Developmental Biology of Pneumocystis carinii, an Alternative View on the Life Cycle of the Parasite*

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Summary. In this paper we present, based on elaborate ultrastructural studies, data on the existence of both intracellular and extracellular stages of Pneumocystis carinii, which result in a proposal of a new life cycle of the parasite. Up to now the formation of daughter cells in thick-walled pneumocysts is supposed to be the only way of multiplication. The present study shows that in rats treated with cortisone acetate the formation of daughter cells also takes place within thin-walled pneumocysts. In our opinion this way of multiplication is important for the understanding of the rapid increase in number of the parasites in an infected lung.

The presence of pneumocysts inside the alveolar epithelial cells suggests that intracellular development of the parasites can occur, but the method of cell penetration, intracellular multiplication and parasite liberation is still unknown.

Moreover our observations for the first time indicate a direct pathogenicity of the parasites in host cells.

Introduction

Pneumocystis is a common parasite in the lungs of vertebrates including man. It can be the cause of an interstitial pneumonia especially in the immuno-suppressed host. The taxonomy and the developmental cycle of the organism are still uncertain. In most studies (Carini and Maciel, 1916; Vanek and Jirovec, 1952; Seifert and Pliess, 1960; Vavra and Kucera, 1970; Campbell, 1972) development is thought to occur extracellularly in the lung alveoli as follows: thin-walled organisms, also called trophic stages (Vavra and Kucera, 1970; Campbell, 1972), develop into thick-walled pneumocysts and within these cysts the so-called intracystic bodies are formed. These intracystic bodies excyst and develop into young thin-walled pneumocysts. Especially electronmicroscopic studies support this view on the developmental cycle of the parasite (Wessel and Ricken, 1958; Vavra and Kucera, 1970; Campbell, 1972). So far intracellular occurrence of pneumocysts in alveolar epithelial cells has been described only by Shively and coworkers (1974). Price and Hughes (1974)

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noted the occurrence of the parasites in alveolar macrophages but these were described as "disorganized degenerated trophozoite debris."

We restudied the various developmental stages of the parasite. From our study we obtained as new information strong arguments pointing to a process of daughter cell formation within thin-walled pneumocysts. Moreover, additional information was obtained about the formation of daughter cells within thick-walled pneumocysts. Also, the important observation of the occurrence of parasites inside the alveolar epithelial cells during the early stages of immunosuppression by cortisone acetate was made.

Materials and Methods

Following the procedure of Weller (1955) for the induction of *Pneumocystis* pneumonia, 6-week-old rats (100–120 g) received subcutaneous injections of 12.5 mg cortisone acetate, four times a week. For the prevention of bacterial infection of the experimental animals 500 mg tetracycline/l was added to the drinking water. The lung tissue of rats treated for 4 weeks and 6 weeks respectively was studied. The decrease in body weight of these rats varied from 40–80 g.

The tissue was processed in the following way: Animals were anesthetized by intraperitoneal injection of pentobarbital. In situ fixation of the lungs was performed according to Weibel (1970): Glutaraldehyde (2%) in 0.05 M phosphate buffer (final osmolarity 320 m.osmol) was slowly instilled in the trachea under a pressure of 2 kPa (20 cm H$_2$O). The trachea was tied, the lungs were removed from the thorax and immersed for another 12 h in the fixation fluid mentioned. After a brief washing in 0.1 M phosphate buffer (320 m.osmol), postfixation of small parts of the lungs was performed with 2% osmium tetroxide during 2 h. Hereafter, the tissue was washed again in the buffer solution, dehydrated and embedded either in Epon 812 or in Paraplast.

Semi-thin and ultra-thin sections of the Epon embedded tissue were made with an LKB ultramicrotome.

For light microscopy the semi-thin sections were stained with toluidine blue. The thin sections were stained with uranyl-acetate and lead citrate, and studied with a Philips EM 300 electron microscope. Especially serial thin sections were extensively analysed. For scanning electron microscopy (SEM) 2-$\mu$m sections of Paraplast embedded tissue were treated with toluene for 10–30 min in order to dissolve the embedding medium. Thereafter the sections were treated with ethanol 100% and critical point dried according to the method of Anderson (1951). The specimens were coated with 20 nm gold. The SEM preparations were studied with a Philips PSEM 500 scanning electron microscope (12 kV, spot size 16–64 nm).

Results

Most of the alveoli of the rats treated for 6 weeks with cortisone acetate, are filled with material that light microscopically shows a honeycomb-like appearance, typical for massive *Pneumocystis* pneumonia. Electron microscopically the alveolar content appears to consist of an exudate, containing parasites, macrophages and cellular debris. At this stage of the infection (i.e., after a 6-week period of cortisone acetate treatment) the parasites are found only in the alveolar lumina. Basically two types of parasites can be distinguished: thin-walled and thick-walled pneumocysts. For the purpose of efficient description these two types are arbitrarily subdivided into several subtypes according to the scheme presented beneath.

Thin-walled pneumocysts

Subtype A: small rounded pneumocyst

B: grown (mature) pneumocyst

C: daughter cell containing pneumocyst