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实验研究

刚地弓形虫RH株速殖子在HeLa细胞系体外培养的实验观察

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摘要
目的 建立稳定高效的刚地弓形虫RH株速殖子(以下简称弓形虫速殖子)体外培养模型。 方法 ①将HeLa细胞（1×10^{4}个）接种置于细胞培养皿内底部的盖玻片，12 h后将纯化的弓形虫速殖子接种于细胞培养皿（1×10^{4}个/皿），继续培养0.5～96 h，观察弓形虫速殖子在HeLa细胞内增殖情况；②将纯化的弓形虫速殖子接种于HeLa细胞后分为2组，一组于37 ℃分别培养24～120 h后，移入25 ℃培养120 h；另组于37 ℃培养48 h后，移入25 ℃分别培养72～168 h，观察不同温度和时间对弓形虫速殖子产率和活虫率的影响；③当培养的HeLa细胞80%发生细胞融合时更换速殖子培养液，同上法12 h后接种纯化的弓形虫速殖子进行培养，当80% HeLa细胞被感染增殖的弓形虫速殖子涨破时，收集、计数弓形虫速殖子，并用其感染（3×10^{6}个/瓶）新的HeLa细胞，观察其连续传代情况；④每隔5代取弓形虫速殖子，腹腔接种昆明小鼠（3×10^{6}个/只），观察小鼠存活时间、评价该体外培养条件对弓形虫速殖子毒力的影响。 结果 接种弓形虫速殖子96 h后HeLa细胞均被感染及涨破，培养弓形虫速殖子30代（3～4 d为1代）增殖稳定，每代获得弓形虫速殖子1×10^7～5×10^{7}个，增殖5～20倍。各代弓形虫速殖子对昆明小鼠的平均致死时间为5.80～6.40 d, 毒力未见减弱(P>0.05)。HeLa细胞接种弓形虫速殖子，于37 ℃培养72 h后，移至25 ℃培养120 h，其产率高达40倍以上，活虫率为90%以上，仅残留极少量Hela细胞。 结论 刚地弓形虫RH株速殖子可在HeLa细胞中增殖并长期稳定传代。

关键词 刚地弓形虫 速殖子 HeLa细胞 体外培养
分类号

In vitro Culture of Tachyzoites of *Toxoplasma gondii* RH Strain in HeLa Cells

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Abstract
Objective To establish a stable and efficient in vitro culture model for tachyzoites of *Toxoplasma gondii* RH strain. Methods Tachyzoites were inoculated into HeLa cells to establish an *in vitro* culture system. The proliferation of tachyzoites was observed under microscope by the method of Giemsa stain. At the same time， the longterm tachyzoites maintenance in HeLa cells was established，and the effect of different temperature and time on the yield and motility of tachyzoites were observed. Results The RH strain tachyzoites were cultured and maintained in HeLa cells. Most HeLa cells were destroyed 96 h after inoculation. In the long-term culture system， the proliferation of tachyzoites was stable and its virulence to mouse showed no decrease. Furthermore， tachyzoites in this system proliferated by 5-20 times and （1-5）×10^7 tachyzoites were harvested. When cultured in HeLa cells at 37 ℃ for 72 h then at 25 ℃ for another 120 h， the tachyzoites proliferated by more than 40 times with a motility rate of over 90%. However， rare HeLa cells left in the medium were found. Conclusion Tachyzoites of *T. gondii* RH strain can be subcultured in HeLa cells for a long time， and high proliferation rate of tachyzoites can be obtained from this *in vitro* culture system.

Key words *Toxoplasma gondii* Tachyzoite HeLa cell *In vitro* culture
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