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Sucker-Like Structures on the Pathogenic Amoeba *Naegleria fowleri*

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Using scanning electron microscopy, we observed sucker-like structures on amoebae of 13 human isolates of *Naegleria fowleri*. The number of suckers per amoeba seemed to vary according to the virulence of the strain. We propose the term amoebastome to describe this unique sucker-like structure of *N. fowleri*.

Naegleria fowleri is a free-living amoeboflagellate and the etiological agent of a fatal human disease known as primary amoebic meningoencephalitis. Although it is a somewhat rare disease, cases of primary amoebic meningoencephalitis have been reported from all continents but Antarctica. Likewise, the amoebae of *N. fowleri* have been isolated from a variety of environmental sources worldwide (8).

The determinants of virulence, invasiveness, and pathogenicity of *N. fowleri* are largely undefined. In vitro studies have implicated toxins and cytopathic enzymes (2, 3, 7, 13), infectious cytopathogenic material (5), and phagocytosis (1, 10). In this report, we describe a novel form of phagocytosis for *N. fowleri* amoebae, one that employs well-organized sucker-like structures.

MATERIALS AND METHODS

Naegleria fowleri amoebae were grown in Nelson medium (12) in 93-mm plastic Leighton tubes with 5-cm^2 plastic cover slips (Costar, Data Packaging Corp., Cambridge, Mass.). At 72 h of culture age (early stationary growth phase), cover slips, with amoebae attached, were removed from the growth medium, rinsed in Page amoeba saline (11), and fixed at room temperature (23°C) for 1 h with 2.5% (vol/vol) glutaraldehyde in Sorensen phosphate buffer (pH 7.2) containing 1% (wt/vol) sucrose. After fixation, cover slips were rinsed in Sorenson buffer, dehydrated in ethanol, critical point dried, mounted on stubs, and coated with gold-paladium. All specimens were examined and photographed in a JEOL 35C scanning electron microscope at 15 kV.

RESULTS

Using scanning electron microscopy, we examined 13 human isolates of *N. fowleri* (see reference 8 for sources and dates of isolation) and observed sucker-like structures on amoebae from all isolates. The number of suckers per amoeba ranged from 1 to, in one instance, 12. Amoebae may have a single sucker with smooth, even margins (Fig. 1) or multiple suckers with irregular margins (Fig. 2). In contrast, we have examined amoebae of three strains of *Naegleria gruberi*, the nonpathogenic relative of *N. fowleri*, and have not observed suckers on any of the organisms.

There appears to be an inverse correlation between the mean number of suckers per amoeba and the virulence of a particular strain for mice (Table 1). For the five strains of *N. fowleri* we have enumerated, the average number of suckers per amoeba ranged from 1.6 in the CJ strain to 0.5 for NF69, with mortality for mice ranging from 0 to 90%, respectively.

The suckers are functional, being employed by the amoebae to engulf food. Figure 3 shows three *N. fowleri* amoebae using their suckers to devour a fourth, presumably dead, amoeba.

DISCUSSION

The presence of suckers on an amoeba is rather unexpected since, by definition, the word amoeba means "change." One would not expect amoebae to have such well-organized surface structures. However, *N. fowleri* is not a typical amoeba. Among other things, it is an amoeboflagellate and a free-living pathogen. The manner in which *N. fowleri* employs its suckers, as shown in Fig. 3, is not unlike the relationship that occurs between predator and prey.

The number of suckers per amoeba varies with the strain or isolate of *N. fowleri*. It may be that the number of suckers per amoeba is a reflection of the length of time an isolate has been maintained in axenic culture, i.e., the longer the time, the greater the number. Strain CJ, with an average of 1.6 suckers per amoeba (Table 1), was isolated in 1967, whereas NF69, with 0.5 sucker per amoeba, was isolated in 1969 (8). However, not all of the isolates have been cultured axenically since primary isolation, so the length of time in axenic culture cannot be correlated with the date of isolation.

We are certain, however, that the presence of suckers is not an artifact of axenic cultivation because we find suckers, albeit fewer, on amoebae (LEE strain) of *N. fowleri* that have been serially mouse passaged, at monthly intervals, 21 times. Figure 1 is a micrograph of LEE strain amoebae at the 16th mouse passage. We also find suckers on amoebae of recent environmental isolates of *N. fowleri*. If the presence of suckers were an artifact of axenic cultivation, we would expect to find similar structures on the amoebae of our axenic stock cultures of *N. gruberi*, and we do not.

