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NUCLEAR CHANGES IN ACANTHAMOEBA DURING PREENCYSTMENT

GUS TOMLINSON
Tennessee State University
Nashville, Tennessee 37203

ABSTRACT

Large intranuclear bodies are visible by transmission electron microscopy in *Acanthamoeba cantellanii* as they prepare to form a cyst wall. The bodies may traverse an enlarged nucleus and be as large as 0.83μm × 10 μm during the preencystment period. Other nuclear changes which occur during preencystment include loss of nucleolar mass, irregular and convoluted nuclear membranes and diffuse distribution of remaining nucleolar material.

INTRODUCTION

Cyst forming amoebae have been suspected as pathogens in mice and humans for many years (Culbertson, 1974). A substantial number of recent reports identifying amoebae with meningoencephalitis in Nigeria (Lawande, *et. al.*, 1979; Lawande, *et. al.*, 1980), meningoencephalitis in Costa Rica (Chinchilla, *et. al.*, 1979), meningoencephalitis in Canada (Scholten, 1979), meningoencephalitis in Austria (Thong, 1980), meningoencephalitis in the United States (Goehle, 1975; Marino, *et. al.*, 1975; Martinez, *et. al.*, 1975 and Yamauchi, *et. al.*, 1976), amebic dysentery in Europe (Prakash, 1974), and cerebral and cerebellar abcesses in the United States (Rinaldi and Murphy, 1979) has confirmed pathogenicity in mammals. Although pathogenic amoebae are usually observed in host tissue in trophozoite form, the capacity of such amoeba to survive both inside and outside their hosts and to be transmitted via air, water, and food to other hosts is directly dependent upon their ability to form thick, highly resistant cyst walls (Cursons, *et. al.*, 1979; Piekarski, 1979; Kadlec, *et. al.*, 1980). *Acanthamoeba castellanii*, which has heretofore been considered to be a relatively inocuous cyst forming amoeba, has now been identifed with skin lesions (Gullet, *et. al.*, 1979), meningoencephalitis (Willaert and Stevens, 1976, Keratitis (Key, *et. al.*, 1980) and virulence in mice (De Jonckheere, 1980). For these reasons and because *Acanthamoeba* can be grown in axenic culture and induced to undergo encystment synchronously (Tomlinson, 1967), this organism was selected for studying cyst wall formation in amoebae.

MATERIALS AND METHODS

Log-phase *Acanthamoeba* were induced to encyst synchronously by suspending them in sterile encystment medium (EM) consisting of a buffered inorganic saline solution which was adjusted to pH 6.8 and aerated (Tomlinson, 1962). Observations were made on aliquots which were collected from a single culture of synchronously encysting cells. Samples were collected at the time the culture was induced to encyst (T_0) and at 2 hour intervals until completion of encystment 30 hours later. The period T_0 to T_{12} is defined as the preencystment period since it precedes cyst wall formation as viewed by phase contrast and electron microscopy. Immediately after collecion, each sample was washed in 0.1 M phosphate buffer pH 6.8 which had been rendered isotonic with potassium chloride. Cells were then concentrated by centrifuging 3 minutes at 500 × g in an international HR-1 centrifuge.

Acanthamoeba were fixed for electron microscopy in 2% glutaraldehyde for 1 hour at 4°C and postfixed in 1% O_sO_4 for 1 hour at 4°C. Specimens were dehydrated in ethanol using two washes of 50%, 70%, 95% and absolute, embedded in Epon 812 and sectioned on an LKB Ultratome with DuPont diamond knives. Specimens were then stained on grids with 1% uranyl acetate for 10 minutes and examined in a Phillips 200 electron microscope operated at 40 KV to 100 KV with double condensors and 20 micron molybdenum apertures in the objective lens.

RESULTS

Low magnification micrographs of sections of *Acanthamoeba* during preencystment show some cells with large intranuclear bodies (Fig. 1)) which almost traverse an enlarged nucleus that is irregular in shape in contrast to the usual spherical nucleus. The nuclear bodies may be as large as 10 μm long and 0.85 μm wide. They were not observed in uninduced cells or mature cysts.

On closer examination, the intranuclear bodies are strand-like in internal design with "Strands" running essentially parallel to the long axis of the body (Fig. 2). Other nuclear changes which were observed during preencystment include loss of nucleolar mass, irregular and convoluted nuclear membranes and diffuse distribution of nucleolar material throughout the nucleus in contrast to a single, spherical nucleolus in *Acanthamoeba* prior to their induction to encyst.

