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Cytopathogenicity of *Naegleria gruberi* for Rat Neuroblastoma Cell Cultures

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Amoebae of *Naegleria gruberi* were cytopathic for cultures of rat neuroblastoma (B-103) cells. *N. gruberi* grew and destroyed B-103 cells at 30°C. As few as one amoeba inoculated per million B-103 cells resulted in cytopathogenicity after extensive growth of *N. gruberi*.

Previous studies have demonstrated that the pathogenic amoeboflagellate *Naegleria fowleri* is cytopathogenic for cultured mammalian cells (2–7, 9). In contrast, previous workers have demonstrated that the free-living species *N. gruberi* is not pathogenic for a variety of experimental animals and have concluded that it is not cytopathogenic for cultured mammalian cells (3–6). We have observed that *N. gruberi* produces cytopathic effects on cultured rat neuroblastoma (B-103) cells under appropriate conditions.

N. gruberi was grown in Balamuth medium composed of 5 g of glucose, 5 g of yeast extract, 10 g of liver digest, 10 g of proteose peptone, 0.12 g of NaCl, 0.142 g of Na_2HPO_4, 0.136 g of KH_2PO_4, 0.004 g of $MgSO_4 \cdot 7H_2O$, and 0.004 g of $CaCl_2 \cdot 2H_2O$ in 1 liter of deionized water. Filter-sterilized hemin was added to a final concentration of 2 μg/ml (1). The amoebae were cultivated in tissue culture flasks (Falcon Plastics, Oxnard, Calif.) at 30°C. Rat neuroblastoma (B-103) cells were obtained from D. Schubert (Salk Institute, San Diego, Calif.). The B-103 cells were grown in Eagle minimal essential medium with Earle balanced salt solution, supplemented with 10% fetal calf serum (Flow Laboratories, Inc., Rockville, Md.). Antibiotics were not added. *N. gruberi* were suspended in Eagle medium and added to a confluent monolayer of B-103 cells in tissue culture flasks to give a multiplicity of infection of one amoeba per target cell unless otherwise indicated. Infected and uninfected B-103 cell cultures were incubated for several days and were observed daily to detect cytopathogenicity in infected cultures or spontaneous deterioration of the target cell monolayer in uninfected cultures. At the end of each incubation period (36 to 300 h), B-103 cell viability was assessed by the trypan blue exclusion assay.

All of the seven strains of *N. gruberi* tested (EGB, NB-1, 1518, S, EGS, NEG, and NG-7) were cytopathogenic for B-103 cells under appropriate conditions. At a multiplicity of infection of one, total destruction of the target cells occurred within 3 to 4 days at 30°C. *N. gruberi* EGB has been chosen for subsequent studies on cytopathogenicity for B-103 cells.

Marked cytopathogenicity was not observed in infected B-103 cultures for several hours but, by light-microscopic examination, the amoebae appeared to be attached to the target cells. After 3 h, about 60% of the nuclei of infected B-103 cells could be stained by trypan blue, whereas less than 10% of the nuclei of uninfected B-103 cells could be stained by trypan blue after 9 days of incubation at 30°C.

The cytopathic action of *N. gruberi* was dependent upon time, temperature, and multiplicity of infection. At 30°C and a multiplicity of infection of one, *N. gruberi* destroyed the B-103 target cells within 72 h; at a multiplicity of infection of one amoeba per 10^6 target cells, more than 300 h was required (Table 1). During this period, the amoebae grew to a population of 10^5 to 10^6/ml. Cytopathogenicity was more rapid and severe at 25°C than at 30, 32, or 37°C. At 37°C, *N. gruberi* was cytopathic at a multiplicity of infection of one, but gross cytopathogenicity was not observed at lower multiplicities. No cytopathic action was detected at 4 or 42°C (Table 2). At 42°C, the B-103 cells detached and became rounded; the added amoebae also became rounded and detached. The infected B-103 cells did not lyse at 42°C, but both infected and uninfected target cells lost the ability to exclude trypan blue after 24 h at 42°C.

Amoebae of *N. gruberi* were closely associated with B-103 target cells, and within 3 h, several neuroblastoma cells were lysed (Fig. 1a). Intact target cells could be observed near lysed B-103 cells. The nuclei of the lysed B-103 cells were intact, although the distribution of chromatin was abnormal. Large vacuoles containing membranous material resembling target cell debris were observed in amoebae near lysed target cells (Fig. 1a and b).

