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Specificity of Antibodies from Human Sera for *Naegleria* Species

FRANCINE MARCIANO-CABRAL,* MICHAEL L. CLINE, AND S. GAYLEN BRADLEY

Department of Microbiology and Immunology, Virginia Commonwealth University, Richmond, Virginia 23298

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Serum samples from adult humans in North Carolina and Pennsylvania were assayed for antibodies against four *Naegleria* species: *N. australiensis*, *N. fowleri*, *N. gruberi*, and *N. lovaniensis*. Agglutinating activities of serum samples from North Carolina subjects were higher for *N. fowleri* than were those from Pennsylvania subjects. The distributions of agglutination titers of human serum samples for *N. australiensis*, *N. gruberi*, and *N. lovaniensis* were heterogeneous. The agglutination capabilities of selected serum samples absorbed with rounded, killed trophozoites of *N. australiensis* and *N. lovaniensis* were distinctly different, as were those of serum samples absorbed with *N. fowleri* and *N. gruberi*. *N. australiensis* and *N. gruberi* shared some agglutinating antigens, as did *N. fowleri* and *N. lovaniensis*. The agglutinating activities of most serum samples correlated with the capability of their immunoglobulin M (IgM) to bind to antigens in extracts of *Naegleria* species but not with the capabilities of their IgG to bind to antigens of *Naegleria* species. Absorption of IgM binding capability with rounded, killed trophozoites established that *N. gruberi* was distinctly different from *N. fowleri* and *N. lovaniensis* but that *N. fowleri* and *N. lovaniensis* shared surface antigens. The proteins in extracts of the four *Naegleria* species were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and tested for their ability to bind immunoglobulins in a serum sample. The antigens of the four species that bound IgM or IgG in the tested serum sample were separated by SDS-PAGE, and when they were incubated with anti-IgM or anti-IgG, they gave distinct profiles. There was one distinct, shared antigen that had a molecular size of 40,000 daltons. Absorption of the test serum with killed, rounded trophozoites did not markedly change the immunoglobulin binding profile for *Naegleria* internal antigens separated by SDS-PAGE and did not remove the shared 40,000-dalton protein(s). These results demonstrate that the four *Naegleria* species have antigenically distinct surfaces and that humans have been individually exposed to antigens of *Naegleria* species.

Sera from young adult humans contain agglutinating activity for *Naegleria fowleri*, but agglutinating activity is negligible in sera from infants (9, 10). The agglutinating activity of human sera for *N. fowleri* is distinctly different from that for *N. gruberi* (10). It is possible however that exposure of humans to other species such as *N. australiensis* or *N. lovaniensis* may elicit antibodies against the more pathogenic *N. fowleri* (1, 4).

Rounded *Naegleria* cells are agglutinated more effectively by human serum than are amoeboid cells (10). Rounded cells, killed and fixed with paraformaldehyde, are agglutinated as well as rounded, live *Naegleria* trophozoites. Rounded, killed cells are the preferred antigen because they are not infectious. The differences in agglutination responses of rounded and amoeboid *Naegleria* cells indicate that they display different surface antigens or different distributions of antigens on the surface, dependent on prior treatment (3).

Previous surveys for *Naegleria* antibodies against *N. fowleri* in human sera have focused on subjects in the southeastern region of the United States (9, 10) and in Australia and New Zealand (4), regions in which primary amoebic meningoencephalitis has occurred. It is conceivable that subjects outside these regions, or with different environmental exposures, or both will have less agglutinating activity and lower antibody levels in their sera than will subjects in areas where primary amoebic meningoencephalitis has been encountered.

In the present study, sera from subjects in Pennsylvania were assayed for ability to agglutinate *N. fowleri*. The antigenic cross-reactions among *N. australiensis*, *N. fowleri*, *N. gruberi*, and *N. lovaniensis* were examined by using agglutinating activity and immunoglobulin binding capability as the criteria for antigenic similarity. Serum samples were absorbed with rounded, killed trophozoites to confirm specificity of the reactions and to identify shared antigens. Our results demonstrate that the four *Naegleria* species have antigenically distinct surfaces and that humans have individually characteristic antibody profiles for the four *Naegleria* species. In general, the sera of the Pennsylvania subjects had lower agglutinating activity for *N. fowleri* than did the sera of the North Carolina subjects.

