered saline (PBS) containing 1g skimmed milk powder in a glass dish at 4 ℃ overnight. Then, it was washed with PBS containing 0.05% Tween-20 (PBST) and incubated overnight at 4 ℃ in 20 ml of 5% bovine serum albumin (BSA) with tris buffer saline tween-20 (TBST) as the diluent and containing 20 μl primary antibody, mouse anti-His antibody (1∶1000 dilution) (788026, Beyotime Biotechnology). After three washings with PBST, the membranes were incubated in anti-mouse IgG-peroxidase conjugate (BA 1050, Boster Biotechnology) at 37 ℃ for 2 h followed by 3-time washings with PBST and then developed with diaminobenzidine tetrahydrochloride (ZL1-9032, Beijing Zhong shan Jinqiao).

4 Enzyme-linked immunosorbent assay (ELISA)

The optimal dilutions of antigens and sera were determined by checkerboard titration. A 100 μl of an optimal dilution of each rSjLAP (25 ng for mice; 100 ng for human) and/or rSjFBPA (200 ng for both mice and human) diluted in 50 mmol/L carbonate buffer (pH 9.6) were added to each well of a polystyrene microtitre plate, and incubated at 4 ℃ overnight. The plate was washed 5 times with PBST and blocked with 100 μl of 5% BSA (diluted in PBS) at 4 ℃ overnight. Then, the plate was washed with PBST and 100 μl of appropriate test and control sera were added. Each serum sample was tested in duplicate. An optimal dilution of anti-mouse IgG (BA 1050, Boster Biotechnology) and anti-human IgG conjugated with horseradish peroxidase (ZB-2304, Zhongshang Bioengineering) for mice sera and human sera respectively was added, 100 μl per well and incubated at 37 ℃ for 1 h. After washings with PBST the color was developed by adding 50 μl of substrate A (17516, Ying Ke Xin Chuang technology) followed by 50 μl of substrate B (96615, Ying Ke Xin Chuang Technology) and then incubated at 37 ℃ for 10 min after which the reaction was stopped by adding 50 μl of hydrochloric acid (0816, Zhongshang Bioengineering) and the absorbance was read at 450 nm using an automatic ELISA reader (ELx808, Bio-Tek, USA).

5 Data analysis

The cut off optical density values were established from the mean plus 2 times the standard deviation of the healthy control. The positive rate and specificity were calculated as described by Galen[14]. Statistical analysis and evaluation were done using GraphPad Prism software and SPSS. Significance was defined in all cases at $P<0.05$.

RESULTS

1 Optimization of protocol

The optical criteria for performing the ELISA assays were detected at different dilutions: as 0.25 ng/μl of rSjLAP at serum dilution of 1∶200, 2 ng/μl of rSjFBPA at serum dilution of 1∶400 and a serum dilution of 1∶100 for both antigens in mice. For human serum samples, 1 ng/μl of rSjLAP and 2 ng/μl of rSjFBPA at serum dilution of 1∶20 were observed. The same serum dilution was detected when the two antigens were combined. The optimal dilution of anti-mouse IgG and anti-human IgG was found to be 1∶30 000. The concentration of the purified proteins ranged from (0.165～0.298) mg/ml for rSjLAP and (0.176～0.335) mg/ml for rSjFBPA.

