Ultrastructural Patterns of Interactions Between the Murine Lung Macrophages and *Cryptococcus neoformans* Yeast Cells of Strains with Different Virulence

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Objectives: to study the peculiarities of interactions between murine alveolar macrophages (**Mg**) and *C. neoformans* yeast cells of the strains with different virulence.

Materials and methods. We studied the low (PKIIFY-881, -1165, -1178) and high virulent (PKIIFY-1090, -1095, -1106) strains of *C. neoformans* isolated from the patients, which were cultured on Sabouraud's agar for 3 days at 37° C. The strains with LD50 > 10⁶ cells/mouse are considered as low virulent and the strains with LD50 = 10² – 6 x 10⁴ cells/mouse as high virulent. The murine were infected by using intravenous injection. For TEM, the pieces of lung at the seventh post-experimental day were fixed in 3% glutaraldehyde (in 0.1 M cacodylate buffer) and post-fixed in 1% osmium tetroxide.

Results. It was found that if **Mg** ingest intact yeast cells, the later can: 1) become completely free from polysaccharide capsules, after that their content undergo lysis, and cell wall debris were extruded from the **Mg** (**1st scenario**); 2) become partly free from their capsules, destroy the phagosomal plasma membrane (Fig. 2 a) and induce destructive processes inside the **Mg** (Fig. 2 b) causing their death (**2nd scenario**); or 3) not lose their capsules and localize inside **Mg** in latent state (**3rd scenario**, fig. 2 h, i). Alveolar **Mg** may also ingest the senescent and dead *C. neoformans* yeast cells, which surrounded by capsule (**4th scenario**); then the last one also step-to-step lose from the stage of ingesting and in phagosome condition (Fig. 2 k-m). In all cases (excluding the cases of latent condition of fungal cells in macrophages) during the phagocytosis of the *C. neoformans* cells the macrophagal plasma membrane developed the systems of the tubules (Fig. 1 d,e), in which the substance of capsular polysaccharide was transported and then accumulated within their lumen in the form of local extention (Fig. 1 g), which then isolated into the macrophage cytosol as microvacuoles. In the phagosome, the system of the plasma membrane tubules, which surround the yeast cell in the form of “crown” (Fig. 1 b-e), the process of the capsular polysaccharide elimination continue.

Fig. 1. Light (a) and transmission electron microscopy (b - o) of **Mg** and *C. neoformans* yeast cells in the murine lung after 7 days of beginning experiments. a, e – PKIIFY-1095; b, c – PKIIFY-1165; d, f – PKIIFY-1178; g - j – PKIIFY-1090; k, l – PKIIFY-881; m - o – PKIIFY-1106. Explanation for this and figure 2: CW – cell wall, DeYC – dead yeast cell; DG – dark granule; DYC – dessicated yeast cell(s); IYC – intact yeast cells, LI – lipid inclusions; M – mito-chondrium(a); Mc – micro-fibrills; Mf – macrophage(s); Mv – microvacuole(s), N – nucleus, Nu – nucleolus, PC – polysaccharide capsule; YC – yeast cell.

Fig. 2. Ultrastructure of the *C. neoformans* yeast cells and **Mg** in the murine lung after 7 days of starting experiments. a, b, d, e – PKIIFY-1106; c, i – PKIIFY-1095; g, m – PKIIFY-881; g, h – PKIIFY-1165; k, l – PKIIFY-1178.

Conclusions:

- 1) after 7 days of beginning experiments, the interactions between the murine alveolar **Mg** and intact *C. neoformans* yeast cells developed by the **3rd scenarios**, whereas the absorption of dying and dead cells - by one (**4th scenario**);
- 2) the dependence of cell-mediated immunity on the stage of development of ingested *C. neoformans* cells is revealed;
- 3) for the first time the presence of dessicated yeast in lung tissue was found and the nature of latent state typical for certain murine **Mg** was discovered;
- 4) the new mechanism of capsular polysaccharide elimination of *C. neoformans* cells by murine **Mg** is described.