MATERIALS AND METHODS

N. fowleri LEE (ATCC 30894) was grown at 37°C in a mixture of equal parts of Nelson medium and Balamuth medium (3). *N. gruberi* EG_B was cultivated in Nelson-Balamuth medium at 30°C. *N. australiensis* PP397 (6) and *N. lovaniensis* Aq/9/1/45D (11) were grown in Nelson-Balamuth medium at 37°C.

Samples of fresh human serum were obtained from the Duke-Watts Family Medicine Center, Durham, N.C., and from Polyclinic Medical Center, Harrisburg, Pa. All sera were collected by centrifugation (1,500 × *g* for 20 min) and stored at −20°C until assayed. Complement was inactivated by heating the samples at 56°C for 30 min.

Amoebae for agglutination assays were harvested from axenic cultures grown in Nelson-Balamuth medium and suspended in Eagle minimum essential medium. The suspensions were chilled to 5°C to round the trophozoites. The rounded *Naegleria* trophozoites were killed and fixed by the addition of paraformaldehyde to a final concentration of 2% (vol/vol). The killed cells were washed three times and suspended in Eagle minimum essential medium. Agglutina-

* Corresponding author.

TABLE 1. Distribution of agglutinating activities for *N. fowleri* and *N. gruberi* of human serum samples from subjects in Pennsylvania and in North Carolina[a]

Source	*Naegleria* species	No. of subjects with agglutinating titer of:			
		<1:2–1:4	1:8	1:16	1:32 or 1:64
North Carolina	*N. fowleri*[b]	3	5	19	4
Pennsylvania	*N. fowleri*[b]	25	10	5	3
North Carolina	*N. gruberi*[c]	9	7	9	6
Pennsylvania	*N. gruberi*[c]	11	18	13	1

[a] Antigens were paraformaldehyde-fixed, rounded trophozoites of *N. fowleri* LEE or of *N. gruberi* EG_B.

[b] Titers for subjects from North Carolina were significantly different than titers for those from Pennsylvania at $P < 0.001$.

[c] Titers not significantly different at $P < 0.05$.

tion assays were conducted in microtiter plates and were scored after 30 min of incubation at 37°C (9, 10). Agglutination titers were expressed as the greatest serum dilution capable of agglutinating the amoebae (1).

Extracts were prepared by subjecting washed *Naegleria* cells to ultrasonic treatment in a cell disruptor (maximum setting, model W-370; Heat Systems-Ultrasonic, Inc.) for 1 min at 4°C. The extract was diluted with an equal volume of lysis solution containing 0.5 M Tris (pH 6.8), 20% glycerol, 10% sodium dodecyl sulfate (SDS), 2% 2-mercaptoethanol, H_2O, and 0.05% (wt/vol) bromophenol blue and was boiled for 5 min (16). The solubilized *Naegleria* extracts were stored at −70°C until used.

Immunoglobulin binding assays were conducted with the *Naegleria* antigen applied as dots on nitrocellulose (dot blots) or after SDS-polyacrylamide gel electrophoretic (SDS-PAGE) separation of the *Naegleria* antigens. For dot blots, the *Naegleria* extract at 1 mg of protein per ml was serially diluted and applied to the nitrocellulose paper as 5-μl drops containing 5.0 to 0.02 μg per dot. The nitrocellulose paper containing the *Naegleria* antigen was incubated for 30 min in 3% bovine serum albumin containing 20 mM Tris, 500 mM NaCl, and 0.05% Tween 20 to block nonspecific binding of serum proteins. For dot blots, serum samples cord 1, DEF, 48, 56, 69, and 85 were used after a 1:40 dilution with 1% bovine serum albumin. After the *Naegleria* antigen was incubated with the serum samples, the dot blots were washed free of unbound serum protein, and the dots were incubated for 2 h with goat anti-human immunoglobulin M (IgM) (mu chain-specific) antibody linked with horseradish peroxidase (diluted 1:500) or goat anti-human IgG antibody linked with horseradish peroxidase (diluted 1:500) (Cappell Laboratories) and then processed for peroxidase activity with an Immun-Blot assay kit (Bio-Rad Laboratories).

