

[1]毛婵,李倩,方瑶,等.类鼻疽伯克霍尔德氏菌感染A549细胞模型的建立[J].第三军医大学学报,2013,35(19):2010-2013.

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Title: Establishment of a *Burkholderia pseudomallei* invaded A549 cell model

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摘要: 目的 建立类鼻疽伯克霍尔德氏菌感染人肺癌上皮细胞A549的细胞模型。 方法 优化类鼻疽伯克霍尔德氏菌感染A549细胞的感染条件(如MOI、感染时间),通过活细胞工作站动态观察、Giemsa染色、激光共聚焦、透射电镜确证胞内感染和宿主细胞的形态变化,通过炎症因子IL-8和TNF- α 的检测,分析病原菌入胞率和宿主反应性,评价该模型中病原菌侵入A549导致多核巨细胞(multinuclear giant cell, MNGC)形成的特点和病理损伤规律。 结果 透射电镜结果显示类鼻疽伯克霍尔德氏菌侵入到胞内的时间最早是4 h。通过Giemsa染色形态观察,类鼻疽伯克霍尔德氏菌感染后最早8 h可观察到典型MNGC的形成。随着感染时间的延长, MNGC形成率和炎症因子IL-8逐渐升高,而TNF- α 在此模型中变化不明显。 结论 成功构建类鼻疽伯克霍尔德氏菌感染A549细胞模型。

Abstract: Objective To establish an infection model of A549 cells by *Burkholderia pseudomallei* in vitro to study its pathogenesis. Methods The purpose of this study was to optimize the methods in infection of A549 cells by *Burkholderia pseudomallei*, such as multiplicity of infection (MOI) and incubation time. The number of intracellular bacteria and the formation of multinuclear giant cells (MNGCs) were visualized by Giemsa staining, live-cell digital video gallery, immunofluorescence assay and transmission electron microscopy. Furthermore, the expression of inflammatory cytokines, interleukin (IL)-8 and tumor necrosis

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factor (TNF- α) were detected. Results Monolayers of A549 cells were co-incubated with *Burkholderia pseudomallei* (MOI=100 : 1) and centrifugated for 5 min at 170 \times g. At 1 hour of post-infection, the extracellular bacteria were killed by the addition of 250 μ g/mL kanamycin. Intracellular bacteria of A549 cells after infection with *Burkholderia pseudomallei* were observed as early as 4 h post-infection. MNGCs were observed in the 8 after infection, and *Burkholderia pseudomallei* induced release of IL-8 from A549 cells in a dose- and time-dependent fashion, whereas TNF- α was not detected. Conclusion A burkholderia pseudomallei invaded A549 cell model is successfully established.

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