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# Immunity to Pathogenic Free-Living Amoebae: Role of Humoral Antibody

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Pathogenic free-living amoebae are common in nature, but few clinical infections by these amoeba have been reported. This has prompted studies of host susceptibility factors in humans. A survey of normal human sera from three New Zealand Health Districts was made; antibodies to pathogenic free-living amoebae were found in all sera, with titers ranging from 1:5 to 1:20 for *Naegleria* spp. and from 1:20 to 1:80 for *Acanthamoeba* spp. The antibodies belonged mainly to immunoglobulin G and immunoglobulin M classes. The presence of a specific neutralizing factor against *Acanthamoeba* spp. but not *Naegleria* spp. was demonstrated. Possible protective mechanisms are discussed.

Pathogenic free-living amoebae (PFLA) have been shown to cause a variety of diseases in both humans and experimental animals, ranging from the acute disease primary amoebic meningo-encephalitis to chronic nonspecific diseases, such as eye infections, respiratory infections, and the newly described humidifier fever (5, 9, 10, 11, 26, 28). Despite the prevalence and ease of isolation of these amoeba from a wide variety of environmental sources (1, 3, 6, 7, 9, 15, 17, 25, 33, 38), surprisingly few clinical infections by these amoebae have been reported. Furthermore, in nearly all reported clinical cases, infection has occurred either because of some underlying predisposing condition (such as immunosuppressive treatment) in immunologically privileged sites in the body (such as in the cornea of the eye) or only sporadically in similarly exposed individuals (9, 13, 37).

This paradox has prompted speculation on the existence of probable host-related susceptibility factors because of the low incidence of infection by PFLA in humans (1, 12, 14, 22, 24, 38). With this in mind, a serological survey of apparently healthy humans was conducted to establish background antibody titers.

## MATERIALS AND METHODS

*Naegleria gruberi* PL200f and *Naegleria fowleri* MsT were obtained from the National Health Institute, Wellington, New Zealand. *Acanthamoeba culbertsoni* A-1 was supplied by the Culture Centre for Algae and Protozoa, Cambridge, England, and *Acanthamoeba castellanii* 1501 was obtained from E. Willaert, Prince Leopold Institute of Tropical Medicine, Antwerp, Belgium. Both *Naegleria* spp. were cultivated axenically in CYM (29), and both *Acanthamoeba* spp. were cultivated axenically in 4.0% Neff medium (32). However, when used as antigens in the indirect fluorescent antibody test, amoebae were cultured monoxenically with *Enterobacter cloacae* on Page amoeba saline agar (30), as amoebae cultured in this way proved to be superior as antigens compared with axenically cultured amoebae. Methods for cell culture have been described by Cursons and Brown (12), as have serological methods (11).

All indirect fluorescent-antibody tests were examined with a Leitz Ortholux microscope equipped with a Leitz fluorescent incident Ploem illuminator and a 100-W quartz halide light source. For excitation we used a KP500 filter consisting of two Leitz KP490 short-wave pass interference filters. The incident illuminator contained a TK510 dichroic mirror and a K515 suppression filter. An additional K530 suppression filter was also used.

Random samples of fresh human sera, including samples from children, were obtained from the Palmerston North, Hamilton, and Rotorua Health Districts in New Zealand. The sera were filter sterilized through an 0.22-μm filter and stored at −20°C after indirect fluorescent-antibody tests were performed. Fresh guinea pig serum was also filtered through a 0.22-μm filter and stored at −70°C as a source of complement.

Neutralization tests were carried out by using standard techniques. Briefly, complement was inactivated at 56°C for 30 min if required. Serum to be tested was diluted in Eagle BHK maintenance medium containing 2% fetal calf serum, 100 U of penicillin per ml and 100 U of streptomycin per ml; $4.0 \times 10^4$ amoebae per ml were added, and the mixture was incubated at 37°C for 60 min with slight agitation. Then 0.5-ml samples of the amoeba-serum mixture were added to 24-h Vero cell cultures maintained in 1.0 ml of Eagle BHK maintenance medium and incubated at 37°C for 8 days. The tubes were checked daily for cytopathic effects (CPE).

