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实验研究

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结核分枝杆菌ESAT6、CFP10基因DNA疫苗对小鼠免疫原性的初步研究

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Preliminary study of Mycobacterium tuberculosis ESAT6 and CFP10 DNA vaccine immunogenicity in mice

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摘要 目的 研究ESAT6、CFP10基因DNA疫苗分别与卡介苗联合免疫小鼠, 以诱导 免疫效果。方法 以构建的真核表达载体pEGFP-N1-ESAT6、pEGFP-N1-CFP10 与卡介苗共同免疫随机分成8组的80只小鼠,分别标记为:生理盐水组(SLYS)、卡介 苗组(KJM)、pEGFP-N1组(P-N1)、pEGFP-N1-ESAT6组(P-N1-E)、pEGFP-N1-CFP10组(P-N1-C)、卡介苗加pEGFP-N1组(K+P-N1)、卡介苗加pEGFP-N1-ESAT6组(K+P-N1-E)和卡介苗加pEGFP-N1-CFP10组(K+P-N1-C)。每只 小鼠皮内注射100µL卡介苗,每只小鼠肌肉注射50µg质粒,每组共注射3次。以纯化 的ESAT6、CFP10蛋白作为抗原,用DOT-ELISA方法检测小鼠血清中的抗体;用 ELISA方法检测小鼠血清中IFN-y的变化。结果 免疫3次后的小鼠血清中检测到针 对CFP10蛋白的抗体, 未检测到针对ESAT6蛋白的抗体。但二者血清中IFN-v水平都 有显著上升, K+P-N1-C和K+P-N1-E免疫3次后, 测得的IFN-γ平均含量为 (107.591±7.3281)pg/mL和(95.7503±9.0184)pg/mL, 显著高于免前、一 免、二免(P<0.01)。结论 结核分枝杆菌ESAT6, CFP10基因DNA疫苗和卡介苗联

合应用后可诱导小鼠产生有效的细胞免疫应答, 为DNA疫苗的研究奠定一定的基

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关键词: 结核分枝杆菌 ESAT6 CFP10 DNA疫苗 卡介苗

Abstract: The objective of the present study was to investigate immune effect by using ESAT6, CFP10 DNA vaccine combined with BCG to immunize mice. DNA vaccine pEGFP-N1-ESAT6 and pEGFP-N1-CFP10 was primarily constructed, and 80 mice were randomly divided into eight groups including saline group (SLYS), BCG group (KJM), pEGFP-N1 group (P-N1), pEGFP-N1-ESAT6 group (P-N1-E), pEGFP-N1-CFP10 group (P-N1-C), BCG plus pEGFP-N1 group (K+P-N1), BCG plus pEGFP-N1-ESAT6 group (K+P-N1-E), and BCG plus pEGFP-N1-CFP10 group (K+P-N1-C). Each mouse was intradermally injected with 100 µL BCG, while each mouse was intramuscularly injected with plasmid pEGFP-N1-ESAT6, pEGFP-N1-CFP10 (50 µg). Each group was injected for three times. Purified ESAT6, CFP10 proteins were used as antigen. DOT-ELISA method was used to detect antibodies in the serum of mice. ELISA method was used to detect the change of the IFN-γ in the serum of mice. Antibodies against CFP10 proteins were detected in mouse serum after three immunizations. Antibody against ESAT6 proteins was not detected. However, IFN-γ levels in serum increased significantly. The average content of the measured IFN-y was significantly higher than that before immunization; the first immunization and the second immunization (P<0.01) was (107.591 ± 7.3281) pg/mL and (95.7503 ± 9.0184) pg/mL after three times immunization with K+P-N1-C and K+P-N1-E. It concluded that Mycobacterium tuberculosis ESAT6 and CFP10 DNA vaccine combined with BCG combination could induce an effective cellular immune response, which lay a foundation for the study of DNA vaccines.

Keywords: Mycobacterium tuberculosis ESAT6 CFP10 DNA vaccine BCG

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