2 Antibody responses to parasite burden in infected mice and absorbance of sera from uninfected mice

The mean A_{450} values of 25 sera from uninfected

mice were 0.156±0.08, 0.150±0.076 and 0.146±0.075 with cut off values of 0.316, 0.302 and 0.296 for rSjLAP, rSjFBPA and both antigens respectively. The serum antibody responses to S. japonicum antigens on the days 3, 7, 10, 20, 30 and 60 post infection are shown in tables 1, 2 and 3. Generally, the patterns of responses were similar. Responses were first detected at day 10 post infection by all the antigen assays. The mean A_{450} titers on this day were 0.415±0.038, 0.535±0.053 and 0.548±0.060 for groups A, B and C respectively by rSjLAP, 0.455±0.056, 0.595±0.033 and 0.608±0.063 for groups A, B and C respectively by rSjFBPA and 0.498±0.077, 0.696±0.104 and 0.621±0.090 for groups A, B and C respectively by rSjLAP and rSjFBPA assay. These values were significantly higher when compared with those of the control mice (P<0.05). Early antibody levels were observed to be frequently and significantly higher in mice infected with 15 or 25 cercariae by all the antigens (P<0.05) (Tables 1, 2 and 3). There was Then a gradual increase in response to all the antigens and peak responses on day 20 in group B (rSjLAP 1.889±0.086, rSjFBPA 1.579±0.041, rSjLAP and rSjBPA assay 2.258±0.394) and group C (rSjLAP 1.741±0.070, rSjFBPA 1.697±0.046, rSjLAP and rSjBPA assay 1.906±0.235) and day 30 in group A (rSjLAP 2491±0254, rSjFBPA 2.089± 0.223, rSjLAP and rSjBPA assay 2.578±0.275) were observed. Hence, the responses to rSjLAP and the combined antigen assay (rSjLAP and rSjFBPA assay) on day 20 were significantly higher in mice of groups B and C than those of groups A (Group B 1.889±0.086 or group C 1.741±0.070>group A 1.362±0.145 for rSjLAP, group B 2.258±0.394 or group C 1.906±0.235>group A 1.413±0.342 for the combined antigen assay) (P<0.05). Also, significant difference was found between groups B and C (Groups B>group C) (P<0.05). Test by rSjFBPA indicated no significant difference between groups B and C (group B 1.579 ± 0.041, group C 1.697±0.046) (P>0.05) but responses of mice in group B and C were significantly different from those of group A (group B 1.579±0.041 or group C 1.697±0.046>group A 1.211±0.249) (P<0.05).

On days 30 and 60, antibody responses were observed to be higher in group 1 than the other groups. Responses of group A to rSjLAP were significantly different from those of groups B and C on days 30 and 60 (day 30 group A 2.491±0.254> group B 2.048±0.057 or group C 1.940±0.056, day 60 group A 2.380±0.194>group B 1.679±0.236 or group C 1.769±0.157) (P<0.05) but no significant difference was observed between groups B and C at the same time (P>0.05). A similar pattern was ob served with rSjFBPA but significant difference was observed between groups B and C only on day 30 (group C 1.81±0.124>group B 1.487±0.333) (P<0.05). Test by the combined antigen assay indicated no significant difference between groups A and B on day 30 and groups B and C on day 60 (day 30 group A 2.578±0.275>group B 2.354±0.347, day 60 group B 2.119±0.345>group C 1.969±0.347) (P>0.05) (Tables 1, 2 and 3). Additionally, statistical analysis of all the antigen assays indicated no significant difference between group A, group B or group C and control mice (group D) on days 3 and 7 (P>0.05) while a significant difference was observed from day 10 to day 60 (P<0.05).

3 Evaluation of rSjLAP and rSjFBPA for the diagnosis of human schistosomiasis

ELISA results for serum samples of confirmed schisto somiasis and uninfected healthy persons were shown in tables 4 and 5. The mean A_{450}±SD value of 90 sera from healthy control for rSjLAP, rSjFBPA and both antigens was 0.188±0.056, 0.173±0.045 and 0.184±0.051 respectively. The cut off value determined from each assay was 0.300 for rSjLAP, 0.263 for rSjFBPA and 0.286 for both antigen assays. As shown in table 4, rSjLAP and both antigen assays had a specificity of 96.7% (87/90) while that of rSjFBPA was 97.8% (88/90). Also, for the diagnosis of acute and chronic schistosomiasis, rSjLAP had positive rates of 97.4% (37/38) and 87.5% (84/96), rSjFBPA had 94.7% (36/38) and 88.5% (85/96) and that of both antigen assays was 94.7% (36/38) and 85.4% (82/96), respectively. Statistical analysis showed no significant difference in the positive rate (P>0.05). The coincidence rate for acute schistosomiasis was 86.8% and that of chronic schistosomiasis was 63.5%. Correlation analysis of both acute and chronic schistosomiasis was found to be significant (P<0.05). Moreover, the re-

Table 1 The value of the serum antibody response of mice to rSjLAP ($\bar{x}\pm s$)

