

实验研究

从大鼠肺灌洗液中分离纯化培养卡氏肺孢子虫

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摘要

目的 建立卡氏肺孢子虫 (*Pneumocystis carinii*, *P.c*) 纯培养株。方法 从肺孢子虫肺炎 (PCP) 大鼠模型离体肺脏的灌洗液中分离 *P.c*, 用改良 IMDM 培养基进行体外培养; 以四胺银染色计数法观察虫体增殖情况; 用 PCR 扩增培养物中 *P.c* 线粒体大亚基 rRNA 基因, 进行基因鉴定; 用透射电镜观察培养虫体的超微结构。结果 用添加 s-腺苷甲硫氨酸 (SAM) 等辅助剂的 IMDM 培养基从 8 只 PCP 大鼠的肺灌洗液中分离出 5 个 *P.c* 纯培养株 (*P.c* Rat1~5)。分离培养的 *P.c* 可进行冷冻保存和复苏培养。培养 72 h 的 *P.c* 包囊可增殖 19~22 倍。形态、超微结构及基因序列分析证实从大鼠肺灌洗液中分离出的培养物是大鼠源性肺孢子虫。结论 从大鼠肺灌洗液中分离培养出 5 株大鼠源卡氏肺孢子虫。

关键词 [卡氏肺孢子虫](#) [培养](#) [分离株](#)

分类号

Continuous Axenic Cultivation of *Pneumocystis carinii* Isolated from the Bronchoalveolar Lavage Fluid of Infected Rat

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Abstract

Objective To establish axenic cultivation of *Pneumocystis carinii* (*P.c*). Methods The organisms of *P.c* were isolated from the bronchoalveolar lavage fluid (BALF) of the rats with *Pneumocystis carinii* pneumonia (PCP) and cultured in a medium which was based on IMDM (GIBCO) supplemented with S-adenosyl-L-methionine, putrescine, N-acetyl glucosamine, putrescine, L-cysteine and L-glutamine, and newborn calf serum. The organisms cultured in the system were identified by observing the morphology of cysts in smears stained with Gomori's methenamine silver nitrate stain (GMS). Ultrastructure of the cysts/trophozoites was examined by transmission electron microscopy. The sequences of mitochondrial large ribosomal DNA subunit of the cultured organisms were compared with the *Pneumocystis carinii* f.sp.ratti variant isolate (GenBank No U20173) and *Pneumocystis carinii* f.sp.hominis (GenBank No M58605). Results Five isolates of *P.carinii* received from BALF of 8 rats with PCP were cultured axenically and continuously in the system. The cultured organisms could be stored in frozen condition and used to reinitiate culture, and were amplified by 19-22 times within 72 h. The morphology, ultrastructure and gene sequencing of the cultured organisms confirmed that the isolated organisms were *P.carinii*. Conclusion Five continuously and axenically cultured isolates of *P.carinii* have been received.

Key words [Pneumocystis carinii](#) [Culture](#) [Isolate](#)

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