For immunoelectrophoretic immunoblotting, 200 μg of *Naegleria* extract protein was loaded onto a one-dimensional uniform SDS-PAGE (12% polyacrylamide) and run as described previously (8, 17). One of each pair of SDS-PAGE lanes was stained with Coomassie brilliant blue (16), and the other was developed for immunoglobulin binding capability. The protein from an SDS-PAGE lane was transferred to nitrocellulose by electrophoresis at 90 mA overnight (13). The SDS-PAGE slabs were stained with Coomassie brilliant blue after transfer of the protein to nitrocellulose to ensure that the proteins had been effectively transferred. For immunoelectrophoretic immunoblotting, a single serum sample (DEF) from a young adult male was used in all immunoglobulin binding assays. After incubation of the electrophoretically separated protein with a 1:40 dilution of serum DEF overnight, the nitrocellulose was washed free of unbound serum, and the immunoglobulin binding bands were incubated with goat anti-human IgG or goat anti-human IgM antibody linked with horseradish peroxidase (diluted 1:500) and processed for peroxidase activity with an Immun-Blot assay kit. Negative controls consisted of samples incubated with goat anti-human serum linked with horseradish peroxidase but not with human serum, and with human serum but not anti-human reagent.

RESULTS

Serum samples from adult humans in North Carolina were assayed for their ability to agglutinate rounded, killed cells of *N. fowleri*. As expected, most of the samples agglutinated *N. fowleri* at a dilution (titer) of 1:16. Serum samples from adult humans in Pennsylvania were less effective in agglutinating *N. fowleri*; most sera had titers of 1:2 or 1:4. The capability of serum samples from both North Carolina and Pennsylvania to agglutinate *N. gruberi* was more varied, with a median titer of 1:8 (Table 1).

The agglutination titers of 30 adult human serum samples from North Carolina were determined with rounded, killed trophozoites of *N. australiensis*, *N. fowleri*, *N. gruberi*, and *N. lovaniensis*. The most frequent agglutination titer with *N. australiensis* or *N. fowleri* was 1:16. The distribution of agglutination titers was heterogeneous with *N. gruberi* or *N. lovaniensis*, with a substantial number of samples having titers of 1:8 and 1:4. The antigenic dissimilarity between *N. australiensis* and *N. lovaniensis* was established with serum samples 3, 7, 11, 12, and 29. There was general congruence of agglutination titers for *N. australiensis* and *N. fowleri*, and for *N. gruberi* and *N. lovaniensis*. There were notable exceptions for each pairwise comparison however; for example, serum sample 13 was selective for *N. lovaniensis* but not *N. gruberi* (Table 2).

The antigenic complexity and uniqueness of the cell surface of trophozoites of the four *Naegleria* species was documented by absorption studies. In all instances, absorption markedly reduced the agglutination titer for the homologous species. Absorption of serum samples with *N. australiensis* lowered the agglutination titer for *N. gruberi*. Absorption of serum samples with *N. fowleri* did not lower the agglutination titer for *N. australiensis* or *N. gruberi*. Absorption of serum samples 15, 22, 23, and 28 with *N. lovaniensis* lowered the agglutination titer for *N. lovaniensis*, *N. fowleri*, and *N. gruberi* but not *N. australiensis*. Conversely, absorption of serum samples 15, 22, 23, or 28 with *N. gruberi* did not lower the agglutination titer for *N. lovaniensis* (Table 3).

The ability of antigens in *Naegleria* extracts to bind antibodies in a selected adult human serum sample (DEF) was determined (Fig. 1). IgM in serum sample DEF, diluted 1:40, was bound by 0.31 μg of protein from *N. australiensis*, 0.04 μg of protein from *N. fowleri*, 0.08 μg of protein from *N. gruberi*, and 0.08 μg of protein from *N. lovaniensis*. The dot blots developed for the binding of IgM gave results congruent with those obtained by agglutination assays; however, the dot blots developed for the binding of IgG gave results unlike those obtained by agglutination assays. The IgG antibodies in the five serum samples tested (48, 56, 69, 85, and cord 1), diluted 1:40, were more effective in detecting *Naegleria* antigens than were the IgM antibodies (Table 4). Moreover, three cord sera lacked IgM antibodies that bound *Naegleria* antigens in dot-blot assays. All three cord sera,

TABLE 2. Specificity of agglutinating activities of human serum samples from North Carolina subjects for *Naegleria* species[a]