Day post infection	No. tested	Absorbance/A_{450} value			
		Group A	Group B	Group C	Group D
1	25	-	-	-	0.156±0.08
3	3	0.227±0.023	0.249±0.011	0.231±0.011	-
7	3	0.269±0.019	0.284±0.004	0.272±0.010	-
10	3	0.415±0.038[a]	0.535±0.053[a]	0.548±0.060[a]	-
14	3	0.811±0.084[a]	1.111±0.141[ab]	0.998±0.055[ac]	-
20	3	1.362±0.145[a]	1.889±0.086[abd]	1.741±0.070[ac]	-
30	3	2.491±0.254[ae]	2.048±0.057[a]	1.940±0.056[a]	-
60	3	2.380±0.194[ae]	1.679±0.236[a]	1.769±0.157[a]	-

Note: vs group D, a P<0.05; vs group A, b P<0.05; vs group A, c P<0.05; vs group C, d P<0.05; vs groups B and C, e P<0.05.

Table 2 The value of the serum antibody response of mice to rSjFBPA ($\bar{x}\pm s$)

Day post infection	No. tested	Absorbance/A_{450} value			
		Group A	Group B	Group C	Group D
1	25	-	-	-	0.150±0.076
3	3	0.184±0.011	0.200±0.026	0.223±0.012	-
7	3	0.211±0.027	0.221±0.022	0.245±0.002	-
10	3	0.455±0.056[a]	0.595±0.033[ab]	0.608±0.063[ac]	-
14	3	0.676±0.063[a]	0.912±0.066[ab]	1.037±0.180[ac]	-
20	3	1.211±0.249[a]	1.579±0.041[ab]	1.697±0.046[ac]	-
30	3	2.089±0.223[ae]	1.487±0.333[a]	1.810±0.124[ad]	-
60	3	1.943±0.078[ae]	1.569±0.119[a]	1.726±0.223[a]	-

Note: vs group D, a P<0.05; vs group A, b P<0.05; vs group A, c P<0.05; vs group B, d P<0.05; vs groups B and C, e P<0.05.

Table 3 The value of the serum antibody response of mice to rSjLAP and rSjFBPA assay ($\bar{x}\pm s$)

Day post infection	No. tested	Absorbance/A_{450} value			
		Group A	Group B	Group C	Group D
1	25	-	-	-	0.146±0.075
3	3	0.174±0.031	0.181±0.024	0.192±0.036	-
7	3	0.196±0.019	0.201±0.013	0.211±0.025	-
10	3	0.498±0.077[a]	0.696±0.104[ab]	0.621±0.090[a]	-
14	3	0.738±0.109[a]	1.201±0.166[abd]	1.052±0.098[ac]	-
20	3	1.413±0.342[a]	2.258±0.394[abd]	1.906±0.235[ac]	-
30	3	2.578±0.275[ae]	2.354±0.347[ad]	2.090±0.219[a]	-
60	3	2.463±0.412[af]	2.119±0.345[a]	1.969±0.347[a]	-

Note: a vs group D, a P<0.05; vs group A, b P<0.05; vs group A, c P<0.05; vs group C, d P<0.05; vs group C, e P<0.05; vs groups B and C, f P<0.05.

sponses of acute and chronic schistosomiasis to ELISA assays were significantly different (Acute 0.896±0.399, chronic 0.582±0.265 for rSjLAP, acute 1.281±0.379, chronic 0.718 ± 0.354 for rSjFBPA, acute 1.709 ±0.394, chronic 0.833 ±0.432 for both antigen assay) (P<0.05). There was a general decrease in the antibody titer of the patients after treatment. In 12 months after treatment it was 0.236±0.212 with rSjLAP, 0.287 ±0.191 with rSjFBPA, 0.235 ±0.120 with both antigens respectively for acute cases. For chronic patients, it was 0.266±0.124, 0.261±0.143 and 0.265±0.140 in 12 months pos-treatment, and 0.204±0.074, 0.176±0.074 and 0.176±0.073 in 2 years, respectively. For healthy control, it was 0.188 ±0.056, 0.173 ±0.450 and 0.184±0.051, respectively. The antibody titer showed no significant difference between the acute or chronic cases after treatment and the control (P>0.05, Table 5). Statistical analysis indicated no significant difference in the sensitivity among the recombinant antigen assays for assessing theroapeutic efficacy (P>0.05).