## RESULTS

**Presence of antibodies to PFLA.** Antibodies to both PFLA and nonpathogenic free-living

amoebae were found in all 93 serum samples tested, with titers ranging from 1:5 to 1:20 for *Naegleria* spp. and from 1:20 to 1:80 for *Acanthamoeba* spp. (Tables 1 through 3). This 100% antibody response has been confirmed by more recent work by R.T.M.C. We detected no differences between different groups and sexes, although cord sera from newborn infants displayed a lower titer than maternal sera. By using class-specific labeled anti-immunoglobulins, it was shown that the antibodies belonged primarily to the immunoglobulin G (IgG) and IgM classes (Table 4). Absorption of human sera with axenically cultured trophozoites abolished fluorescent staining, which ruled out false-positive reactions due to rheumatoid factors. The observation of antibodies belonging to the IgG class is not surprising because IgG replaces initially formed IgM antibodies and has the longest half-life of the immunoglobulin classes. However, the finding of antibodies belonging to the IgM class with specific titers which closely paralleled the titers of IgG antibodies suggests recent contact with PFLA.

**Presence of specific neutralizing factor against PFLA in normal human sera.** To evaluate further the specificity of the antibodies detected against PFLA in normal human sera, random neutralization tests for pathogenic *Naegleria* spp. and *Acanthamoeba* spp. were performed in Vero cell cultures with both normal human and hyperimmune rabbit sera. Table 5 shows the specificity of the antibodies as judged by in vitro neutralization tests when cell cultures were used as indicators of pathogenicity. No neutralization was obtained with either unheated, complement-inactivated hyperimmune rabbit sera or human sera for *N. fowleri*. On the other hand, fresh unheated adult sera neutralized *A. culbertsoni* at a titer of 1:10 to 1:20,

TABLE 1. *Presence of antibodies to PFLA in human sera from the Palmerston North Health District, New Zealand*