DISCUSSION

Since large intranuclear bodies save been observed in *Acanthamoeba* only during preencystment, it seems likely that their appearance is related to preparation to form cyst wall. One of the major structural components of *Acanthamoeba* cyst walls is cellulose (Tomlinson, 1962). *De novo* synthesis of cellulose from intracellular precursors begins in late preencystment (Tomlinson, 1967) and seems to correlate in time with the appearance of the "Nuclear bodies" observed in this study. It is tempting to speculate that the two events are related. This possibility is under investigation at the present time.

Non-nuclear changes were also observed in this study. Among them were ultrastructural changes in mitochondria, an increase in the number of secretion granules and cytoplasmic vacuoles, and conspicuous lipid droplets throughout the cell. These changes and changes in lysosomes and microbodies as cyst wall formation procedes will be the subject of a later report.

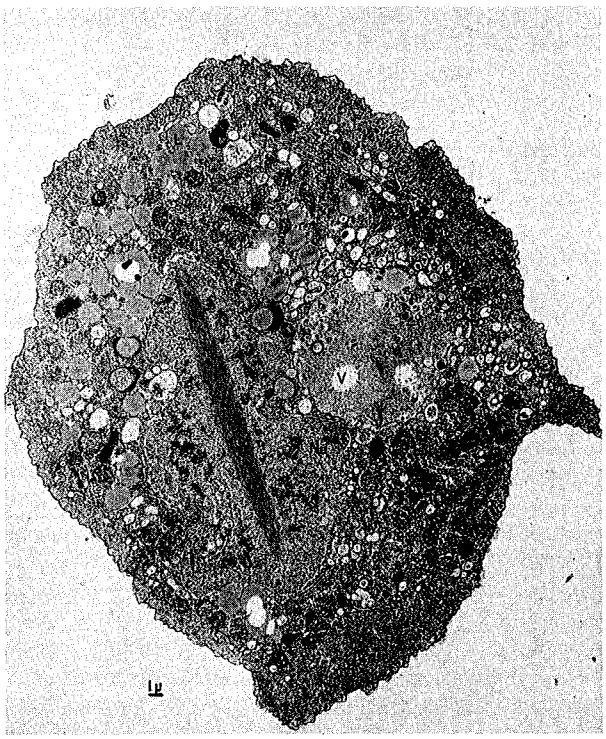

FIG. 1. *A section of Acanthamoeba during preencystment. The arrows point to an intranuclear body surrounded by diffuse nucleolar granules. L, lipid; V, vacuole; Nu, nucleus. X 14000.*

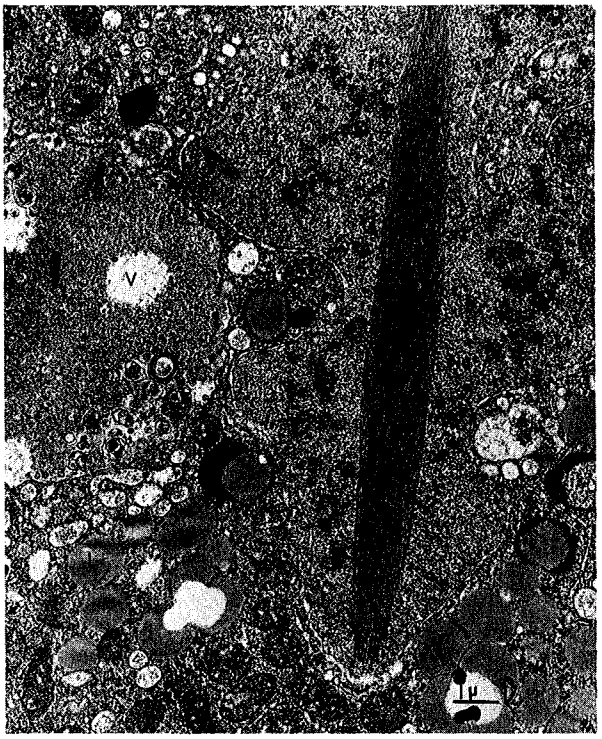

FIG. 2. *A section of Acanthamoeba during encystment at higher magnification. The arrows point to the same intranuclear body as in Fig. 1. X 35000.*

LITERATURE CITED

Chinchilla, M., Castro, E., and Partilla, E., 1979 Free Living Amoeba that can Cause Meningoencephalitis: 1st Findings in Costa Rica. Rev. Lat. Am. Microbiol. 21 (3): 135-42.

