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论著

# 大鼠感染血清免疫筛选弓形虫速殖子cDNA文库

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摘要

目的为弓形虫病疫苗的研制提供新的抗原分子。方法用弓形虫RH株速殖子感染大鼠,分离其血清作为探针筛选弓形虫cDNA文库,对阳性克隆的插入片段分别进行PCR扩增及DNA序列测定。结果从cDNA文库4×105~5个噬菌斑中筛选出13个阳性克隆,其插入片段大小分别为0.45～2.4kb。对L1、L2、L4和L5四个克隆进行测序,将所得序列查询基因库,结果,克隆L2与弓形虫P24主要抗原基因序列相同,L4与蔗糖丙酮酸磷酸激酶具有同源性,L1无任何相匹配的序列,为未曾报告过的新基因(GenBank登录号为AY180109),命名为T.g-R1。T.g-R1编码134个氨基酸的非跨膜蛋白。PROSCAN分析显示T.g-R1含有2个蛋白激酶C磷酸化位点,2个酪蛋白激酶Ⅱ磷酸化位点,1个肉豆酸酰化位点,1个微体细胞C端靶信号。L5为一小片段,无完整编码读框。结论阳性克隆的筛选和鉴定为抗弓形虫病疫苗的研制提供又一途径。

关键词 弓形虫 cDNA文库 免疫筛选 大鼠

分类号



# Immunological Screening of Toxoplasma Tachyzoite cDNA Expression Libraries with Serum from Infected Rats

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Abstract

Objective To screen and identify the potential candidates for the development of toxoplasmosis vaccine. Methods Rats were infected with Taxoplasma gondii ( T. gortdii) RH strain and their sera were used as a probe to screen T. gondii tachyzoite cDNA expression libraries. The positive clones were analyzed by PCR amplification and DNA sequencing. Results Thirteen positive clones were obtained from about 4 × 105 phage plagues after three rounds of screening. The size of the inserts ranged from 0.45 kb to 2.4 kb. A BLAST search of all available sequence databases using the partial sequences from four positive clones (LI, L2, L4, L5) showed that the-sequence of L2 clone was identical with T. gondii P24 major antigen gene (TgP24). Clone L4 had a high homology with Saccharum officinarum pyruvate orthophosphate dikinase. There is no significant hit of any sequences to clone L1, suggesting that L1 could be a novel gene(GenBank accession number AY180109), named T. g-R1, which encodes a non-transmembrane protein with 134 amino acid open reading frame. PROSCAN analysis of the T. g-R1 amino acid sequence showed that this gene product contains two protein kinase C phosphorylation site, two casein kinase Ⅱ phosphorylation site, one N-myristoylation site and one microbodies C-terminal targeting signal. Clone L5 was a small partial fragment. Conclusion The identification of positive clones provides a possible way for the development of toxoplasmosis vaccine.

Key words Toxoplasma gondii cDNA library immunoscreening rat

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