| Serum | Type | Sex[a] | Blood group | Titer of human sera against: | | | |
|---|---|---|---|---|---|---|---|
| | | | | *N. fowleri* MsT | *N. gruberi* P1200f | *A. culbertsoni* A-1 | *A. castellanii* 1501 |
| $A_1$ | Mother | | O negative | 1:10 (I-II)[b] | 1:20 (I-II) | 1:20 (II-III) | 1:40 (I-II) |
| $A_2$ | Cord | | O positive | 1:5 (I-II) | 1:10 (I-II) | 1:5 (II-III) | 1:10 (I-II) |
| $B_1$ | Mother | | O positive | 1:10 (I-II) | 1:20 (I-II) | 1:20 (II-III) | 1:40 (I-II) |
| $B_2$ | Cord | | B positive | 1:5 (I-II) | 1:10 (I-III) | 1:5 (III-IV) | 1:10 (II-I) |
| $C_1$ | Mother | | A negative | 1:10 (I-II) | 1:20 (I-II) | 1:20 (II-III) | 1:40 (I-II) |
| $C_2$ | Cord | | O positive | 1:5 (II-III) | 1:10 (I-II) | 1:5 (II-III) | 1:10 (I-II) |
| $D_1$ | Mother | | O negative | 1:10 (I-II) | 1:20 (I-II) | 1:20 (II-III) | 1:40 (I-II) |
| $D_2$ | Cord | | O negative | 1:5 (II-III) | 1:10 (I-II) | 1:5 (II-III) | 1:10 (II-III) |
| $E_1$ | Mother | | A positive | 1:10 (II-III) | 1:20 (I-II) | 1:20 (III-IV) | 1:40 (II-III) |
| $E_2$ | Cord | | A positive | 1:5 (I-II) | 1:10 (I-II) | 1:5 (II-III) | 1:10 (I-II) |
| $F_1$ | Mother | | A positive | 1:10 (I-II) | 1:20 (II-III) | 1:20 (III-IV) | 1:40 (II-III) |
| $F_2$ | Cord | | O positive | 1:5 (I-II) | 1:10 (II-III) | 1:5 (III-IV) | 1:10 (II-III) |
| $G_1$ | Mother | | O negative | 1:10 (I-II) | 1:20 (I-II) | 1:20 (II-III) | 1:40 (I-II) |
| $G_2$ | Cord | | O positive | 1:5 (I-II) | 1:10 (I-II) | 1:5 (II-III) | 1:10 (I-II) |
| $H_1$ | Mother | | A negative | 1:10 (I-II) | 1:20 (I-II) | 1:20 (II-III) | 1:40 (I-II) |
| $H_2$ | Cord | | A positive | 1:5 (I-II) | 1:10 (I-II) | 1:5 (II-III) | 1:10 (I-II) |
| I | | M | A positive | 1:10 (II-III) | 1:20 (II-III) | 1:20 (III-IV) | 1:40 (II-III) |
| J | | M | A positive | 1:10 (II-III) | 1:20 (II-III) | 1:20 (IV) | 1:10 (III-IV) |
| K | | M | B positive | 1:10 (I) | 1:20 (I) | 1:20 (II-III) | 1:40 (I-II) |
| L | | F | NG[c] | 1:10 (II-III) | 1:20 (II-III) | 1:20 (II-III) | 1:40 (II-III) |
| M | | M | NG | 1:10 (II-III) | 1:20 (II-III) | 1:20 (II-III) | 1:40 (II-III) |
| N | | F | NG | 1:10 (I) | 1:20 (II-III) | 1:20 (II-III) | 1:40 (II-III) |
| O | | F | NG | 1:10 (I-II) | 1:20 (I-II) | 1:20 (II-III) | 1:40 (I-II) |
| P | | F | NG | 1:10 (I-II) | 1:20 (I-II) | 1:20 (II-III) | 1:40 (I-II) |
| Q | | M | AB | 1:10 (I-II) | 1:20 (I-II) | 1:20 (II-III) | 1:40 (II-III) |
| R | | M | NG | 1:10 (I-II) | 1:20 (I-II) | 1:20 (II-III) | 1:40 (II-III) |
| S | | M | NG | 1:10 (I) | 1:20 (II-III) | 1:20 (III-IV) | 1:80 (II-III) |
| T | | F | NG | 1:10 (II-III) | 1:20 (II-III) | 1:20 (II-III) | 1:40 (II-III) |
| U | | M | NG | 1:10 (I) | 1:20 (I-II) | 1:20 (II-III) | 1:40 (II-III) |
| V | | F | NG | 1:10 (I-II) | 1:20 (I-II) | 1:20 (II-III) | 1:40 (II-III) |

[a] M, Male; F, female.

[b] Values in parentheses indicate the following intensities of fluorescence: IV, very high; III, high; II, low; I, very low.

[c] NG, Not given.

TABLE 2. *Presence of antibodies to PFLA in human sera from the Hamilton Health District, New Zealand*

| No. of sera with common reaction | Type | Sex | Titer of human sera against: | | | |
|---|---|---|---|---|---|---|
| | | | *N. fowleri* MsT | *N. gruberi* P1200f | *A. culbertsoni* A-1 | *A. castellanii* 1501 |
| 20 | NG[a] | NG | 1:10 (I-II)[b] | 1:20 (I-II) | 1:20 (II-III) | 1:40 (II-III) |
| 6 | NG | NG | 1:10 (I) | 1:20 (I-II) | 1:20 (II-III) | 1:40 (II-III) |
| 2 | NG | NG | 1:10 (I-II) | 1:20 (I-II) | 1:20 (III-IV) | 1:40 (III-IV) |
| 1 | NG | NG | 1:10 (II-III) | 1:20 (I-II) | 1:20 (III-IV) | 1:40 (III-IV) |
| 1 | NG | NG | 1:10 (I-II) | 1:20 (II-III) | 1:20 (II-III) | 1:40 (II-III) |

[a] NG, Not given.
[b] Values in parentheses indicate the following intensities of fluorescence: IV, very high; III, high; II, low; I, very low.

