

实验报道

重组恶性疟原虫醛缩酶鉴定及其单克隆抗体的制备

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摘要

目的 鉴定重组表达的恶性疟原虫醛缩酶 (ALD), 制备针对此酶的单克隆抗体。方法 用PCR法扩增恶性疟原虫海南株ALD基因, 经大肠埃希菌表达并纯化的ALD免疫BALB/c小鼠, 腹腔注射免疫3次, 每次间隔2周, 加强免疫后3d取免疫小鼠脾细胞制备单克隆抗体。同时用获得的免疫血清进行间接荧光抗体试验 (IFAT) 和蛋白质印迹 (Western blotting) 分析。结果 ELISA检测表明, 小鼠能产生较高的针对

ALD免疫应答, 3次免疫后血清中特异性抗体滴度达 $1:10^5$, IFAT显示免疫血清能特异性识别疟原虫体内的抗原; Western blotting分析显示免疫血清识别的疟原虫蛋白相对分子质量 (Mr) 约41 000; 所制备的免疫血清与人红细胞内醛缩酶无交叉反应。经ELISA检测 3次, 筛选获得7株分泌针对ALD的单克隆抗体的杂交瘤细胞株, 其中3株分泌的单克隆抗体能识别培养的恶性疟原虫; 抗体亚型鉴定结果显示均为IgG1型。结论 本实验构建并表达了重组疟原虫糖酵解醛缩酶, 并获得特异性的单克隆抗体。

关键词 [恶性疟原虫](#) [醛缩酶](#) [基因表达](#) [单克隆抗体](#)

分类号

Identification of Recombinant Aldolase of *Plasmodium falciparum* and its Monoclonal Antibody Preparation

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Abstract

Objective To identify the recombinant aldolase (ALD) of *Plasmodium falciparum*, and to develop monoclonal antibodies (McAbs) against the recombinant ALD. Methods ALD gene was amplified by PCR from genomic DNA of FCC1/HN strain, and expressed in *E.coli* DH5 α . BALB/c mice were immunized with the recombinant ALD of *P. falciparum* via celiac injection for 3 times with 2 weeks interval. Three days after a booster injection, spleen cells of the immunized mice were used for producing McAbs. The immune serum was tested by IFAT and Western blotting. Results BALB/c mice immunized with purified aldolase protein developed strong immune response to the antigen, and the titer of specific antibody reached $1:10^5$ in all immune sera after the third immunization. Moreover, immune sera specifically recognized the cultured *P. falciparum*. Western blotting showed that the immune sera recognized specifically a Mr 41 000 band of crude malaria antigen. No cross-reaction with human red cells was detected. Seven positive hybridoma cell lines were obtained after 3 rows of selection. All the McAbs' subclasses belong to IgG1. IFAT showed that only 4 McAbs could recognize the cultured *P. falciparum*. Conclusion Plasmodial aldolase has been successfully expressed and purified, and the established hybridoma cell lines can secrete McAbs specific to the aldolase of *P. falciparum*.

Key words [Plasmodium falciparum](#) [6-bisphosphate aldolase](#) [Gene expression](#) [Monoclonal antibody](#)

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