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论著

犬钩虫特异性抗原基因的克隆与表达

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摘要

目的 寻找可诱发宿主保护性免疫力的犬钩虫特异性抗原。 方法 用犬钩蚴免疫获得保护性免疫力的家犬血清,免疫筛选犬钩虫期幼虫λZAPcDNA文库,阳性克隆基因经测序,在质粒PUC18、PET28中进行一系列亚克隆,重组pET28C质粒诱导表达并对表达产物进行SDS-PAGE和Westernblotting分析。 结果 从cDNA文库中筛获5个相同的阳性克隆,携带的犬钩虫抗原(AcAg)外源基因片段在原核体系(pET28C)中表达分子量为43kDa的融合蛋白。Westernblotting分析该重组蛋白可与筛库的犬血清反应。 结论 AcAg为新的犬钩虫特异性抗原,其基因与美丽纤杆线虫(Caenorhabditiselegans)基因unc-89同源性为35%。该抗原诱发宿主保护性免疫力及作为疫苗的潜力值得进一步研究。

关键词 犬钩虫抗原 免疫筛选 克隆表达

分类号

Cloning and Expression of Specific Antigen Genes of Ancylostoma caninum

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Abstract

Objective To search for the gene encoding specific antigen of Ancylostoma caninum that induces host's protective immunity. Methods A lambda ZAPⅡ cDNA library of A.canium was screened with sera from dogs immunized subcutaneously with hookworm larvae(L3). After sequencing, insert gene (AcAg) from positive clones was ligated into PUC18 and PET28C. Recombinant pET28C plasmid was induced to expressed protein in the E.coli BL21. The characteristic of recombinant protein is analyzed by SDS-PAGE and Western bloting assay. Results Five positive clones were obtained, and proved to be the same. The insert gene (AcAg) in pET28C vector expressed a recombinant protein of about 43 kDa. Using Western bloting assay, this protein was recognized by the sera from dog immunized with Ancylostoma caninum third stage infected larvae and used for screening library. Conclusion The AcAg, which exhibits 35% homologous to Caenorhabditis elegans gene unc-89, is a novel specific antigen of A.caninum. Its ability to elicit the protective immunity and the probability of the recombinant protein as a vaccine need to be further evaluated.

Key words hookworm antigen cloning and expression

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