TABLE 3. *Presence of antibodies to PFLA in human sera from the Rotorua Health District, New Zealand*

| No. of sera with common reaction | Type | Sex[a] | Titer of human sera against: | | | |
|---|---|---|---|---|---|---|
| | | | *N. fowleri* MsT | *N. gruberi* P1200f | *A. culbertsoni* A-1 | *A. castellanii* 1501 |
| 10 | NG[a] | 6 M, 3 F, 3 NG | 1:10 (I-II)[b] | 1:20 (I-II) | 1:20 (I-III) | 1:40 (II-III) |
| 5 | NG | 4 M, 1 F | 1:10 (I-II) | 1:20 (I-II) | 1:20 (II-III) | 1:40 (I-II) |
| 2 | NG | 1 M, 1 F | 1:10 (I-II) | 1:20 (I-II) | 1:20 (I-II) | 1:40 (I-II) |
| 2 | NG | 1 M, 1 NG | 1:10 (I) | 1:20 (I-II) | 1:20 (II-III) | 1:40 (II-III) |
| 1 | NG | F | 1:10 (I) | 1:20 (I-II) | 1:20 (I-II) | 1:40 (I-II) |
| 1 | NG | F | 1:10 (I-II) | 1:20 (II-III) | 1:20 (I-II) | 1:40 (II-III) |
| 1 | NG | M | 1:10 (I-II) | 1:20 (I-II) | 1:20 (I-II) | 1:40 (II-III) |
| 1 | NG | F | 1:10 (I-II) | 1:20 (II-III) | 1:20 (II-III) | 1:40 (III-IV) |
| 1 | NG | F | 1:10 (I-II) | 1:20 (I-II) | 1:20 (II-III) | 1:40 (III-IV) |
| 1 | NG | M | 1:10 (I-II) | 1:20 (I-II) | 1:20 (I-II) | 1:40 (III-IV) |
| 1 | NG | M | 1:10 (I-II) | 1:20 (I-II) | 1:20 (III-IV) | 1:40 (III-IV) |
| 1 | NG | NG | 1:10 (II-III) | 1:20 (I-II) | 1:20 (II-III) | 1:40 (I-II) |
| 1 | NG | NG | 1:10 (I) | 1:20 (I) | 1:20 (II-III) | 1:40 (III-IV) |

[a] M, Male; F, female; NG, not given.
[b] Values in parentheses indicate the following intensities of fluorescence: IV, very high; III, high; II, low; I, very low.

TABLE 4. *Presence of class-specific antibodies to PFLA in human sera*

| Adult human serum | Titer | Antigen | Fluorescence with:[a] | | | |
|---|---|---|---|---|---|---|
| | | | Anti-human IgG, IgM, and IgA | Anti-human IgG | Anti-human IgM | Anti-human IgA |
| 952 | 1:10 | *N. fowleri* MsT | I | II | I-II | |
| | 1:20 | *N. gruberi* P1200f | I-II | II | II | |
| | 1:40 | *A. castellanii* 1501 | II | I-II | II | I |
| | 1:80 | *A. culbertsoni* A-1 | I | I-II | I-II | I |
| 956 | 1:10 | *N. fowleri* MsT | I | I-II | I-II | |
| | 1:20 | *N. gruberi* P1200f | II | II-III | II | |
| | 1:40 | *A. castellanii* 1501 | I | I | I | I |
| | 1:80 | *A. culbertsoni* A-1 | I | II | II | I |
| 960 | 1:10 | *N. fowleri* MsT | II | II | II | |
| | 1:20 | *N. gruberi* P1200f | II | II-III | I-II | |
| | 1:40 | *A. castellanii* 1501 | II | II-III | I-II | I |
| | 1:80 | *A. culbertsoni* A-1 | II | II-III | I-II | I |

[a] III, High intensity of fluorescence; II, low intensity of fluorescence; I, very low intensity of fluorescence.

