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【论著】

恶性疟原虫 AMA-1 基因变异区在大肠杆菌中的诱导表达

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【摘要】 **目的**　恶性疟原虫（*P. f.*）AMA-1 蛋白抗原在大肠杆菌中的表达。 **方法**　以 FCC1/HN 基因组 DNA 作为模板 PCR 扩增 AMA-1 基因变异区，扩增产物以 *Bam*H Ⅰ 和 *Hind* Ⅲ 双酶酶切后作为插入片段，与具有相同粘性末端的表达质粒 pQE-40 连接，并用 DNA 自动测序仪测定 AMA-1 DNA 片段的序列。取含重组表达质粒的重组菌株以 IPTG 进行诱导表达，表达产物以 SDS-PAGE 电泳和以兔抗 AMA-1 抗血清进行 Western blotting 分析鉴定。 **结果**　FCC1/HN AMA-1 基因变异区 DNA 序列长度为 506 bp，预计编码 168 个氨基酸。Western blotting 分析确认诱导后的 SG13009/AMA 1 表达产物在分子量约 23.0 kDa 处出现 1 条与兔抗 AMA-1 抗血清特异反应的条带。 **结论**　FCC1/HN AMA-1 基因变异区在大肠杆菌中获得表达，Western blotting 分析表明该蛋白片段含有特异抗原表位。

【关键词】 **恶性疟原虫；AMA-1 基因变异区；PCR；克隆；大肠杆菌；基因表达**

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Induced Expression of the Variable Region of AMA-1 from *Plasmodium falciparum*

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【Abstract】 **Objective**　To express the variable region of AMA-1 gene from *Plasmodium falciparum* in *Escherichia coli*. **Methods**　Genomic DNA of FCC1/HN was used as template and the variable region of AMA-1 gene was amplified by polymerase chain reaction (PCR). The PCR products were digested by endonuclease *Bam*H Ⅰ and *Hind* Ⅲ, cloned into pBlu2KSP. The nucleotide sequences of the variable region of AMA-1 gene were determined by sequencing. The AMA-1 gene fragment was subcloned into plasmid pQE, expressed in *E. coli* and induced by IPTG. The fusion product as identified by SDS-PAGE gel electrophoresis and Western blotting were proceeded with anti-AMA-1 sera from rabbit. **Results**　The size of the variable region of AMA-1 gene from FCC1/HN was 506 bp and encoded 168 amino acids. On SDS-PAGE gel dyed with Coomassie brilliant blue R250, no specific protein band can be discerned, but Western blotting proceeded with anti-AMA-1 sera from rabbit demonstrated that the specific protein band was about 23.0 kDa. **Conclusion**　The variable region of AMA-1 gene from FCC1/HN was able to be expressed in *E. coli* and analysis of Western blotting demonstrated that the AMA-1 fussion protein contained specific antigenic epitopes.

【Key words】 *Plasmodium falciparum*, variable region of AMA-1, PCR, cloning, *Escherichia coli*, gene expression

恶性疟原虫（*P. f.*）AMA-1 是重要的无性血液期候选疫苗抗原之一。已用不同的表达系统分别表达了脆弱疟原虫[1]、间日疟原虫[2]、夏氏疟原虫 AMA-1[3]，表达产物可激发猴和小鼠产生针对 AMA-1 的抗体应答。由于大肠杆菌仍是目前外源蛋白基因表达最有用的宿主菌之一[4]，本试验将 *P. f.* AMA-1 基因变异区片段克隆于融合表达载体 pQE，并以 IPTG 进行诱导表达。

材料与方法

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1　材料

FCC1/HN，由中国预防医学科学院寄生虫病研究所体外培养。质粒 pQE-40、*Escherichia coli* SG13009 由本教研室疟疾分子疫苗实验室保存。

2　方法

2.1　PCR 反应　参考有关文献[5]以 Chelex-100 方法抽提 FCC1/HN 基因组 DNA 作为 PCR 反应模板；根据 *P. f.* AMA-1 序列[6]自行设计 1 对引物 P1 与 P2，分别在引物 5′ 端加 *Bam*H Ⅰ、*Hind* Ⅲ 酶切位点和保护碱基：

P1：5′-CGCGGATCCGGAACTCAATATAGACTTCC-3′

P2：5′-CCCAAGCTTAAATTCTTTCTAGGGCAAAC-3′