4 Cross reaction

Sera form C. sinensis infection showed a cross reaction of 15.2% (5/33) for rSjLAP, 12.1% (4/33) for rSjFBPA and 9.2% (3/33) for the combined antigens; P. westermani had a cross reaction of 15.0% (6/40),

Table 4 ELISA result for schistosomiasis japonica in human

Serum sample	No. tested	rSjLAP		rSjFBPA		rSjLAP and rSjFBPA assay	
		No. positive	Positive rate/%	No. positive	Positive rate/%	No. positive	Positive rate/%
Acute cases	38	37	97.4 (37/38)	36	94.7 (36/38)	36	94.7 (36/38)
Acute cases 12 months after treatment	36	7	19.4 (7/36)	10	27.8 (10/36)	5	13.8 (5/36)
Chronic cases	96	84	87.5 (84/96)	85	88.5 (85/96)	82	85.4 (82/96)
Chronic within 12 months after treatment	36	12	33.3 (12/36)	11	30.5 (11/36)	13	36.1 (13/36)
Chronic cases in 2 years after treatment	64	4	6.3 (4/64)	5	7.8 (5/64)	4	6.3 (4/64)
Healthy control	90	3	3.3 (3/90)	2	2.2 (2/90)	3	3.3 (3/90)

Table 5 The value of the serum samples of schistosomiasis japonica in human($\bar{x}\pm s$)

Serum sample	No. tested	Absorbance/A_{450} value		
		rSjLAP	rSjFBPA	rSjLAP and rSFBPA assay
Acute cases	38	0.896±0.399[a]	1.281±0.379[abd]	1.079±0.394[ac]
Acute cases 12 months after treatment	36	0.236±0.212	0.287±0.191	0.235±0.120
Chronic cases	96	0.582±0.265[a]	0.718±0.354[ab]	0.833±0.432[ace]
Chronic cases 12 months after treatment	36	0.266±0.124	0.261±0.143	0.265±0.140
Chronic cases in 2 years after treatment	64	0.204±0.074	0.176±0.074	0.176±0.073
Healthy control	90	0.188±0.056	0.173±0.045	0.184±0.051

Note: vs control, a $P<0.05$; vs AS or CS tested by rSjLAP only, b $P<0.05$; vs AS or CS tested by rSjLAP only, c $P<0.05$; vs AS tested by rSjLAP and rSjFBPA assay, d $P<0.05$; vs CS tested by rSjFBPA only, e $P<0.05$.

Table 6 ELISA result for other parasite infections

Serum sample	No. tested	Cross reactivity rate/%		
		rSjLAP	rSjFBPA	rSjLAP and rSjFBPA assay
C. sinensis	33	15.2 (3/33)[a]	12.1 (4/33)[a]	9.2 (3/33)[a]
P. westermani	40	15.0 (6/40)[a]	12.5 (5/40)[a]	15.0 (6/40)[a]
Hookworms	37	8.1 (3/37)[a]	8.1 (3/37)[a]	8.1 (3/37)[a]
Healthy control	90	3.3 (3/90)	2.2 (2/90)	3.3 (3/90)

Note: vs the healthy control, a $P<0.05$.

12.5% (5/40) and 15.0% (6/40), respectively; and hookworm infection had a cross reaction of 8.1% (3/37) for the 3 assays (Table 6).

DISCUSSION

Schistosome infection, either in man or domestic animals, elicits strong humoral response. Purified recombinant FBPA and LAP were reported to have high sensitivity and specificity and perform better especially in patients with low intensities of infections. Also, they have been reported to provide information of the duration of infection, protective immunity and evaluation of treatment. FBPA is an enzyme which catalyzes the reversible cleavage of fructose 1, 6-phosphate to form dihydroxyacetone phosphate and glyceraldehydes-3-phosphate in the glycolytic metabolic pathway during gluconeogenesis[15]. Hence, it is needed for energy production. FBPA has been detected in serum samples from hosts with Candida albicans[16], Plasmodium falciparum[17], Onchocerca volvulus[18] and Schistosoma mansoni[19]. Also, recombinant FBPA has been observed to provide significant protection in experimental animals infected with S mansoni[20]. It has been observed to interact with SHP-1 which contributed to macrophage dysfunction in Leishmania donovani[21].