TABLE 5. *Use of hyperimmune rabbit and normal human sera in neutralizing N. fowleri MsT and A. culbertsoni A-1 in Vero cell cultures using an inoculum of $10^4$ cells per ml*

| Antigen | Antiserum treatment | Titer | IFAB titer[a] | CPE on day:[b] | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| MsT | Control (no antiserum) | | | | II | IV | | | | |
| | Rabbit MsT heated at 56°C for 30 min | 1:10 | 1:500 (III)[c] | | II | IV | | | | |
| | Rabbit MsT heated at 56°C for 30 min + GPC[d] | 1:10 | | | II | IV | | | | |
| | Rabbit MsT unheated | 1:10 | | | II | IV | | | | |
| | Human pooled unheated | 1:10 | 1:10 (II) | | II | IV | | | | |
| A-1 | Control (no antiserum) | | | | II | IV | | | | |
| | Rabbit A-1 heated at 56°C for 30 min | 1:10 | 1:500 (III) | | | I | I | II | II | III |
| | | 1:20 | | | | II | II | III | III | IV |
| | | 1:40 | | | II | IV | | | | |
| | Rabbit A-1 heated at 56°C for 30 min + GPC | 1:10 | 1:500 (III) | | | I | I | II | III | IV |
| | | 1:20 | | | I | II | II | III | IV | |
| | | 1:40 | | | II | IV | | | | |
| | Rabbit A-1 unheated | 1:10 | 1:500 (III) | | | | I | II | II | III |
| | | 1:20 | | | | I | I | II | III | III |
| | | 1:40 | 1:40 | | I | III | IV | | | |
| | Rabbit 1501 heated at 56°C for 30 min | 1:10 | 1:500 (I) | I | II | IV | | | | |
| | Rabbit 1501 heated at 56°C for 30 min + GPC | 1:10 | 1:500 (I) | I | II | IV | | | | |
| | Rabbit 1501 unheated | 1:10 | 1:500 (I) | I | II | III | IV | | | |
| | | 1:20 | | I | II | IV | | | | |
| | $A_1$ human heated at 56°C for 30 min | 1:10 | 1:20 (II) | | II | IV | | | | |
| | $A_2$ human heated at 56°C for 30 min | 1:10 | 1:5 (II) | | II | IV | | | | |
| | $A_1$ human unheated | 1:10 | 1:20 (II) | | | | | | II | IV |
| | $A_1$ human unheated | 1:20 | 1:20 | | | | | II | IV | |
| | | 1:40 | | | I | III | IV | | | |
| | | | | | I | | | | | |
| | $A_2$ human unheated | 1:10 | 1:5 (II) | | II | IV | | | | |
| | $B_1$ human heated at 56°C for 30 min | 1:10 | 1:20 (II) | | II | IV | | | | |
| | $B_2$ human heated at 56°C for 30 min | 1:10 | 1:5 (III) | | II | IV | | | | |
| | $B_1$ human unheated | 1:10 | 1:20 (II) | | | | | | | |
| | | 1:20 | | | | | | | | II |
| | | 1:40 | | | I | III | IV | | | |
| | $B_2$ human unheated | 1:10 | 1:5 (II) | | | III | | | | |
| | Pooled human unheated | 1:10 | 1:20 (II) | | | | | | | |
| | | 1:20 | | | | | | | | |
| | | 1:40 | | | | | II | IV | | |
| | | 1:80 | | | I | II | IV | | | |
| | Pooled human heated at 56°C for 30 min | 1:10 | 1:20 (II) | | II | IV | | | | |
| | Pooled human unheated | 1:10 | 1:20 (III) | | | | | | | |
| | | 1:20 | | | | | | | | |
| | | 1:40 | | | | | | | | II |
| | | 1:80 | | | I | II | III | IV | | |
| | Pooled human heated at 56°C for 30 min | 1:10 | 1:20 (III) | | II | IV | | | | |
| | Pooled human heated at 56°C for 30 min + GPC | 1:10 | 1:20 (III) | | II | IV | | | | |

