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研究报告

O型口蹄疫病毒VP1 T细胞、B细胞表位双拷贝基因与大肠杆菌LTB、STI肠毒素基因融合表达产物的免疫应答

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摘要

合成O型口蹄疫病毒VP1蛋白中与细胞免疫（21--40表位肽）及体液免疫（141--160表位肽）相关的基因序列2020VP1，运用基因工程技术构建了含有肠毒素大肠杆菌LTB、STI基因及观赏兽2020VP1的融合表达载体r2020-B-2020-STI，转化宿主菌 菌21(DE3) RIL后的表达产物经SDS-PAGE分析，结果显示重组融合蛋白的分子量约为45 kDa，表达量较高。ELISA实验结果显示，融合蛋白能与霍乱毒素（cholera toxin）CTB抗体特异结合。动物实验表明，融合蛋白能够诱发兔体产生较强的FMDV中和抗体，免疫豚鼠在低浓度FMDV刺激下能够产生特异性T淋巴细胞增殖反应，说明融合蛋白能诱导机体产生FMDV特异性细胞及体液免疫反应，同时，融合蛋白免疫雌鼠能够抵抗大肠杆菌腹泻毒株攻击，免疫兔体能够产生STI中和抗体，且融合蛋白不具STI毒性，证明融合蛋白具有良好的LTB、STI免疫原性，实验结果表明，此融合蛋白具有开发成为口蹄疫及肠毒素腹泻联合疫苗的应用价值。

关键词 口蹄疫病毒;T细胞表位;B细胞表位;肠毒素大肠杆菌LTB STI;免疫应答

分类号 Q933

The Immune Responses of the Fusion Protein Including Two Copies of T-cell and B-cell Epitopes of Food-and-mouth Disease Virus VP1 Type O and LTB and STI Enterotoxins of Escherichia coli

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Abstract

<P>A nucleotides fragment 2020VP1 related to T-cell epitope (aa21-40) and B-cell epitope (aa141-160) of VP1 of Food-and-mouth disease virus（FMDV）type O was synthesized, and the recombined expression vector r2020-LTB-2020-STI was correctly constructed, which was used to express the fusion protein consisted of two copies of aa21-40, aa141-160 of FMDV VP1 and LTB and STI Enterotoxins of Escherichia coli. The fusion protein with molecular weight of about 45kD was successfully expressed in E.coli BL21(DE3) RIL at high level and confirmed by SDS-PAGE. The purified fusion protein could be specifically recognized by CTB antibody. The purified protein was then used to vaccinate guinea pigs, rabbits and Balb/c mice at 6-8 week age, and immune responses were finally observed. The fusion protein could induce proliferation of spleen T cells in vaccinated
guinea pigs and elicit a high level of neutralizing antibody in rabbits. It could be concluded that the fusion protein
could activate FMDV-specific cellular immune-response and humoral immune-response simultaneously. At the same time, the vaccinated mice could survive challenge of Enterotoxigenic Escherichia coli C83902, and the sera of vaccinated rabbits could neutralize the STI toxin. Moreover, the fusion protein had no STI toxicity, indicating that the fusion protein had LTB and STI immunogenicities. Therefore, it can be concluded that the fusion protein could be explored as a potentially efficient FMDV and ETEC vaccine.
</P>

Key words

FMDV；VP1；T-cell epitope；B-cell epitope；Enterotoxigenic Escherichia coli；LTB；STI；immune responses

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