



结核分枝杆菌Rv0440 CTL表位肽的免疫原性研究

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Immunogenicity of CTL epitopes in Mycobacterium tuberculosis Rv0440 protein

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摘要

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摘要 目的 体外鉴定结核分枝杆菌(Mycobacterium tuberculosis, Mtb)Rv0440

蛋白序列中表位肽362-370 aa和369-377 aa的HLA-A*0201限制性CD8⁺ CTL表位的免疫原性, 为基于表位的结核疫苗研究提供实验依据。方法 根据T2细胞HLA-A*0201分子与多肽结合力分析实验结果, 选取结核分枝杆菌Rv0440蛋白质氨基酸序列中对HLA-A*0201分子高亲合力的Rv0440-1(362-370 aa, KIQERLAKL)和Rv0440-2(369-377 aa, KLAGGVAVI)作为候选表位肽。用候选表位肽刺激PPD(+++)健康志愿者外周血单个核细胞(peripheral blood mononuclear cells, PBMC)检测细胞分泌IFN- γ 的水平。用候选表位肽诱导特异性CTL细胞, 检测特异性CTL细胞对负载表位肽的T2细胞的杀伤活性, 观察Rv0440-1和Rv0440-2的HLA-A*0201限制性CD8⁺ CTL表位的免疫原性。结果 ELISPOT实验结果显示, 表位肽Rv0440-1能够明显诱导HLA-A*0201(+), PPD(+++)健康志愿者PBMC分泌IFN- γ ($P < 0.05$); 且表位肽Rv0440-1负载DC诱导的CTL在效靶比为10:1时对负载相应表位肽的T2细胞的特异性杀伤活性高于对照组($P < 0.05$); 与对照组相比, 表位肽Rv0440-2没有诱导能力。结论 表位肽Rv0440-1(362-370 aa,

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KLQERLAKL)具有良好的免疫原性,是有效的结核分枝杆菌的HLA-A*0201限制性CTL表位。

关键词: 结核分枝杆菌 表位肽 CD8⁺CTL 特异性杀伤活性

Abstract: The immunogenicity of HLA-A*0201 restricted CD8⁺CTL epitopes 362-370 aa and 369-377 aa in Mycobacterium tuberculosis (Mtb) Rv0440 protein were identified in this study, so as to provide evidence for epitope-based study for tuberculosis(TB) vaccine. T2 cell line was used to assay the affinity between the predicted peptides and HLA-A*0201 molecules. Based on peptides with high binding affinity to HLA-A*0201 molecules, Rv0440-1(362-370 aa, KLQERLAKL) and Rv0440-2(369-377 aa, KLAGGVAVI) were chosen to be the candidate epitopes. The secreting IFN- γ release of peripheral blood mononuclear cells (PBMC) was investigated. The specific CTLs were induced from PBMC of HLA-A*0201 (+) and PPD (+++) in healthy donors by the candidate peptides. In vitro cytotoxicity of peptide-induced CTL was determined to screen HLA-A*0201 restricted CD8⁺CTL epitopes from those candidates. The result showed that Rv0440-1 significantly induced the HLA-A*0201 (+) and PPD (+++) of donors' PBMC to secrete IFN- γ by ELISPOT ($P < 0.05$). And when the proportion of effective cells to target cells (E: T) was 10 : 1, CTL induced by dendritic cells (DC) loaded with Rv0440-1 had higher cytotoxicity to T2 target cells ($P < 0.05$), while Rv0440-2 had no significant inducing activity. So the conclusion is Rv0440-1(362-370 aa, KLQERLAKL) might be the effective HLA-A*0201 restricted CTL epitope.

Keywords: Mycobacterium tuberculosis epitope peptides CD8⁺ cytotoxic T-lymphocytes specific cytotoxicity

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