Serum sample	Agglutinating titer for:			
	N. australiensis	*N. fowleri*	*N. gruberi*	*N. lovaniensis*
1	1:64	1:64	1:16	1:16
2	1:16	1:16	1:8	1:4
3	1:32	1:16	1:16	1:4
4	1:32	1:16	1:8	1:8
5	1:16	1:16	1:8	1:4
6	1:16	1:16	1:16	1:4
7	1:32	1:8	1:16	1:4
8	1:32	1:16	1:16	1:16
9	1:32	1:16	1:16	1:32
10	1:8	1:4	1:2	1:16
11	1:32	1:16	1:16	1:4
12	1:64	1:16	1:8	1:4
13	1:8	1:8	1:2	1:32
14	1:8	1:16	1:8	1:4
15	1:16	1:32	1:32	1:16
16	1:4	1:4	1:4	1:8
17	1:16	1:16	1:8	1:16
18	1:32	1:16	1:4	1:8
19	1:16	1:16	1:16	1:16
20	1:16	1:16	1:16	1:16
21	1:32	1:16	1:8	1:8
22	1:16	1:16	1:32	1:16
23	1:32	1:32	1:64	1:16
24	1:16	1:8	1:4	1:8
25	1:8	1:4	1:4	1:4
26	1:16	1:8	1:4	1:4
27	1:16	1:8	1:4	1:8
28	1:16	1:16	1:32	1:32
29	1:8	1:64	1:8	1:64
30	1:64	1:16	1:64	1:16

[a] Antigens were paraformaldehyde-fixed, rounded trophozoites of *N. australiensis* PP397, *N. fowleri* LEE, *N. gruberi* EG_B, or *N. lovaniensis* Aq/9/1/45D. Data are from four assays of each serum sample.

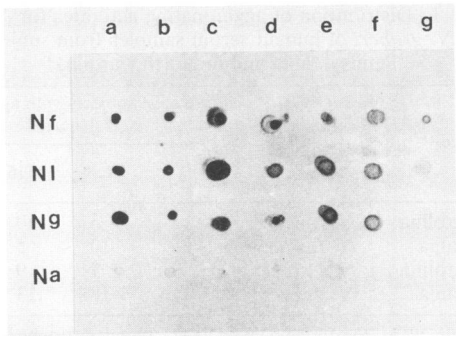

FIG. 1. Dot blot demonstration of the ability of antigens in extracts of *N. fowleri* (Nf), *N. lovaniensis* (Nl), *N. gruberi* (Ng), and *N. australiensis* (Na) to bind antibodies in an adult human serum sample (DEF). The extracts were serially diluted (twofold dilutions) and dotted on nitrocellulose paper: lanes a (5.0 μg of protein) through g (0.08 μg of protein). The nitrocellulose strips were incubated with serum DEF diluted 1:40. Bound immunoglobulin was detected with peroxidase linked to anti-human IgM.

however, contained IgG antibodies that bound *Naegleria* antigens. Results for one cord serum sample are presented in Table 4. Absorption of serum DEF with either *N. fowleri* or *N. lovaniensis* removed the IgM binding activity for each of these two species but not for *N. australiensis* or *N. gruberi*. Absorption of serum DEF with *N. gruberi* removed IgM binding capability for *N. gruberi* or *N. australiensis* but not for *N. fowleri* or *N. lovaniensis* (Table 5).

The proteins in extracts of the four *Naegleria* species were subjected to SDS-PAGE. The protein profile, as revealed by Coomassie blue staining was distinct for each species (Fig. 2). The majority of the proteins were smaller than 50,000 daltons. The SDS-PAGE slabs were stained with Coomassie blue before and after blotting, and selected nitrocellulose sheets were stained with amido black after blotting to document that the proteins had been effectively transferred. Both molecular weight standards and *N. fowleri* proteins

TABLE 3. Absorption of agglutinating activities of human serum samples specific for *Naegleria* species

Serum sample	Sample absorbed with[a]:	Agglutinating titer for:			
		N. australiensis	*N. fowleri*	*N. gruberi*	*N. lovaniensis*
15		1:16	1:32	1:32	1:16
	N. australiensis	1:2	1:16	1:8	1:8
	N. fowleri	1:32	1:4	1:32	1:8
	N. gruberi	1:8	1:16	1:2	1:16
	N. lovaniensis	1:8	1:2	1:4	1:2
22		1:16	1:16	1:32	1:16
	N. australiensis	1:4	1:8	1:4	1:8
	N. fowleri	1:32	1:4	1:16	1:4
	N. gruberi	1:8	1:16	1:4	1:16
	N. lovaniensis	1:16	1:4	1:4	1:4
23		1:32	1:32	1:64	1:16
	N. australiensis	1:4	1:16	1:4	1:16
	N. gruberi	1:16	1:16	1:4	1:16
	N. lovaniensis	1:16	1:4	1:4	1:4
28		1:16	1:32	1:32	1:32
	N. fowleri	1:32	1:4	1:16	1:16
	N. gruberi	1:8	1:16	1:4	1:16
	N. lovaniensis	1:16	1:4	1:4	1:4

[a] Human serum samples were absorbed with paraformaldehyde-fixed, rounded *Naegleria* cells (5×10^6/ml). The sera were absorbed five times for 1 h at 37°C.