Culbertson, D. G., 1974 Pathogenic Soil Amoebas: Summary of Experimental and Clinical Developments, *in* Ninth International Congress on Tropical Medicine, Vol II, P16, G. Papaevangelou, Ed. Congress Publications Committee: Athens, Greece.

Cursons, R. T., Brown, T. J., and Hyne, B. E., 1979 Primary Amoebic Meningoencephalitis in an Indoor Heat-Exchange Swimming Pool. NZ Med. J. 90 (646); 330-31.

De Jonckheere, J. F., Growth Characteristics, Cytopathic Effect in Cell Culture and Virulence in Mice of 36 type Strains Belonging to 19 Different *Acanthamoeba* Spp. Appl. Environ. Microbiol. 39 (4); 681-85.

Goehle, R. M., 1975 Amoebic Encephalitis. J. Pediatr. 87 (1): 149.

Gullett, J. Mills, J., Hadley, K., Podemski, B. 1979 Disseminated Granulomatous *Acanthamoeba* Infection Presenting as an Unusual Skin Lesion. Am. J. Med. 67 (5): 891-96.

Kadlec, U., Sky, A. J. and Cerva, L., 1980 Virulent *Naegleria fowleri* in Indoor Swimming Pool. Folia. Parasitol. (Praha) 27 (1): 11-17.

Key, S. N., Willaert, E., and Stevens, A. R., 1980 Keratitis Due to Acanthamoeba castellanii: A Case Report. Arch. Opthamol. Mar; 98(3):475-9

Lawande, R. U., John, I., Dobbs, R. H. and Egler, L. J., 1979 A Case of Primary Amebic Meningoencephalitis in Zaria, Nigeria. Am. J. Clin. Pathol. 71 (5): 591-94.

Lawande, R. U., MacFarlane, J. T., and Weir, W. R., 1980 A Case of Primary Amebic Meningoencephalitis in a Nigerian Farmer, Am. J. Trop. Med. Hyg. 29 (1): 21-25.

Marino, J. T., Jr., Sotelo-Avilo, C and Taylor, F. M., 1975 Amplification of Primary Amoebic Meningoencephalitis. J. Pediatr. 86 (1): 160-161.

Martinez, A. J. and Duma, R. J., 1975 Experimental Amoebic Meningoencephalitis Due to Free Living Amoebas. J. Neuropathol. Exp. Neurol. 34 (1): 76-77.

Piekarski, G., 1979 Amoebas in Drinking Water and in Public Baths. Immun. Infekt. 7 (2) 39.

Prakash, C., 1974 Laboratory Methods in the Diagnosis of Amoebiasis, *in* Epidemiology, Therapeutics and Clinical Correlations, XII P179. G. Padilla Ed. Charles C. Thomas, Publishers, Springfield, Ill.

Rinaldi, I., and Murphy, D., 1979 Primary Amebic Meningoencephalitis with Cerebral and Cerebellar Abcesses: Case Report. NeuroSurg. Nov. 5 (5): 607-10.

Scholten, T., 1979 Soil Amoeba in Canada. Can. Med. Assoc. J. Feb. 3; 120 (3): 264, 267-8.

Thong, Y. H., 1980 Primary Amoebic Meningoencephalitis: Fifteen Years Later. Med. J. Aust. Apr. 19; 1 (8): 352-4.

Tomlinson, G. and Jones, E. 1962 Isolation of Cellulose from the Cyst Wall of a Soil Amoeba. Biochem. Biophys. Acta 63; 194-200.

Tomlinson, G., 1967. The Glyoxylate Pathway in *Acanthamoeba*. J. Protozool. 14 (1): 114-16.

Willaert, E., and Stevens, A. R., 1976 Indirect Immunofluorescent Identification of *Acanthamoeba* causing Meningoencephalitis. Pathol. Biol. 24 (8): 545-47.

Yamauchi, T., McKee, T. W., Euler, A. R. and White, P. C. 197 Amedic Meningoencephalitis in Arkansas. J. Ark. Med. Soc. Sep. 76 (4): 164-68.