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中国寄生虫学与寄生虫病杂志 » 2011, Vol. 29 » Issue (2) :81-86 DOI:

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日本血吸虫金属蛋白酶基因的克隆和表达及其对小鼠的免疫保护性研究

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Cloning and Expression of Metalloprotease Gene from *Schistosoma japonicum* and its Immunoprotective Efficiency

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摘要

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摘要 目的 克隆和表达日本血吸虫金属蛋白酶(metalloprotease)编码基因并纯化表达产物,观察其在成虫体内定位及在小鼠的免疫试验。方法 根据表达序列标签(EST)测序的结果设计引物,从含有金属蛋白酶基因的日本血吸虫cDNA克隆(SJM2CFB04,简称SjB04)中扩增得到该编码基因片段,亚克隆至原核表达载体pET28a中表达,应用组氨酸标签亲和层析法纯化表达产物,蛋白质印迹(Western blotting)分析其免疫原性。应用间接免疫荧光法,观察金属蛋白酶在血吸虫成虫体内的分布。将18只C57BC/6小鼠随机分成两组,实验组用SjB04重组蛋白(25 μg/只)免疫小鼠,共3次,每次间隔2周;对照组用佐剂免疫(50 μl/只)。末次免疫后2周,每鼠腹部感染40±2条日本血吸虫尾蚴,攻击后第37天起连续收集6 d小鼠粪便,计算每克粪虫卵数和减卵率;第42天剖杀小鼠,门静脉灌注收集成虫并计算减虫率。结果 获得了SjB04基因(ORF 528 bp)的编码序列,构建了原核表达载体,并在大肠埃希菌中表达,经组氨酸标签亲和层析法获得纯化重组蛋白SjB04。免疫荧光法检测结果显示,SjB04主要定位于日本血吸虫成虫的肠管表皮。Western blotting分析结果显示,该纯化重组蛋白SjB04能被感染兔血清和免疫兔血清识别,在相对分子质量(M_r) 29 800处出现一清晰条带。用该纯化蛋白免疫C57BC/6小鼠后,减虫率为27.1%,粪减卵率为57.8%。结论 克隆获得的日本血吸虫SjB04重组蛋白主要定位于日本血吸虫成虫的肠管表皮,该蛋白可明显减少感染日本血吸虫的C57BC/6小鼠的粪卵排出量。

关键词: 日本血吸虫 金属蛋白酶 免疫保护力

Abstract: Objective To clone and express a metalloprotease gene of *Schistosoma japonicum*, purify the expressed protein, and investigate the induced immune response in mice and its localization in the parasite. Methods Specific primers were designed according to the EST sequence and used for amplification of the encoding sequence from the *S. japonicum* cDNA clone containing *S. japonicum* metalloprotease. The gene was subcloned into pET-28a plasmid and expressed, and the recombinant protein was purified with HisoTag affinity chromatography. Western blotting was used to analyze the immunogenicity. Eighteen C57BC/6 mice were divided into two groups. Mice in group A were immunized each with 25 μg purified recombinant SjB04 at every 2 weeks for 3 times. Mice in group B received only adjuvant as control. Each mouse was challenged by (40±2) cercariae at the third week after the last immunization. Fecal samples were collected for 6 days from 37th days after challenge. Eggs per gram feces and rate of egg reduction were calculated. *S. japonicum* adult worms were collected from infected mice, and used for preparing frozen sections and indirect immunofluorescence staining with specific polyclone antibody to *S. japonicum* metalloprotease. Results The metalloprotease gene SjB04 was cloned, sequenced and expressed. The immunofluorescence localization showed that SjB04 protein distributed mainly in the intestinal epithelium of the adult worm. The recombinant protein was specifically recognized by the *S. japonicum*-infected rabbit sera, showing that the expressed product possessed antigenicity. Mice immunized with the recombinant protein revealed a reduction in number of adult worms, eggs in feces by 27.1% and 57.8%, respectively. Conclusion The recombinant protein of *S. japonicum* metalloprotease has been obtained with M_r 36 500. The protein locates in the intestinal epithelium of adult worm. Immunization with the SjB04 protein induces significant reduction of fecal eggs.

Keywords: *Schistosoma japonicum* Metalloprotease Immune protection

引用本文:

徐斌1, 鞠川1,3, 卢艳2, 莫筱谨1, 冯正1, 许学年1, 胡薇1*. 日本血吸虫金属蛋白酶基因的克隆和表达及其对小鼠的免疫保护性研究[J] 中国寄生虫学与寄生虫病杂志, 2011, V29(2): 81-86

XU Bin1, JU Chuan1,3, LEI Yan2, MO Xiao-Jin1, FENG Zheng1, HU Hua-Nian1, HU Wei1*. Cloning and Expression of Metalloprotease Gene from *Schistosoma japonicum* and its Immunoprotective Efficiency[J], 2011, V29(2):81-86

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