论著

刚地弓形虫入侵细胞过程中细胞骨架和Ca²⁺浓度变化

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目的 探讨弓形虫入侵不同类型细胞过程中胞内游离Ca²⁺ 浓度及细胞骨架的变化。 方法 常规方法制备 刚地弓形虫RH株速殖子悬液,分别感染吞噬性细胞(小鼠单核巨噬样细胞J774A.1)和非吞噬性细胞(人脐静脉内皮细胞HUVEC)。光学显微镜观察弓形虫感染情况及细胞骨架抑制剂秋水仙素、松胞菌素 D对感染率的影响。用荧光显微镜观察弓形虫速殖子入侵J774A.1、HUVEC过程中细胞微丝和微管变化。用激光共聚焦显微镜检测弓形虫速殖子入侵过程中宿主细胞游离Ca²⁺ 浓度变化。 结果 正常 J774A.1胞内游离Ca²⁺ 浓度为102.0%±6.2%。弓形虫感染后 2 min游离Ca²⁺ 浓度升高,测得荧光强度为 305.2%±21.5%,感染后30~40 min最高荧光强度为1219.7%±58.4%,显著高于基础值(P<0.01)。而经磷脂酶C抑制剂U73122预处理的J774A.1,Ca²⁺ 浓度无明显变化(P>0.05);虫体入侵过程中J774A.1微丝结构发生凝集。微丝结构抑制剂松胞菌素D(P<0.01)和微管结构抑制剂秋水仙素(P<0.05)均可明显降低弓形虫感染率。弓形虫入侵HUVEC过程中Ca²⁺ 浓度变化不明显(P>0.05),宿主细胞微丝、微管结构亦无明显变化。松胞菌素D和秋水仙素对弓形虫入侵HUVEC的能力影响均较小(P>0.05)。 结论 弓形虫入侵吞噬性细胞J774A.1过程中胞内游离Ca²⁺ 浓度显著升高,细胞骨架微丝结构发生凝聚,而入侵非吞噬性细胞HUVEC过程中胞内游离Ca²⁺ 浓度和细胞骨架均无明显变化。

关键词 <u>刚地弓形虫</u> <u>人脐静脉内皮细胞</u> <u>小鼠单核巨噬样细胞</u> <u>细胞骨架</u> <u>Ca²⁺</u>. 分类号

Change of Cytoskeleton and Variance of Ca2+ in Cultured Cells During the Invasion of *Toxoplasma gondii*

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Objective To explore the change of cytoskeleton and the variance of Ca²⁺ in cultured cells during the invasion of Toxoplasma gondii. Methods Tachyzoites suspensions were gathered by routine method and used to infect phagocytic cells (J774A.1) and nonphagocytic cells (HUVEC) . The ability of T. gondii invading into the cells and the influence of cytoskeleton inhibitor, colchicine and cytochalasin D, were observed by microscopy. The rearrangement of cytoskeleton of cells was observed by fluoromicroscopy. By using laser scanning confocal microscope, the variance of Ca^{2+} in J774A.1 and HUVEC was detected. Results Ca²⁺ increased greatly in J774A.1 during the invasion of *T.gondii* (P<0.01) and PLC inhibitor, U73122, could block the increase of Ca^{2+} (P>0.05). The microfilaments of J774A.1 were agglomerated during the invasion of *T. gondii*. Cytoskeleton inhibitor, cytochalasin D (P < 0.01) and colchicine (P < 0.05) significantly reduced the infection rate of J774A.1 cells. No considerable change of Ca²⁺ in HUVEC was found (P>0.05) during the invasion and cytoskeleton was not changed. Cytochalasin D and colchicine showed little effect on the infection rate of HUVEC. Conclusion The concentration of Ca²⁺ increases greatly and cytoskeleton (mainly the microfilament) has been rearranged in phagocytic cell during the invasion of T. gondii, while both of them show no significant change in non-phagocytic cell.

Key words Toxoplasma gondii HUVEC J774A.1 Cytoskeleton Ca²⁺

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