[a] IFAB, Indirect fluorescent antibody.

[b] CPEs were scored as follows: I, early CPE; II, pronounced CPE; III, very pronounced CPE; IV, total degeneration of monolayer. Manifestations of CPE included rounding of Vero cells and degeneration, refractility, and finally loss of the monolayer.

[c] Values in parentheses indicate the following intensities of fluorescence: I, very low; II, low; III, high; IV, very high.

[d] GPC, Guinea pig complement.

whereas complement-inactivated adult or cord sera did not. Furthermore, the addition of fresh guinea pig complement did not affect the results, nor did the use of hyperimmune rabbit anti-*A. culbertsoni* serum with a specific indirect fluorescent-antibody titer of 1:1,000 although some delay in the formation of CPE with the homologous serum was observed (Table 5).

## DISCUSSION

The observation of widespread occurrence of antibodies to PFLA in human sera may simply be a reflection of the ubiquitous distribution of free-living amoeba in the environment which interact with the immune systems of humans, or it may represent cross-reacting antibodies to an as-yet-undefined antigen. It is well known that antibodies cross-reacting between different genera of intestinal and pharyngeal bacteria exist in human sera and that autoantibodies, especially rheumatoid factors, are responsible for fluorescent antibody false-positive results in human sera (for example, with *Toxoplasma*). Notwithstanding this, the loss of fluorescent staining after antibodies have been absorbed with amoeba trophozoites, the difference in antibody titers, and the presence of a heat-labile neutralizing factor specific for *A. culbertsoni* but not *N. fowleri* suggest at least some degree of specificity. Previous reports of antibodies to PFLA in human sera are shown in Table 6.

The results shown in Tables 1 through 3 show that whereas titers to *Acanthamoeba* spp. ranged from 1:5 to 1:80, titers to *Naegleria* spp. were only between 1:5 and 1:20. Generally, the intensities of fluorescence and antibody titers to the pathogen were similar to those of nonpathogens, and no discrimination was observed between different blood groups or between sexes. Serum samples from children may be responsible for some of the lower titers. This serological cross-reactivity between pathogenic and nonpathogenic amoebae is probably a result of antigenic similarity (21, 39). The results in Table 4 suggest that these antibodies belong to the IgG and IgM classes. Since only IgG crosses the placenta, maternal IgG antibodies are probably responsible for the positive reactions with cord sera, as it is unlikely that newborn infants would have had prior contact with PFLA or enough time to synthesize their own antibodies. The finding of specific IgM antibodies in adult sera was surprising in view of the relatively short half-life of IgM and the fact that as the immune response unfolds, IgM antibodies are replaced by IgG antibodies. The titer and intensity of the fluorescence of the IgM antibodies closely paralleled the titer and intensity of the fluorescence of the IgG antibodies, and this may be due to either recent infection or persistent exposure to free-living amoebae. In this context it is interesting to note that *Naegleria* spp., like other protozoa (such as *Leishmania*, *Toxoplasma*, *Entamoeba*, and *Trypanosoma*), have the ability to remove antibody bound to their surface membranes by endocytosis (20) and that this could influence the IgM response.

