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补体成分Ficolin-A抗小鼠感染伯氏疟原虫的研究

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Study on Ficolin-A against Infection of Plasmodium berghei in Mouse Model

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摘要 【摘要】 目的 探讨免疫系统补体成分Ficolin-A抗伯氏疟原虫（Plasmodium berghei）感染的效果。 方法 克隆扩增伯氏疟原虫裂殖子表面蛋白MSP119基因，构建pGEX-KG-MSP119质粒。将pGEX-KG-Ficolin-A质粒和pGEX-KG-MSP119质粒分别转染至大肠埃希菌（E. coli）BL21，1 mmol/L异丙基硫代-β-D-半乳糖苷（IPTG）诱导表达，谷胱甘肽琼脂糖凝胶4B柱纯化重组蛋白，十二烷基硫酸钠-聚丙烯酰胺电泳（SDS-PAGE）和蛋白质印迹（Western blotting）鉴定蛋白表达情况。40只小鼠随机均分为5组，Ficolin-A蛋白组和MSP119蛋白组小鼠每次注射蛋白20 μg，MSP119蛋白+Ficolin-A蛋白组小鼠每次注射2种蛋白各20 μg，PBS对照组和GST对照组小鼠每次各注射PBS 200 μl和GST 20 μg，各组小鼠每2周免疫1次，共免疫4次。末次免疫后2周，各组小鼠腹腔注射感染伯氏疟原虫的红细胞300 μl，分别于注射感染后第2、4、6、8和10天每组各取3只小鼠，尾静脉采血涂片，吉氏染色后，显微镜下计数感染的红细胞，计算疟原虫密度。感染疟原虫后第20天统计各组小鼠的存活数量。 结果 测序结果表明，pGEX-KG-Ficolin-A和pGEX-KG-MSP119质粒构建成功。SDS-PAGE和Western blotting结果表明，Ficolin-A融合蛋白的相对分子质量（Mr）约为69 000，MSP119融合蛋白约Mr 41 000，均与预测值相符。动物实验结果显示，感染后第2、4、6、8和10天，MSP119蛋白+Ficolin-A蛋白组小鼠的疟原虫密度均略低于其他4组，至感染后第10天，疟原虫密度为（22.2±1.7）%，略低于MSP119蛋白组［（33.4±2.7）%］、Ficolin-A蛋白组［（36.2±3.1）%］、GST对照组［（43.8±4.8）%］和PBS对照组[（45.3±3.6）%]，但差异均无统计学意义（P>0.05）。感染后第20天，PBS对照组8只小鼠均死亡，Ficolin-A蛋白组存活小鼠数量（3只）与GST对照组（2只）比较，差异无统计学意义（P>0.05）；MSP119蛋白+Ficolin-A蛋白组存活小鼠数量（6只）显著高于GST对照组（P<0.05）。 结论 补体成分Ficolin-A对降低小鼠疟原虫密度效果不明显，联合MSP119使用可提高小鼠感染疟原虫后的生存机会。

关键词： Ficolin-A 伯氏疟原虫 裂殖子表面蛋白1羧基端Mr 19 000片段

Abstract： 【Abstract】 Objective To evaluate the effect of Ficolin-A, a lectin complement against Plasmodium berghei in mice model. Methods The Mr 19 000 fragment of merozoite surface protein-1 of P. berghei（MSP119） was cloned and then subcloned into the vector pGEX-KG. The recombinants of pGEX-KG-Ficolin-A and pGEX-KG-MSP119 were transformed into Escherichia coli BL21, and followed by expression of the protein induced by 1 mmol/L IPTG. The fusion protein was purified by affinity chromatography using Glutathione Sepharose 4B, and then identified by SDS-PAGE and Western-blotting. Five mouse model groups were treated with PBS, GST, Ficolin-A, MSP119, or Ficolin-A+MSP119, respectively. Each group had eight mice. Mice in Ficolin-A or MSP119 groups were injected with 20 μg Ficolin-A or MSP119 protein each time, respectively. Mice in Ficolin-A+MSP119 group were injected with 20 μg Ficolin-A and 20 μg MSP119 each time. Mice in control groups were injected with 200 μl PBS or 20 μg GST, respectively. All the mice received four immunizations at 2-week intervals. Two weeks after the last immunization, all the mice were inoculated with 300 μl Plasmodium berghei-infected red blood cells. On day 2, 4, 6, 8, and 10 post-infection, blood samples were collected from three mice of each group, and the Giemsa stained-blood films were microscopically examined. Density of malaria parasites was calculated. The survival rate was evaluated on day 20 post-infection. Results The recombinant vectors of pGEX-KG-Ficolin-A and pGEX-KG-MSP119 were constructed. Purified fusion proteins, Ficolin-A-GST and MSP119-GST, were obtained. Western blotting analysis indicated that the relative molecular mass of fusion proteins Ficolin-A-GST and MSP119-GST was about Mr 69 000 and Mr 41 000. Animal experiments showed that on day 10 after infection, the parasite density in Ficolin-A+MSP119 group ［（22.2±1.7）%］ was slightly lower than that of the groups MSP119 ［（33.4±2.7）%］, Ficolin-A [（36.2±3.1）%］, GST ［（43.8±4.8）%］ and PBS [（45.3±3.6）%], but the difference was not statistically significant（P>0.05）. No mouse survived in PBS group on day 20 after infection. There was no significant difference in number of survival mice between Ficolin-A group (3 mice) and GST group (2 mice). Six mice survived in Ficolin-A+MSP119 group, which was significantly more than that of GST group （P<0.05）. Conclusion Ficolin-A cannot significantly suppress parasite density. However， Ficolin-A+MSP119 can increase the survival rate of Plasmodium berghei-infected mice.

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