On the other hand, LAP is made of two members of the M1 and M17 peptidase families, which cleaves N-terminal residues from proteins and peptides. It is involved in cell maintenance, growth, development and defence[22,23]. LAP has been identified in all the developmental stages of S. mansoni[24]. It has also been reported to provide protection against Fasciola hepatica[25] and Cryptosporidium parvum infections[26].

Our study has revealed that rSjFBPA and SjLAP were recognized by the sera of the experimentally

infected mice with different intensities of S. japonicum infection and patients with schistosomiasis japonica. The results revealed that, IgG antibody responses to rSjLAP, rSjFBPA or the combined antigens in the infected mice first occurred and detected on day 10 post infection regardless of the intensity of infection. However, early response was clearly associated with the intensity of infection, as determined by all the assays (rSjLAP, rSFBPA, or rSjLAP and rSjFBPA). Also, antibody responses after reaching their peak were relatively stable and this was similar for all the antigens tested in the infected groups. Significantly higher response was found in light infection compared with those with heavy infection. Moreover, for the negative control group from which cut off values were determined, two false positive were detected by the assays yielding a specificity of 92%. On the other hand, Peng et al [27] reported a specificity of 100% in water buffaloes in the diagnosis of S. japonicum infection.

In order to further evaluate these assays under clinical conditions, 270 patients with schistosomiais and 90 healthy donors from schistosomiais-free areas were tested. All the assays revealed high positive rates for both acute and chronic patients. Also, coincidence rates of both acute and chronic schistosomiasis by the assays were relatively high but correlation analysis indicated no complementary value in diagnosis. Also, the absorbance titer of the antibodies against the antigens declined within 12 months and more than two years after treatment with praziquantel. These results were in consistent with those reported previously by Zhong et al[10]. The reaction of rSjLAP and or rSjFBPA in this study was rapid. Only two false positives were found in the normal control groups when tested by rSjFBPA and three false positive when tested by both rSjLAP and the combined antigens. However, our results revealed that all the assays showed certain cross reactivity with C. sinensis, P. westermani or hookworm infection.

IgG antibody response in experimentally infected mice may be detected as early as day 10 post-infection by the assays. Hence these assays may be used in the diagnosis of both light and heavy S. japonicum infections. However, further study is needed on the histopathological change in relation to the immunological response of the infected hosts by using the recombinant protein-based ELISA. Also, the assays could be used to evaluate the therapeutic efficacy in schistosomiasis treatment. Based on their positive rate and specificity, rSjLAP and rSjFBPA are potential candidates for the diagnosis of S. japonicum infection. However, further research is needed for better reagents and assays to avoid existing shortcomings such as the cross reactivity with other helminthes.

References

[1] WHO. The control of schistosomiasis: second report of the WHO Expert Committee[R]. World Health Organ Tech Rep Ser, 1993, 830: 1-86.

[2] Ross AG, Sleigh AC, Li Y, et al. Schistosomiasis in the People's Republic of China: prospects and challenges for the 21st century[J]. Clin Microbiol Rev, 2001, 14(2): 270-295.

[3] WHO. Schistosomiasis: Number of people treated in 2008[J]. Week Epidemiol Rec, 2010, 85 (18): 157-164.

[4] Manzella A, Ohtomo K, Monzawa S, et al. Schistosomiasis of the liver[J]. Abdom Imaging, 2008, 33(2): 144-150.

[5] Gryseels B, Polman K, Clerinx J, et al. Human schistosomiasis [J]. Lancet, 2006, 368(9541): 1106-1118.

[6] Berhe N, Medhin G, Erko B, et al. Variations in helminth faecal egg counts in Kato-Katz thick smears and their implications in assessing infection status with Schistosoma mansoni [J]. Acta Trop, 2004, 92(3): 205-212.

[7] Engels D, Chitsulo L, Montresor A, et al. The global epidemiological situation of schistosomiasis and new approaches to control and research[J]. Acta Trop, 2002, 82(2): 139-146.

[8] Steinmann P, Keiser J, Bos R, et al. Schistosomiasis and water resources development: systematic review, meta-analysis, and estimates of people at risk[J]. Lancet Infect Dis, 2006, 6 (7): 411-425.

[9] Salem N, Balkman JD, Wang J, et al. In vivo imaging of schistosomes to assess disease burden using positron emission tomography (PET)[J]. PLoS Negl Trop Dis, 2010, 4(9): e827.

