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弓形虫MIC8基因与基因佐剂IL-12共免疫小鼠的保护性研究

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Evaluation on the Immune Response Induced by DNA Vaccine Encoding MIC8 Co-Immunized with IL-12 Genetic Adjuvant against Toxoplasma gondii Infection

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摘要 目的 观察弓形虫MIC8基因和基因免疫佐剂白细胞介素（IL-12）共免疫小鼠所诱导的免疫保护性。方法 将编码弓形虫MIC8的基因插入到真核表达质粒中pc DNA3.1中，构建真核表达质粒pcMIC8，并转染至HeLa细胞，蛋白免疫印迹分析（Western blotting）检测重组蛋白的表达情况。80只昆明小鼠随机均分为5组，分别为对照组（PBS组，pcDNA3.1空质粒组，pcIL-12质粒组），pcMIC8质粒组以及pcMIC8+pcIL-12质粒共免疫组，共免疫组以pcMIC8质粒和pcIL-12质粒各100 μg（溶于100 μl PBS）肌注昆明小鼠后腿部，共免疫3次，每次间隔2周，PBS组，pcDNA3.1空质粒组，pcIL-12质粒组和pcMIC8质粒组分别注射等量的PBS，pcDNA3.1空质粒，pcIL-12质粒和pcMIC8质粒。间隔免疫，免疫前及初次免疫后13 d，27 d，41 d和55 d，用ELISA测小鼠血清中抗体IgG 和IgG2a的表达水平。末次免疫后4周，ELISA法测各实验组小鼠脾脏脾细胞培养液上清中γ干扰素（IFN-γ）和IL-4的表达水平。末次免疫后3周，各组小鼠腹腔注射拟种103 弓形虫RH株速殖子，观察小鼠受攻击感染后的存活时间。 结果 Western blotting显示，重组质粒pcMIC8能在HeLa细胞中表达，蛋白相对分子质量（Mr）约为74 000。初次免疫后55d，共免疫组小鼠血清IgG（0.51±0.028），IgG2a（0.261±0.04）抗体水平高于pcMIC8质粒组（0.409±0.031，0.159±0.031）和对照组（P<0.05），IgG1抗体在各实验组之间未见有显著差异。末次免疫后4周，共免疫组小鼠脾脏脾细胞上清中IFN-γ含量（1083.27±925.34）显著高于pcMIC8质粒组（497.65±98.15）和对照组（PBS 47.18±2.73，pcDNA3.1 50.08±4.62，pcIL-12 118.15±12.73）（P<0.05）。各组IL-4的水平差异无统计学意义（P>0.05）。攻击感染后，共免疫组小鼠存活时间（15 d）较pcMIC8组（10 d）和对照组（PBS 5d，pcDNA3.1 6d，pcIL-12 8d）显著延长（P<0.05）。 结论 弓形虫MIC8基因与基因佐剂IL-12共免疫小鼠能增强其免疫反应。

关键词：
DNA疫苗 弓形虫抗原MIC8 基因佐剂IL-12

Abstract: Objective To examine the immunoprotection effect induced by MIC8 DNA vaccine co-immunized with a plasmid encoding murine IL-12（pcIL-12）as an adjuvant in mice against the challenge of Toxoplasma gondii. Methods The gene sequence encoding MIC8 of T. gondii RH strain was inserted into eukaryotic expression vector pcDNA3.1 to construct the pcMIC8 expression plasmid. The recombinant plasmid was transfected into HeLa cells to test its expression and the recombinant protein was then characterized by Western blotting. Eighty Kunming mice were randomly divided into 5 groups（16 per group）: 3 control groups（PBS, pcDNA3.1, and pcIL-12）, pcMIC8 group, and pcMIC8 plus pcIL-12 group. Mice in the pcMIC8 plus pcIL-12 group were co-injected intramuscularly at a dosage of 100 μl each of pcMIC8 and pcIL-12 suspended in 100 μg sterile PBS. Mice in other groups were inoculated with PBS, pcDNA3.1, pcIL-12, and pcMIC8 respectively following the same protocol. All the mice received three immunizations at 2-week intervals. Serum samples were collected on day 0, 13, 27, 41, and 55 before each inoculation for determining antibody IgG, IgG subclass IgG2a. Four weeks after the final immunization, IFN-γ and IL-4 levels in splenocytes cultures from immunized mice were detected by ELISA. The mice were challenged with 103 tachyzoites of the virulent T. gondii RH strain three weeks after the last immunization to observe the survival time. Results Western blotting showed that the protein extracts in HeLa cells upon transfection with pcMIC8 were effectively expressed in cells. The levels of IgG（0.51±0.028）and IgG2a（0.261±0.04）(on day 55) in mice immunized with pcMIC8 plus pcIL-12 were higher than pcMIC8 group(497.65±98.15) and control groups (PBS 47.18±2.73, pcDNA3.1 50.08±4.62, pcIL-12 118.15±12.73）（P<0.05）. There was no significant difference in the level of IgG 1 and IL-4 among the five groups（P>0.05）. After a lethal challenge of T. gondii RH strain, the survival time in mice immunized with pcMIC8 plus pcIL-12（15 d）was prolonged in comparison to that of pcMIC8（10 d） and control groups（PBS 5 d, pcDNA3.1 6 d, pcIL-12 8 d）（P<0.05）. Conclusion The immune responses induced by the combined use of the recombinant plasmid encoding MIC8 of T. gondii with murine IL-2 gene adjuvant can be enhanced.

Keywords: DNA vaccine Toxoplasma gondii Antigenic MIC8 Gene adjuvant IL-12

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