NOTES

Cytopathogenicity of Naegleria fowleri for Rat Neuroblastoma Cell Cultures: Scanning Electron Microscopy Study

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Neuroblastoma cells were inoculated with Naegleria fowleri Lee and examined for cytopathology at various periods post-inoculation by scanning electron microscopy. By 18 h post-inoculation, approximately 50% of neuroblastoma cells were nonviable, as evidenced by trypan blue exclusion and light microscopic examination. This cytopathology resulted from piecemeal consumption of target cells mediated by a sucker apparatus extending from the surface of N. fowleri.

Naegleria fowleri, the causative agent of primary amoebic meningoencephalitis in humans, is cytopathogenic for a variety of cultured mammalian cells (1, 2, 4–6, 10, 11). The proposed mechanism(s) of the cytopathic action of this organism for mammalian cells include active phagocytosis of cells by pseudopod formation (11), trogocytosis or repeated nibbling by trophozoites (1, 2), and secretion of cytotytic substances by N. fowleri (4). Previous transmission electron microscopy studies in this laboratory have established that amoebae ingest portions of mammalian cells without causing lysis during 12 h of cocultivation (10).

In the present study, we utilized scanning electron microscopy to demonstrate that N. fowleri amoebae induce cytopathogenesis in rat neuroblastoma (B103) cultures by focal ingestion of target cells. This ingestion is mediated by a sucker structure which extends from the amoeba.

N. fowleri Lee (ATCC-30894) was isolated by E. C. Nelson from the cerebrospinal fluid of a patient with fatal primary amoebic meningoencephalitis (7). Amoebae were grown axenically in Nelson medium (12) in tissue culture flasks (Falcon Plastics, Oxnard, Calif.) at 37°C.

Rat neuroblastoma (B103) cells were obtained from D. Schubert (The Salk Institute, San Diego, Calif.) and grown in Eagle minimal essential medium with Hanks balanced salt solution supplemented with 10% fetal calf serum. Antibiotics were not added. N. fowleri was suspended in Eagle medium and then added to a subconfluent monolayer of B103 cells which had been seeded on 15-mm round glass cover slips. Infected cell cultures, uninoculated control cultures, and N. fowleri cultures maintained in Eagle minimal essential medium with Hanks balanced salt solution were incubated for 24 h in a CO2 incubator. Replicate cultures of each sample were assessed for cell viability by trypan blue exclusion. Cultures were harvested after 3, 6, 12, 18, and 24 h and processed for scanning electron microscopy.

Samples for scanning electron microscopy were fixed in situ by replacing the culture medium with warm (37°C) 2% glutaraldehyde in 0.1 M sodium-cacodylate-hydrochloride buffer (pH 7.2) containing 0.1 M sucrose. After fixation at 37°C for 30 min, the cover slip cultures were stored at 4°C for 24 h. Cultures were postfixed in 1% OsO4 in 0.15 M cacodylate buffer at room temperature for 90 min. After a rinse in 0.15 M cacodylate buffer, cultures were dehydrated in a graded series of ethanol, subjected to critical-point drying, and sputter-coated with a layer of gold (3). Cover slips were examined with a Hitachi HS-500 scanning electron microscope operating at an accelerating voltage of 20 kV.

The B103 nerve cells in uninfected cultures typically were spindle shaped and had relatively smooth surfaces. Cytoplasmic extensions of each cell reached out to and made contact with adjacent cells. In cultures inoculated with N. fowleri, the protozoa could be readily distinguished from B103 cells by their amoeboid appearance and by the presence of pseudopodia-like structures bearing a sucker (Fig. 1B and C). In cultures examined 3 to 6 h post-inoculation, N. fowleri was observed attached to B103 cells (Fig. 1B). Cytopathic effects on the neuroblasto-
ma target cells were not evident up to 6 h post-inoculation, although portions of the target cell appeared to be drawn into the sucker apparatus (Fig. 1, arrow) of the amoeba. Significant damage to neuroblastoma cells was evident 18 h after inoculation (Fig. 1C). The cytoplasmic extensions of the nerve cells were absent. In addition, numerous crater-like depressions were present over the entire surface of the neuroblastoma cell. B103 cells with these craters generally had amoebae either attached to their surface or in close proximity. At 24 h post-inoculation, B103 cells had most of their cytoplasm consumed; a cratered, cellular skeleton remained (Fig. 1D).

FIG. 1. Scanning electron micrographs of B103 neuroblastoma cells cultured in the absence or presence of *N. fowleri* Lee. (A) Uninfected B103 cells are spindle shaped and exhibit cytoplasmic extensions which contact adjacent cells. (B) B103 cells at 6 h post-inoculation exhibit *N. fowleri* in contact with the nerve cell body and cytoplasmic extensions. Note that a portion of the nerve cell cytoplasm appears to be ingested into the *N. fowleri* sucker apparatus (arrow). (C) B103 cells at 18 h post-inoculation exhibit numerous crater-like indentations on the cell surface. Note the presence of the sucker structure on *N. fowleri* (arrow). (D) At 24 h post-inoculation, a cratered skeletal remnant of a B103 cell is evident. Bars, 5 μm.
when these amoebae destroys fowleri medium exhibited the mechanism of target cells. However, the ingestion of portions of the neuroblastoma cell cytoplasm. This unique sucker appears to be part of the normal structure of N. fowleri amoebae, since it was observed on amoebae from axenic cultures as well as on amoebae cocultivated with B103 neuroblastoma cells. Thus, the sucker apparatus does not appear to be specifically induced when amoebae come in contact with mammalian cells.

Other investigators who utilize scanning electron microscopy to study N. fowleri have failed to observe such structures, probably because of the fixation procedures employed (8; A. J. Martinez, E. C. Nelson, D. G. Fultz, and R. Geerlings, Proc. 33rd Ann. Meet. Electron Microsc. Soc. Am., p. 652). The procedure employed for fixation of N. fowleri proved of utmost importance in elucidating the presence of the sucker apparatus on amoebae. Sucker structures were not observed on the surfaces of amoebae when cold (4°C) fixation procedures were utilized. However, when freshly prepared 2% buffered glutaraldehyde warmed to 37°C was used as the primary fixative, the sucker structures were readily demonstrated. This procedure minimizes distortions of the surface and retraction of surface structures.

Surface extensions called food cups have been observed on the surface of Entamoeba histolytica (9). The nature and function of these food cups are unknown.

In summary, N. fowleri amoebae induce cytopathology via piecemeal ingestion of target cells, which is mediated by a sucker-like apparatus. This ingestion process does not result in immediate lysis of the target cell. However, the nature of the cell-to-cell interaction which occurs between amoebae and target cells within the sucker structure remains to be defined.

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LITERATURE CITED