[10] Zhong ZR, Zhou HB, Li XY, et al. Serological proteome-oriented screening and application of antigens for the diagnosis of schistosomiasis japonica[J]. Acta Trop, 2010, 116(1): 1-8.

[11] Jin YM, Lu K, Zhou WF, et al. Comparison of recombinant proteins from Schistosoma japonicum for schistosomiasis diagnosis [J]. Clin Vaccine Immunol, 2010, 17(3): 476-480.

[12] Cheng PC, Tsaihong JC, Lee KM. Application of recombinant Sjc26GST for serodiagnosis of Schistosoma japonicum infection in water buffalo (Bos buffelus)[J]. Vet Parasitol, 2007, 150 (4): 314-320.

[13] Zhu SC, Zhao ZR, Lei L, et al. Immunoscreening of schistosomulum cDNA library of Schistosoma japonicum and preliminary identification of membrane-associated protein abundant in tegument [J]. Chin J Parasitol Parasit Dis, 2006, 24(2): 106-110. (in Chinese)
(朱绍春, 赵志荣, 雷黎, 等. 日本血吸虫童虫文库免疫筛选及其高丰度表达膜蛋白初步鉴定[J]. 中国寄生虫学与寄生虫病杂志, 2006, 24(2): 106-110.)

[14] Galen RS. Predictive value and efficiency of laboratory testing [J]. Pediatr Clin North Am, 1980, 27(4): 861-869.

[15] Patron NJ, Rogers MB, Keeling PJ. Gene replacement of fructose-1,6-bisphosphate aldolase supports the hypothesis of a single photosynthetic ancestor of chromalveolates[J]. Eukaryot Cell, 2004, 3(5): 1169-1175.
[16] Pitarch A, Diez-Orejas R, Molero G, et al. Analysis of the serologic response to systemic Candida albicans infection in a murine model[J]. Proteomics, 2001, 1(4): 550-559.
[17] Srivastava IK, Schmidt M, Certa U, et al. Specificity and inhibitory activity of antibodies to Plasmodium falciparum aldolase [J]. J Immunol, 1990, 144(4): 1497-1503.
[18] McCarthy JS, Wieseman M, Tropea J, et al. Onchocerca volvulus glycolytic enzyme fructose-1,6-bisphosphate aldolase as a target for a protective immune response in humans[J]. Infect Immun, 2002, 70(2): 851-858.
[19] Harrop R, Coulson PS, Wilson RA. Characterization, cloning and immunogenicity of antigens released by lung-stage larvae of Schistosoma mansoni[J]. Parasitology, 1999, 118(Pt 6): 583-594.
[20] Marques HH, Zouain CS, Torres CB, et al. Protective effect and granuloma down-modulation promoted by RP44 antigen a fructose-1, 6-bisphosphate aldolase of Schistosoma mansoni[J]. Immunobiology, 2008, 213(5): 437-446.
[21] Nandan D, Tran T, Trinh E, et al. Identification of Leishmania fructose-1, 6-bisphosphate aldolase as a novel activator of host macrophage Src homology 2 domain containing protein tyrosine phosphatase SHP-1[J]. Biochem Biophys Res Commun, 2007, 364(3): 601-607.
[22] Matsui M, Fowler JH, Walling LL. Leucine aminopeptidases: diversity in structure and function[J]. Biol Chem, 2006, 387(12): 1535-1544.
[23] Rinaldi G, Morales ME, Alrefaei YN, et al. RNA interference targeting leucine aminopeptidase blocks hatching of Schistosoma mansoni eggs [J]. Mol Biochem Parasitol, 2009, 167(2): 118-126.
[24] Auriault C, Pierce R, Cesari IM, et al. Neutral protease activities at different developmental stages of Schistosoma mansoni in mammalian hosts [J]. Comp Biochem Physiol, 1982, 72(3): 377-384.
[25] Acosta D, Cancela M, piaenza L, et al. Fasciola hepatica leucine aminopeptidase, a promising candidate for vaccination against ruminant fasciolosis[J]. Mol Biochem Parasitol, 2008, 158(1): 52-64.
[26] Akili D, Heidari M, Welter LM, et al. Characterization of a factor from bovine intestine that protects against Cryptosporidium parvum infection[J]. Vet Parasitol, 2006, 142 (1-2): 168-172.
[27] Peng SY, Lee KM, Tsaihong JC, et al. Evaluation of recombinant fructose-1, 6-bisphosphate aldolase ELISA test for the diagnosis of Schistosoma japonicum in water buffaloes[J]. Res Vet Sci, 2008, 85(3): 527-533.
(Received: 2011-05-26 Editors: YU Sen-hai, ZHANG Zheng-yan)