Similarly, suckers are not an artifact of fixation of axenically cultured amoebae, e.g., the possibility that a sucker may be an artifact produced, upon fixation, by a conventional food vacuole just below the membrane surface. Suckers have clearly defined ridge-like margins and are far too large to be collapsed membranes over food vacuoles. If suckers were merely the product of fixation, then similar structures should be present on axenically grown similarly fixed amoebae of *N. gruberi*.

We find the inverse correlation between the number of suckers per amoeba and virulence for mice to be a provocative observation because one would expect greater virulence

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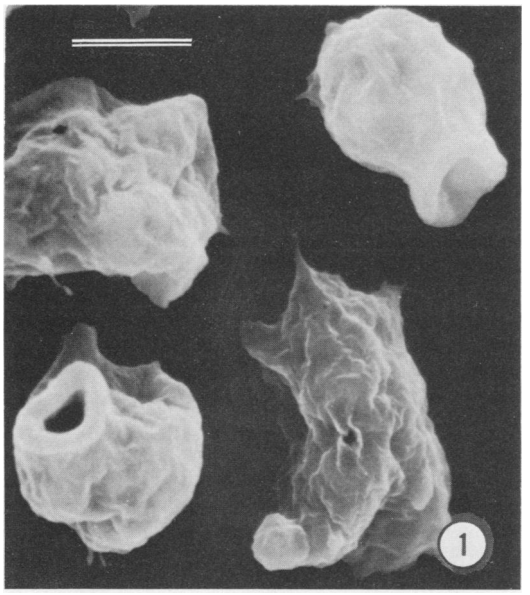

FIG. 1. *N. fowleri* (LEE M-16 strain) amoebae from axenic culture. Two amoebae have single smooth-edged suckers. The M-16 denotes that the strain had been serially mouse passaged 16 times, at monthly intervals, to retain virulence. Bar = 5 μm.

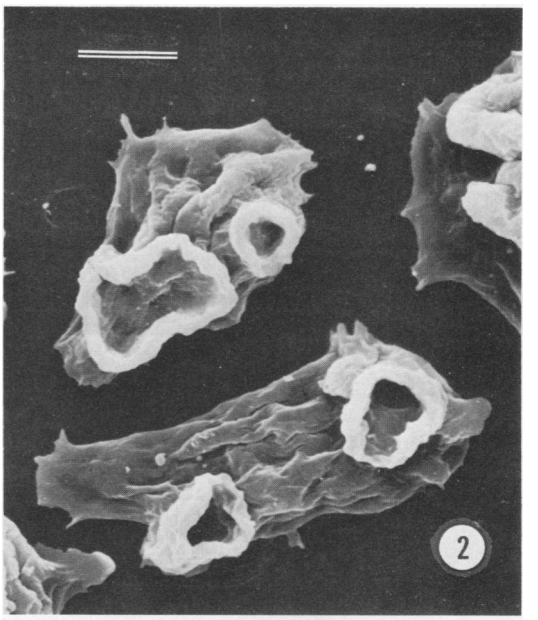

FIG. 2. *N. fowleri* (0359 strain) amoebae, from axenic culture, with multiple suckers having irregular margins. Bar = 5 μm.

to be associated with increased number of suckers, if these structures were involved in pathogenesis. The data suggest that the more virulent the strain, the fewer suckers present and, presumably, the less phagocytic (engulfment) activity taking place. We also know that increased axenic cultivation decreases amoeba virulence (4, 6); hence, the number of suckers may, again, be reflecting the length of time in axenic culture.

