论著

细粒棘球绦虫Eg95抗原基因疫苗体外瞬时表达及对小鼠诱导的免疫应答

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目的 检测细粒棘球绦虫Eg95抗原基因疫苗(pcDNA3 Eg95)体外瞬时表达,探讨其诱导小鼠的体液和细胞免疫效果。 方法 pcDNA3 Eg95经脂质体转染HeLa细胞瞬时表达。逆转录聚合酶链反应 (RTPCR)检测Eg95抗原信使RNA(mRNA)在HeLa细胞中的表达,酶联免疫吸附测定(ELISA)和蛋白质印迹法(Westernblot ting)检测Eg95蛋白的瞬时表达情况。pcDNA3 Eg95基因疫苗肌肉注射免疫 BALB/c小鼠,ELISA检测IgG和IgG2a水平,四甲基偶氮唑盐试验(MTT法)检测免疫小鼠T淋巴细胞增殖反应。 结果 RTPCR检测结果显示,pcD NA3 Eg95瞬时表达组有Eg95抗原基因mRNA表达,ELISA和 Westernblotting检测结果表明,可在HeLa细胞中特异性表达Eg95蛋白。用pcDNA3 Eg95基因疫苗免疫BALB/c小鼠,第3周出现特异性IgG免疫应答,持续升高至第10周,显著高于对照组。从第2周开始,小鼠血清IgG2a应答即为阳性,且长时间(至第10周)维持较高水平,与pcD NA3空质粒组比较,其差异具有非常显著性意义(P<0.01)。用原核表达的Eg95重组蛋白刺激免疫小鼠脾细胞,有明显的T细胞增殖反应。pcDNA3 Eg95基因疫苗免疫组刺激指数明显高于pcDNA3空质粒组(P<0.01)。结论 pcDNA3 Eg95基因疫苗可诱发小鼠产生特异的体液免疫和细

关键词 细粒棘球绦虫 抗原 基因疫苗 基因表达 免疫应答

分类号

Transient Expression of Echinococcus granulosus Eg95 DNA Vaccine and Induction of Immune Response in Mice

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Ahstract

Objective To detect the in vitro expression of pcDNA3 Eg95 and to observe the immunological effect of the Eg95 DNA vaccine in mice. Methods The eukaryotic recombinant plasmid pcDNA3 Eg95 was transfected into HeLa cells with liposome mediated method. RT PCR, ELISA and Western blotting were used to analyze the expression of Eq95 mRNA and Eq95 protein, respectively. The BALB/c mice were immunized by pcDNA3 Eg95. Anti Eg95 IgG and IgG2a in murine serum were determined by ELISA. The proliferation activity of spleen T lymphocytes was tested using MTT assay. Results Using RT | PCR method, the expression of Eg95 mRNA was confirmed in vitro. The results of ELISA and Western blotting showed that there was a specific Eg95 protein, which can be specifically recognized by anti sera of Eg95 prokaryotic expressing protein in pcDNA3 Eg95 transfected HeLa cell lysis. The specific IgG was induced during the 3rd week and continued to increase until week 10. IgG2a was detected after 2 weeks and maintained a higher level till week 10. There was a significant difference of IgG2a level between pcDNA3 Eg95 immunized group and pcDNA3 control(P<0.01). In spleen T cell proliferation response, the stimulation index (SI) in pcDNA3 Eg95 group was higher than that of vector control(P<0.01). Conclusion Eg95 DNA vaccine can induce significant cellular and humoral immune response in mice. Key words Echinococcus granulosus Antigens DNA vaccine Gene expression Immune response

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