文章编号:1000-7423(2011)-05-0347-02

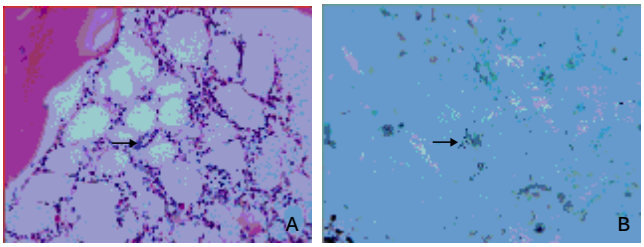
【病例报告】

肝穿刺活检诊断内脏利什曼病 2 例

刘真真，吕晓菊

中图分类号：R531.6 文献标识码：D

病例 1，患者，男，30 岁，四川省巴中市人，长期居住于四川省南充市南部县。患者因高热(39.5 ℃)、畏寒、头痛和腹泻等症状，于 2005 年 8 月 16 日入住四川大学华西医院。查体：心、肺未见异常，肝下缘剑突下 10 cm、肋下 6 cm 可扪及，质中，脾于肋下 8 cm 可扪及，质中。乙肝、丙肝和 HIV 标志物均为阴性，实验室检查白细胞 1.80×10⁹/L，中性粒细胞 72%，嗜酸粒细胞 3.5%，血红蛋白 92 g/L，血小板 57×10⁹/L，总胆红素 14.3 μmol/L，谷丙氨酸转氨酶 67 IU/L，谷草转氨酶 86 IU/L，血清白蛋白 29.3 g/L，球蛋白 29.1 g/L。结核抗体阴性。疟原虫厚血膜涂片阴性。胸部 CT 未见异常，腹部增强 CT 示肝大，巨脾，骨髓涂片未见异常。骨髓活检示骨髓造血组织增生尚活跃，以粒细胞系统为甚，六胺银和过碘酸雪夫(PAS)染色未查见病原微生物。RK39 免疫层析试纸条检测利什曼病(-)，结核菌素（PPD）皮试(-)，多次血培养结核菌均阴性。给予左氧氟沙星 0.4 g 静脉滴注，每天 1 次，共 14 d，头孢哌酮/舒巴坦 2.0 g 静脉滴注，每天 3 次，共 8 d。治疗期间患者体温无下降，外周血白细胞(1.46～2.77)×10⁹/L，嗜酸粒细胞(3.5～5.5)%，血红蛋白(85～101) g/L，血小板(67～114)×10⁹/L，谷丙氨酸转氨酶(97～257) IU/L，谷草转氨酶(88～175) IU/L。骨髓穿刺涂片 2 次均提示查见少量淋巴样、浆细胞样组织细胞，其中 1 次见 1 个比较典型异常组织细胞，骨髓活检示个别核仁明显异常的细胞，骨髓组织增生偏低下，疑为恶性组织细胞病（图 1A）。再行肝穿刺活检示 1～2 小灶区肝细胞和窦壁细胞中见可疑病原菌（疑似利什曼原虫），HE 染色和银染色不能确定病原体（图 1B）。再次行 RK39 免疫层析试纸条检测利什曼病“可疑阳性”。遂给予葡萄糖酸锑钠(每疗程总量 9 g，8 d 为 1 疗程)诊断性治疗2 疗程，患者体温降至 38 ℃左右，但停药后再次上升，给予两性霉素 B 40 mg 静脉滴注，每天 1 次，使用第 12 天患者体温完全正常至出院。随访半年无复发。



A：骨髓涂片(HE 染色，×400)；**B：肝穿刺活检**(六胺银染色，×1000)。

图 1 患者活检图

作者单位：四川大学华西医院感染性疾病中心，成都 610041

(下转第 352 页)