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杜氏利什曼原虫无鞭毛体蛋白基因重组质粒的免疫原性研究

李金福1，陈建平2 *，田玉2，杨志伟2，马莹2，胡孝素2

1 贵阳医学院寄生虫学教研室，贵阳 550004；2 四川大学华西基础医学与法医学院寄生虫学教研室，成都 610041

Immunogenicity of the Recombinant Plasmid of *Leishmania donovani* Amastin Gene

LI Jin-fu1，CHEN Jian-ping2 *，TIAN Yu2，YANG Zhi-wei2，MA Ying2，HU Xiao-su2

1 Department of Parasitology，Guiyang Medical College，Guiyang 550004，China；2 Department of Parasitology，School of Preclinical and Forensic Medicine，Sichuan University，Chengdu 610041，China

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摘要 目的 研究杜氏利什曼原虫无鞭毛体蛋白基因重组质粒pcDNA3.1-amastin的免疫原性。 方法 将18只雌性BALB/c小鼠随机分为实验组和对照组，每组9只。两组分别肌肉注射重组质粒pcDNA3.1-amastin和空质粒pcDNA3.1（+） （50 μg/只），2周后同法加强免疫1次。加强免疫后第7、14和21天每组各取小鼠3只，内眦采血，分离血清，间接ELISA法测定血清中抗原特异性抗体水平。随后脱颈处死小鼠，无菌取脾，分离脾细胞，用刀豆球蛋白A刺激培养，3-（4，5-二甲基噻唑-2）-2，5-二苯基四氮唑溴盐（MTT）法检测脾淋巴细胞增殖活性和细胞毒性T淋巴细胞（CTL）杀伤活性。双抗体夹心ELISA法检测脾淋巴细胞培养上清中γ干扰素（IFN-γ）、白细胞介素-2（IL-2）和IL-4的水平。 结果 加强免疫后第7、14和21天，实验组均检测到特异性IgG抗体，效价在1 ： 640以上，而对照组未检测到IgG抗体（$P<0.01$）；实验组脾淋巴细胞增殖活性刺激指数分别为4.28±0.51、5.01±0.60和4.39±0.50，均高于对照组（$P<0.01$）；实验组脾淋巴细胞培养上清中IFN-γ含量分别为（42.06±4.26）、（66.02±6.02）和（58.29±3.75） pg/ml，IL-2含量分别为（38.21±5.11）、（64.79±8.67）和（52.69±7.15） pg/ml，均高于对照组（$P<0.01$），两组均未检测到IL-4；实验组脾淋巴细胞CTL杀伤活性分别为（42.20±5.96）%、（63.66±5.44）%和（52.24±4.56）%，均高于对照组（$P<0.01$）。 结论 杜氏利什曼原虫无鞭毛体蛋白基因重组质粒pcDNA3.1-amastin免疫小鼠后可诱导其产生特异的体液免疫应答和Th1型细胞免疫应答。

关键词： 杜氏利什曼原虫 无鞭毛体蛋白 基因疫苗 免疫原性

Abstract： Objective To investigate the immunogenicity of recombinant plasmid pcDNA3.1-amastin with *Leishmania donovani* amastin gene. Methods Eighteen female BALB/c mice were randomly divided into experimental group and control group. Mice in experimental group and control group were intramuscularly injected with 50 μg recombinant plasmid pcDNA3.1-amastin and blank plasmid vector pcDNA3.1（+）， respectively, and then received equivalent dose of plamid after 2 weeks. On days 7, 14, and 21 after the second immunization， serum samples were collected from 3 mice each group. The mice were then sacrificed, spleens were removed and splenocytes were collected. Serum antibody level was determined by indirect ELISA. Splenocyte proliferation responses and cytotoxicity of spleen-derived lymphocytes were analyzed by MTT colorimetry after stimulation with ConA. Level of IFN-γ， IL-2 and IL-4 in the splenocyte culture supernatants was determined by double antibody sandwich ELISA. Results On days 7, 14, and 21 after the second immunization， specific IgG antibody （more than 1 ： 640） was found in experimental group, but not in the control（$P<0.01$）； stimulation index （SI） of spleen cells in experimental group （4.28±0.51, 5.01±0.60, and 4.39±0.50） was higher than that of control group （$P<0.01$）; the level of IFN-γ［（42.06±4.26），（66.02±6.02），and （58.29±3.75） pg/ml］ and IL-2 ［（38.21±5.11），（64.79±8.67），and （52.69±7.15） pg/ml］ in splenocyte culture supernatants of experimental group was higher than that of control group （$P<0.01$）; IL-4 was not found in the two groups; cytotoxicity of spleen-derived lymphocytes in experimental group ［（42.20±5.96）%，（63.66±5.44）%，and （52.24±4.56）%］ was stronger than that of control （$P<0.01$）. Conclusion The recombinant plasmid pcDNA3.1-amastin can induce specific humoral and Th1 type cellular immune responses in mice.

Keywords： *Leishmania donovani* Amastin DNA vaccine Immunogenicity

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