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论著

日本血吸虫22.6kDa抗原T细胞表位的重组、表达及初步鉴定

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摘要

目的 鉴定日本血吸虫中国大陆株22.6kDa抗原(Sj22.6)的T细胞表位。 方法 用计算机软件预测Sj22.6分子的T细胞表位,设计并合成其编码核苷酸,定向克隆入融合表达载体pET32c(+),转化大肠杆菌BL21感受态细胞,经酶切及测序鉴定出重组克隆。阳性克隆经IPTG诱导表达,表达产物用NTA柱纯化。用纯化后的表位肽融合蛋白体外刺激C3H小鼠脾单个核细胞,3HTdR掺入法检测其增殖。 结果 用计算机软件预测获得Sj22.6抗原的4个候选表位肽,并成功克隆入pET32c(+)。其重组体均可表达分子量约20kDa的融合蛋白,用NTA柱纯化后经12%聚丙烯酰胺凝胶电泳(SDSPAGE)显示单一条带。其中P4、P5、P6融合蛋白能有效刺激小鼠脾单个核细胞增殖。 结论 从Sj22.6抗原中初步鉴定出P4、P5、P63个T细胞表位

关键词 日本血吸虫 22.6kDa抗原 T细胞表位 鉴定

分类号

Preliminary Identification of T Cell Epitopes on 22.6kDa Antigen of Schistosoma japonicum

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Abstract

Objective To identify the T cell epitopes on 22.6kDa antigen of Schistosoma japonicum (Sj22.6). Methods The primary structure of Sj22.6 molecule was analysed using various predictive algorithms and a panel of 4 peptides were acquired. Their oligonucleotides were designed, synthesized and inserted into the multiple cloning site of plasmid pET-32c(+). The recombinant plasmids were transformed into E.coli BL21 and identified by endonuclease digestion and sequencing. The positive clones containing the recombinant plasmids could express specific fusion proteins (trx-epitope, MW≈20kDa) induced by IPTG. The fusion protein with 6×His could be coupled with NTA resin specifically, and purified by elusion of the column with buffer containing imidazole. The purified fusion proteins were incubated with splenocytes of C3H mice and then, the proliferation of splenocytes was determined by~3H-TdR incorporation assay. Results The recombinant plasmids were constructed successfully and the positive clones containing the recombinant plasmids expressed specific fusion proteins. Three of the purified fusion proteins (P4、P5、P6) could stimulate the lymphocyte proliferation. Conclusion Three T cell epitopes on Sj22.6 antigen were identified.

Key words Schistosoma japonicum 22.6 kDa antigen T cell epitope identification

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