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伯氏疟原虫ANKA株抗哌喹系小鼠模型免疫学分析

海南医学院科学实验中心，海口 571101

Immunological Analysis of Piperaquine-resistant Murine Model of *Plasmodium berghei* ANKA Strain

Scientific Experiment Center，Hainan Medical University，Haikou 571101，China

摘要 目的 观察伯氏疟原虫（*Plasmodium berghei*，*Pb*）ANKA抗哌喹（PQR）系小鼠模型的免疫学特征。 方法 64只昆明小鼠随机分为3组，A组和C组各16只，B组32只（其中16只用于观察存活天数）。A组和B组小鼠分别经腹腔感染伯氏疟原虫ANKA株哌喹敏感系（*Pb*PQS）和抗性系（*Pb*PQR）红内期原虫1×107个（200 μl血），C组（健康对照组）注射等量生理盐水。每组于感染后4、8、12和16 d各取4只小鼠，取尾血制薄血膜镜检，计算红细胞原虫感染率（简称原虫率）。脱颈处死小鼠，无菌取脾制备脾淋巴细胞悬液，用CCK?鄄8法测定各组小鼠脾淋巴细胞经刀豆球蛋白A （Con A）刺激后的增殖反应，用Griess法和ELISA分别测定脾淋巴细胞培养上清中NO含量和γ干扰素（IFN-γ）水平。另取10只昆明小鼠，每鼠腹腔接种*Pb*PQR系原虫约1×10^7个，待原虫率上升后下降，典型的原虫转变为蓝染细胞时，腹腔感染*Pb*PQS系原虫（1×10^6个）进行攻击感染，观察小鼠原虫率和小鼠存活情况。 结果 A组小鼠平均存活（9.0±3.0） d，感染后6～12 d原虫率均>50%，出现严重贫血。感染后16 d，B组小鼠全部存活，原虫率为（26.66±2.54）%。A、B两组小鼠的脾淋巴细胞经Con A刺激后增殖显著，感染后12 d，分别为0.65±0.08和0.86±0.20（*P*<0.01）。脾淋巴细胞培养上清中，NO含量随感染时间延长而上升，感染后12 d，A、B和C组分别为（48.80±3.49）、（54.80±2.17）和（7.80±0.71） μmol/L，三者比较差异有统计学意义（*P*<0.01）。A组脾淋巴细胞培养上清中IFN-γ水平随感染时间延长而上升，感染后12 d达到最高，为（752.20±39.49） pg/ml，B组于感染后8 d升至峰值［（855.80±33.65） pg/ml］，感染后12 d降至（620.20±27.11） pg/ml；感染后8 d和12 d，A组和B组IFN-γ水平的差异有统计学意义（*P*<0.01）。用*Pb*PQS系攻击感染*Pb*PQR系小鼠模型后10 d，原虫率为（2.44±2.07）%，随之逐渐消失，感染后40 d未检出虫体，无小鼠死亡。 结论 伯氏疟原虫ANKA株哌喹抗性系感染小鼠的脾淋巴细胞增殖水平、NO水平和IFN?鄄?酌含量均显著高于*Pb*ANKA株PQS系感染小鼠，可诱导小鼠产生一定保护性免疫反应。

关键词： 伯氏疟原虫 哌喹抗性 免疫

Abstract: Objective To analyze the immunological characteristics of murine model of piperaquine sensitive （PQS） line and resistant （PQR） line of *Plasmodium berghei* （*Pb*） ANKA strain. Methods 64 Kunming mice were divided into three groups, 16 in each of groups A and C， 32 in group B（16 of 32 were used for observing suvival days）. Each mouse in groups A and B was infected with 1×10^7 erythrocytic stage parasites of *Pb*PQS and *Pb*PQR，respectively. Mice in group C were injected with the same volume of normal saline. On days 4，8，12 and 16 after inoculation，4 mice from each group were sacrificed. Blood samples were collected for thin blood smear examination，and parasitemia rate calculated. Spleens were removed and spleen lymphocytes suspension prepared. Spleen lymphocytes were stimulated with ConA，and cell proliferation was measured by Cell Counting Kit-8 （CCK-8） assay. Nitrogen oxide （NO） and IFN-γ level of spleen cell culture supernatants were detected by the Griess reagent and ELISA methods，respectively. Another 10 mice were each inoculated with 1×10^7 parasites of *Pb*PQR line，and the mice were then challenged with lethal PQS line when the parasites turned into blue stained cells. The parasitemia and survival days were recorded. Results The average survival time of group A was （9.0±3.0） d，the parasitemia rate was over 50% at 6-12 days post-infection with severe anemia. On 16th day post-infection，no death was recorded in group B with a parasitemia rate of （26.66±2.54）%. After ConA stimulation，the proliferation of spleen lymphocytes in groups A （0.65±0.08） and B （0.86±0.20） at 12 days after infection was significantly higher than that of group C （0.18±0.03） （*P*<0.01）. NO level in spleen cell culture supernatant increased with prolonged infection time. On 12th day post-infection，NO level of groups A［（48.80±3.49） μmol/L］ and B［（54.80±2.17） μmol/L］ was higher than that of group C ［（7.80±0.71） μmol/L］ （P<0.01）. IFN-γ concentration in spleen lymphocytes culture supernatant increased with prolonged infection time. The highest IFN-γ level of group A was （752.20±39.49） pg/ml on 12th day post-infection，while in group B it was （855.80±33.65） pg/ml on 8th day after infection，then decreased on 12th day ［（620.20±27.11） pg/ml］. IFN-γ level showed a significant difference between groups A and B （*P*<0.01）. In 10 days after challenge，the parasitemia rate in PQR group was up to （2.44±2.07）%，and gradually disappeared. No parasite was detected on 40th day after challenge and no mice died. Conclusion The proliferation of spleen cells，NO and IFN-γ levels of spleen lymphocytes culture supernatant in PbANKA strain PQR line are significantly higher than that of PQS line. PbPQR line can induce

certain protective immunoreaction.

Keywords： *Plasmodium berghei* Piperaquine resistance Immunity

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