Our preliminary observations indicate that lysis of target cells by amoebae required con-

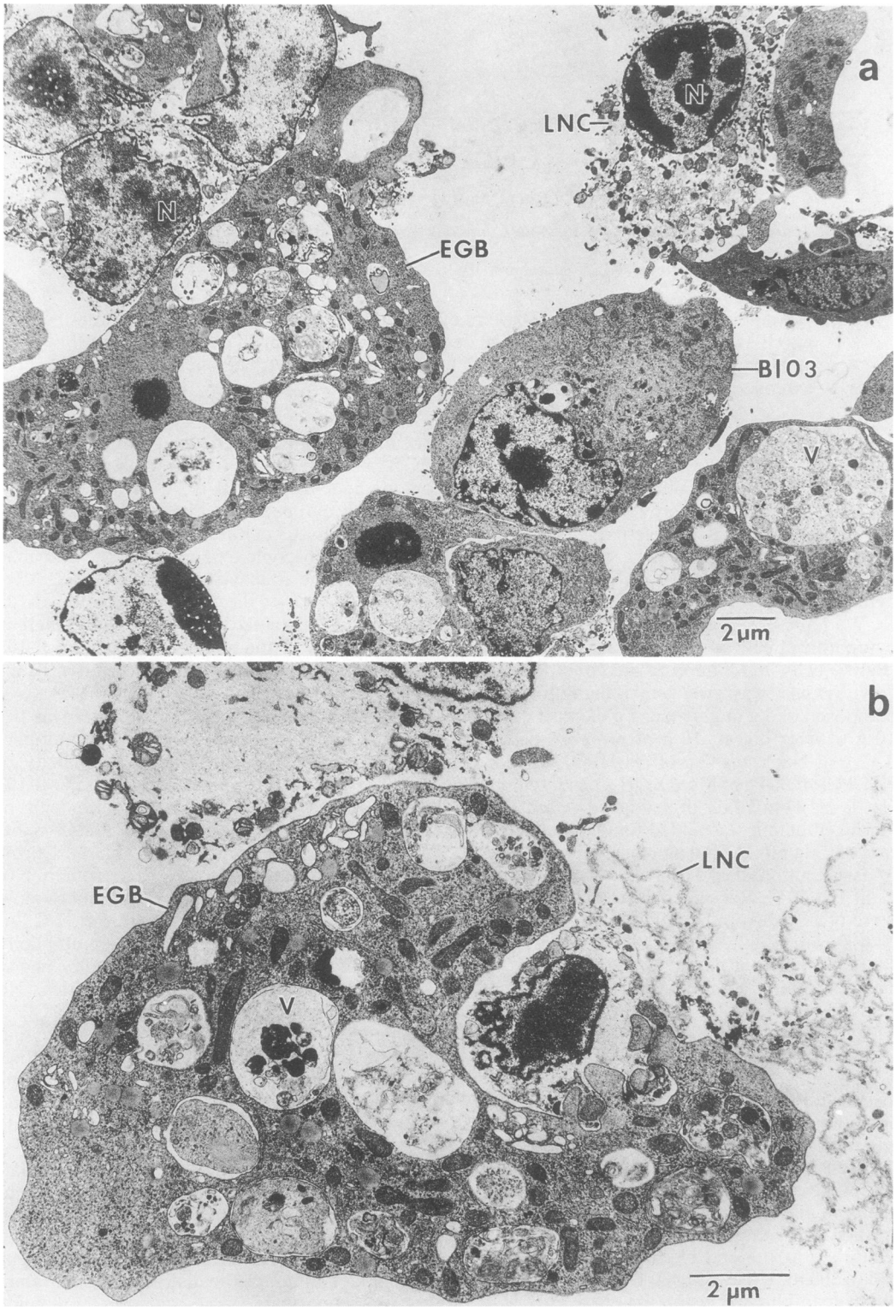

FIG. 1. Lysis of rat neuroblastoma cells by *N. gruberi* within 3 h at 30°C. (a) Both lysed neuroblastoma cells (LNC) and intact neuroblastoma cells (B-103) were observed near *N. gruberi* amoebae (EGB). The nuclei (N) of lysed B-103 cells remained intact. Vacuoles (V) containing debris resembling lysed target cells were found in several amoebae (a and b). Amoebae appeared to ingest B-103 cellular debris by phagocytosis (b). Scale marker = 2 μm.

TABLE 1. Influence of multiplicity of infection on the time required for complete destruction of B-103 cells by *N. gruberi* EGB[a]

No. EGB/no. B-103	Days for cytopathogenicity
1:1	3
1:10	4
$1:10^2$	7
$1:10^3$	9
$1:10^4$	11
$1:10^5$	13
$1:10^6$	14

[a] Cultures of B-103 cells were infected with EGB at the multiplicity of infection indicated and incubated at 30°C. The progress of cytopathogenicity was monitored by light-microscopic examinations at 12-h intervals.

TABLE 2. Influence of incubation temperature on the time required for complete destruction of B-103 cells by *N. gruberi* EGB[a]

Temperature (°C)	Days for cytopathogenicity
4	—[b]
25	1.5–2
30	3
32	4
37	3–5

[a] Cultures of B-103 cells were infected with EGB at a multiplicity of infection of one and incubated at the temperature indicated. The progress of cytopathogenicity was monitored by light-microscopic examinations at 12-h intervals.

[b] No cytopathogenicity detected.

tact. Accordingly, lysis was probably not the result of toxic products or enzymes released into the medium. The amoebae appeared to ingest target cell constituents after lysis (Fig. 1b). *Entamoeba histolytica* also appears to kill target cells by a contact-dependent process (8).

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