TABLE 6. *Previous reports of amoeba antibodies in human sera*

| Amoeba | Serological test | Titer | Reference |
|---|---|---|---|
| *A. castellanii* | Complement fixation | 1:40–1:160 | 8 |
| *Acanthamoeba* spp. | Complement fixation | 1:5–1:40 | 19 |
| *N. gruberi* | Gel diffusion | Neat | 18 |
| *N. fowleri* | Radio-immunoassay | 1:45–1:398 | 35 |
| *A. castellanii* | IFAB[a] | Neat–1:1,000 | 28 |
| *A. polyphaga* | Gel diffusion | Neat | 28 |

[a] IFAB, Indirect fluorescent antibody.

The detection of a factor present only in fresh adult human sera capable of inhibiting CPE in Vero cell cultures caused by *A. culbertsoni* (Table 5) suggests that normal human sera contain some "natural antibody" to *Acanthamoeba* spp. (2, 27). This view is supported by the fact that this factor possesses properties typical of those exhibited by natural antibodies. These properties include the following: (i) its action was independent of antibody titer (1:10 to 1:20 compared with 1:40 to 1:80); (ii) it was found only in adults and not in cord sera; (iii) it was specific for *A. culbertsoni* and not for *N. fowleri*; and (iv) it was heat labile (exposure to 56°C for 30 min inactivated it).

No neutralization was observed when heated or unheated hyperimmune rabbit antisera were used, nor was any neutralization observed against *N. fowleri*. The addition of fresh guinea pig complement did not affect any of the results. The finding of an amoebicidal factor against *Acanthamoeba* spp. in fresh adult sera has been reported previously by Culbertson (10). He, like Carter (4), also found that fresh normal human sera were amoebicidal for *N. fowleri*. This discrepancy between the neutralization results reported by Carter and Culbertson (4, 10) and the results found in this study probably result from differences in assay conditions. Carter reported that his highest titer for immobilization and lysis of $10^2$ to $10^3$ amoebae per ml was 1:8. However, the lowest assay titer used in the neutralization of $10^4$ amoebae per ml in Vero cell cultures was

1:10. Neutralizing factors in human sera against other protozoans, such as *Trichomonas vaginalis* and *Trypanosoma brucei*, have also been reported (23, 34). In the case of *T. brucei*, the trypanocidal factor was shown to be heat labile, to be present in both plasma and serum at equivalent levels, not to be removed by absorption with IgG fractions of antisera against human IgM or $\alpha_2$ macroglobulin, and to have a molecular weight of about $5 \times 10^5$ (31). It is thus thought that this acanthamoebicidal factor present in human serum may explain the very low incidence of infections in humans. Furthermore, it has been demonstrated that passively transferred immune sera with an agglutination titer of 1:256 could protect mice to some degree against a lethal intranasal inoculation of *N. fowleri* (36). Although the mechanism of this protective immunity was not known, it was hypothesized that such immune serum could act either as an opsonin promoting phagocytosis or as an antitoxin. Some support for the latter hypothesis is given by the observations that, although hyperimmune rabbit antisera prepared from soluble antigens of mechanically disrupted trophozoites failed to lyse trophozoites or prohibit the formation of CPE in Vero cell culture in the presence of complement, hyperimmune rabbit antisera prepared against phospholipase 2-lysophospholipase enzymes isolated from the supernatants of axenically grown *N. fowleri* (16) were able to both prohibit and delay the formation of CPE in Vero cell culture, depending on their concentrations (R.T.M. Cursons, Ph.D. thesis, Massey University, Palmerston North, New Zealand, 1978).

Whatever the antigenic stimulation (specific or nonspecific) responsible for the formation of antibodies against PFLA, these antibodies could be involved in the generalized resistance of humans to these potentially pathogenic organisms. The discovery that free-living protozoa are able to infect humans and animals has revolutionized the concept of parasitism, and it is possible that these antibodies may protect by promoting opsonization and subsequent phagocytosis of amoebae, by immobilizing and/or agglutinating amoebae, by prohibiting surface adsorption, by fixing complement, or by neutralizing amoebic toxins.

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## LITERATURE CITED

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