The only paper we are aware of that describes a similar surface structure for amoebae is one in which small disks, referred to as food cups, have been reported on the surface of an amoeba of *Entamoeba histolytica*, the pathogenic intestinal amoeba of man (9). Brown (1) refers to the nibbling activity of *N. fowleri* amoebae on cultured mouse embryo

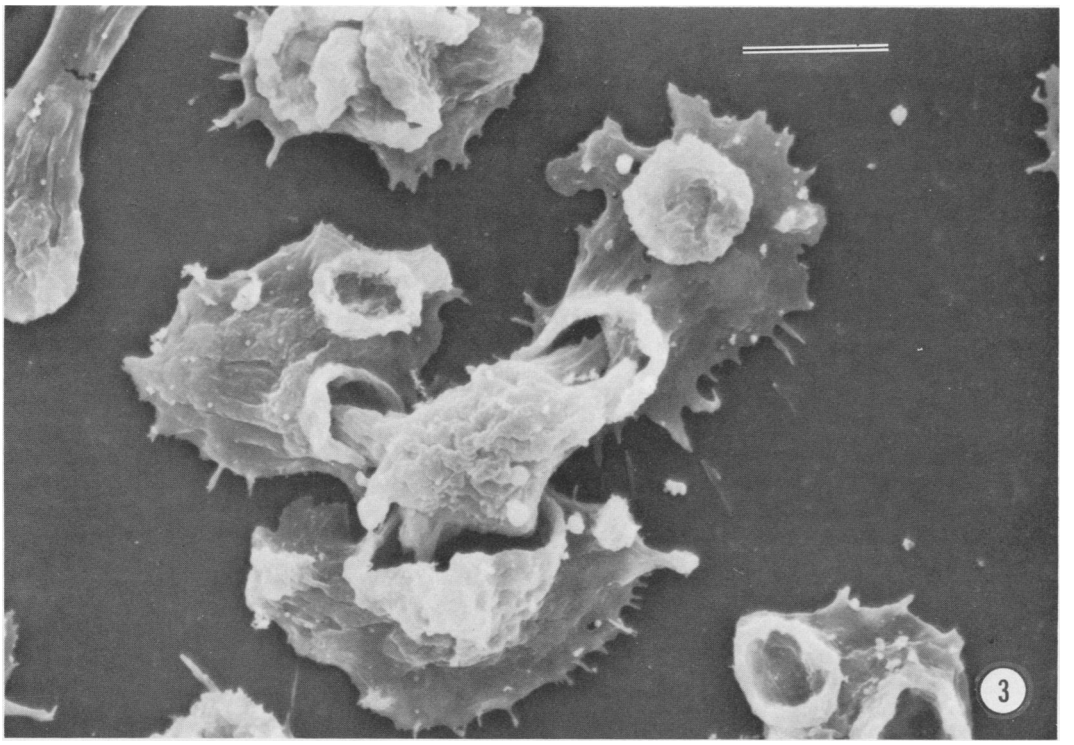

FIG. 3. Three *N. fowleri* (CJ strain) amoebae, from axenic culture, attacking and beginning to devour or engulf a fourth, presumably dead, amoeba. Bar = 10 μm.

TABLE 1. Comparison by strain of the average number of suckers per amoeba of *N. fowleri* and virulence for mice

Strain of amoeba	Mean no. of suckers per amoeba[a]	Mortality (%) for mice inoculated intranasally[b]
CJ	1.6 ± 0.80	0
TY	1.4 ± 0.67	10
LEE	1.0 ± 0.71	20
NF66	0.6 ± 0.42	75
NF69	0.5 ± 0.41	90

[a] The total number of suckers on 100 amoebae per strain was counted, and the average number of suckers per amoeba was calculated.

[b] The numbers in this column are from Table 3 (p. 117) of reference 8. Groups of 20 male DUB/ICR mice (13 to 18 g) were inoculated intranasally with 5×10^3 amoebae per mouse for each of the five strains of *N. fowleri* listed. Cumulative percent dead (mortality) was recorded to 28 days after inocuation.

cells as trogocytosis, from the Greek meaning "to nibble." The papers by Brown (1) and Marciano-Cabral et al. (10) have remarkable transmission electron micrographs (plate XXVIII and Fig. 3, respectively) showing trogocytosis which, in hindsight and with the perspective of scanning electron microscopy, most certainly are amoeba suckers drawing in or engulfing portions of target cells in a manner similar to that which we show in our Fig. 3.

Neither food cups nor trogocytosis seems appropriate for describing the appearance or the forceful engulfing employed by the suckers we have observed. "Sucker," too, remains inadequate. The word cytostome is used to identify the mouth-like openings present in certain protozoa. However, cytostomes are fixed openings, whereas the suckers we have described are not. Therefore, we propose the term amoebastome to describe the sucker-like structures that *N. fowleri* amoebae use in this novel form of phagocytosis.

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