The discovery of naked cluster particles of Parachlamydia and its developmental mechanism  LI Qinxue, HANG Qing-wu SHEN Jie, LI Zi-hua. School of Public Health, Fudan University, Shanghai 200032, China

Abstract  Objective  To study the survival and developmental morphology of Parachlamydia BN9 within Acanthamoeba. Methods  The morphology of BN9 within Acanthamoeba was studied by inverted phase contrast microscope, electron microscope, Gimenez and AO-staining with amoebal coculture. Results  The endosomal maturation-blocked were formed after the egress of BN9. Two developmental stages-elementary and reticulate bodies were both observed within the vacuoles. The reticulate bodies multiplicated by binary fission were located mainly within the vacuoles while the elementary bodies can also be located in the plasma individually. The naked cluster particles were observed after the trophozoites cytolysis with Gimenez-staining. The light infectious trophozoites could encyst and elementary bodies could survive within the mature cysts. Conclusion  The egress of BN9 could form the endosomal maturation-blocked which was presented in two developmental stages-elementary and reticulate bodies. It exhibited the cytolsin activity that could lyse the infectious trophozoites and were expelled in the vesicles. A few light infected amoeba could encyst with survival elementary bodies in the plasma.

Key words  Parachlamydia, Acanthamoeba, Electron microscopy
叶酸
氯高铁血红素
双蒸水
小牛血清
缓冲液
校正
棘阿米巴滋养体处于对数生长期，分别于4、8和12天成囊胞后，用吸管吹吸液体，使贴壁阿米巴脱落于液体中，然后取培养液，离心，弃上清后，将沉淀加培养液悬浮成密度约为1×10^6个/ml，进行玻片细胞涂片，然后进行吉姆尼兹染色，在显微镜下观察。

电镜观察: 同时分别于4、8、12天取培养液，离心后用戊二醛固定，超薄切片后透射电镜观察。

结果
副衣原体在卡氏棘阿米巴内的生活发育: 电镜下超微结构可见在阿米巴中存在两种密度明显不同的形态，颗粒在滋养体内可形成吞噬内体，其中密度较低的为网质体，可表现为不同的形态，有椭圆形、半月形、不规则形，大小约为2-3 μm，也可与网织体共存于吞噬体中。

染色后形态学观察: 在阿米巴共培养后期，我们观察到大部分感染的阿米巴滋养体出现溶解，副衣原体表现为成堆的大小不等的裸露圆形颗粒，在吉姆尼兹染色中，呈蓝紫色，其分布正好占满整个阿米巴原虫轮廓。

讨论
实验中，可见副衣原体存在明显的二个生活周期形态，即网织体和原体期，这与衣原体属的生活史相同，在电镜下可见致密的原体被阿米巴吞噬并在胞内形成吞噬体，可见原体在吞噬泡中发育成疏松的具有代谢能力的网织体，并经二分裂方式繁殖，分裂后的网织体再发育成致密的原体，这与等的副衣原体的发育假设相同，而在电镜下还可见有月牙体阶段，可能为副衣原体发育的介于网织体和原体间的生活期，也具有感染能力。
endosomal maturation-blocked EMB


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