



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

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

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摘要

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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 (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 (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

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 (A450) on this day were

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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 rSjFBPA and 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.

Keywords: *Schistosomiasis japonica* Immunodiagnosis Leucine aminopeptidase Fructose-1, 6-bisphosphate aldolase

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