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论著

伯氏疟原虫红内期融合抗原的构建、表达及其免疫原性研究

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摘要

目的 合成伯氏疟原虫红内期融合抗原基因PbCP-2.9，在毕赤酵母真核系统中表达其产物，并进行免疫原性分析。 方法 选取与恶性疟原虫红内期融合抗原基因PfCP-2.9具有同源性的伯氏疟原虫AMA1（Ⅲ）和MSP1-19序列，融合形成PbCP-2.9基因。基因序列经密码子优化，在毕赤酵母中分泌表达。60只BABL/c小鼠均分为6组，其中蛋白免疫组3组，分别用PbCP-2.9蛋白与福氏佐剂、ISA206和IMS1312佐剂乳化后，皮下注射免疫小鼠，抗原免疫剂量20 μg/只·次，注射体积200 μl，共免疫3次，每次间隔2周。佐剂对照组3组，以 PBS代替免疫抗原同法免疫。免疫前及每次免疫后1周鼠尾取血，分离血清。用ELISA和IFAT方法检测血清中特异性抗体的滴度及其与天然抗原的反应结果。 结果 PbCP-2.9基因在毕赤酵母中分泌表达出Mr约26 400的 PbCP-2.9蛋白，其与抗伯氏疟原虫红内期原虫的血清能进行特异性反应；ELISA检测PbCP-2.9蛋白免疫组结果表明，福氏佐剂组第2次免疫后特异性抗体滴度为(52.62±11.26)，第3次免疫后为(94.50±52.84)；ISA206 组第2次免疫后为(7.59±5.61)，第3次免疫后为(25.60±16.92)；IMS1312组第2次免疫后为(9.41±8.86)，第3次免疫后为(28.92±12.98)。福氏佐剂组第2次免疫后特异性抗体滴度分别为ISA206 组的6.9和IMS1312组的5.6倍（F=81.06，P<0.01），第3次免疫后分别为ISA206 组的3.7和IMS1312组的3.3倍（F=13.29，P<0.01）。IFAT检测结果显示，经PbCP-2.9免疫的鼠血清与Pb ANKA株虫体表面抗原有阳性反应。 结论 PbCP-2.9基因在毕赤酵母中高效表达，重组抗原免疫原性强，其免疫血清能识别伯氏疟原虫天然抗原。

关键词 伯氏疟原虫 红内期 融合抗原 基因表达 免疫原性

分类号

Construction and Expression of *Plasmodium berghei* Chimeric Protein in *Pichia pastoris* and its Immunogenicity in Mice

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Abstract

Objective To produce an erythrocytic stage chimeric protein of *Plasmodium berghei* in *Pichia pastoris* and evaluate its immunogenicity. Methods The DNA sequences of AMA1（Ⅲ） and MSP1-19 from *P. berghei* homologous to the corresponding sequences of *P. falciparum* chimeric antigen 2.9 (*PfCP*-2.9) were fused to generate a chimeric gene，designated as PbCP-2.9. The resulting gene was redesigned using Pichia preferential coden usage and expressed in *P. pastoris* in the secreted form. The recombinant protein was purified by Ni-NTA affinity chromatography. Three groups each with 10 BALB/c mice were immunized subcutaneously with 20 μg of purified PbCP-2.9 antigen formulated in Freund's adjuvant，Montanide ISA720 and Montanide IMS 1 312，respectively. Three control groups each with 10 mice received only adjuvants emulsified with PBS. All the mice received three immunizations at 2-week intervals with the same dose of antigen. Serum samples were collected at pre-immunization and one week after each immunization，and were analyzed for specific antibodies by ELISA and reaction with natural *P. berghei* proteins by IFAT. Results The PbCP-2.9 antigen with *M*r 26 400 was successfully expressed in *P. pastoris* in secreted form. The recombinant protein can be recognized by the serum against blood stage parasites of *P. berghei*. High antibody responses were detected in all three PbCP-2.9-immune groups of mice by ELISA. However，mice immunized with PbCP-2.9 antigen in Freund's adjuvant produced higher antibody titers than those with PbCP-2.9 antigen in Montanide ISA 206 and Montanide IMS 1312 adjuvants. The mean antibody titer in Freund's adjuvant was 6.9-fold higher than in Montanide ISA 206 adjuvant and 5.6-fold higher than in Montanide IMS 1312 adjuvant after the second immunization （F=81.06，P<0.01). In addition，after the third immunization the mean antibody titer in Freund's adjuvant was 3.7-fold higher

than in Montanide ISA 206 adjuvant and 3.3-fold higher than in Montanide IMS 1312 adjuvant（F=13.29，P<0.01). The results from IFAT assay demonstrated that the immune sera recognized the surface proteins of *P. berghei* parasites. Conclusion The codenoptimized PbCP-2.9 gene has been constructed and expressed in *P. pastoris*. The chimeric antigen is highly immunogenic in mice and the immune sera can interact with natural proteins of *P. berghei* parasite.
Key words *Plasmodium berghei* Erythrocytic stage Chimeric protein Gene expreesion Immunogenicity
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