TABLE 4. Correlation of agglutination and dot blot titers for IgM antibodies against *Naegleria* spp.

Serum sample	Titer by assay for:											
	N. australiensis			*N. fowleri*			*N. gruberi*			*N. lovaniensis*		
	Agg[a]	IgM[b]	IgG[b]	Agg	IgM	IgG	Agg	IgM	IgG	Agg	IgM	IgG
Cord 1	<1:2	>5.0	5.0	<1:2	>5.0	>5.0	<1:2	>5.0	1.25	<1:2	>5.0	>5.0
48	<1:2	>5.0	0.04	1:16	1.25	0.63	1:16	0.63	0.08	1:16	0.63	0.02
56	1:4	5.0	0.08	1:32	0.63	0.04	1:16	1.25	0.08	1:4	2.5	0.63
69	1:8	0.63	0.02	1:16	0.16	0.63	1:16	0.32	0.02	1:32	0.08	0.04
85	1:4	2.5	0.32	1:8	1.25	0.32	1:16	0.63	0.32	1:8	2.5	0.16

[a] Agg, Agglutinin assay. Greatest serum dilution that agglutinated fixed, rounded trophozoites.
[b] IgM, Immunodot IgM assay; IgG, immunodot IgG assay. Dot blot titers are expressed in terms of the lowest amount of antigen (micrograms of protein per dot) that gave an observable peroxidase reaction. (Antigen dilutions were 5.0 to 0.02 μg of protein per dot.)

were reproducibly transferred to nitrocellulose (Fig. 3). The antigens that bound IgG or IgM in serum sample DEF after SDS-PAGE of the extracts of each species also gave distinct profiles. The banding profiles for a given species, stained by using anti-IgG or anti-IgM as the secondary antibody, were similar, although there were both qualitative and quantitative differences. The banding profiles of extracts of *N. fowleri* and *N. lovaniensis* stained with anti-IgG or anti-IgM had noticeable similarities (Fig. 4). There was one shared antigen stained with anti-IgG or anti-IgM which had a molecular size of 40,000 daltons in all four *Naegleria* species. Absorption of serum DEF with killed, whole amoebae of any of the four species did not remove the anti-IgG binding activity for the 40,000-dalton protein. Absorption with whole *N. australiensis* amoebae did not change the IgG banding profile for the antigens separated by SDS-PAGE of extracts of the four *Naegleria* species, whereas absorption with *N. lovaniensis* cells removed some bands of *N. lovaniensis* and *N. fowleri* (Fig. 5). Absorption of serum with rounded, fixed *N. fowleri* or *N. lovaniensis* cells, however, removed essentially all of the IgM banding profile for *N. fowleri* and *N. lovaniensis* antigens. It should be noted that there were no *Naegleria* antigens that bound IgM in cord serum.

DISCUSSION

We have reported previously that serum samples from adults living in North Carolina or Virginia possessed agglutinating activity for *N. fowleri* (9, 10). In the previous studies, and in a limited series reported here, the median agglutination titer of North Carolina serum samples for *N. fowleri* was 1:16. The median agglutination titer of Pennsylvania serum samples for *N. fowleri* was 1:4. One interpretation for the different agglutinating activities of the two populations is that the Pennsylvania population sample has been exposed to *N. fowleri* antigens less extensively than has the North Carolina or Virginia population. Variation in exposure could reflect either the distribution of the *Naegleria* antigens in the environment or differences in contact between the humans and the antigen reservoirs. *Naegleria* amoebae have been isolated from lakes in Pennsylvania (12) as well as from lakes in several southern states (7). The Pennsylvania population selected may be less inclined to water sports in lakes and ponds than are the North Carolina and Virginia populations.

The capabilities of sera from different human subjects to agglutinate *Naegleria* spp. are distinctive. It is probable that most subjects were exposed to specific antigens for more than one species. Nearly all of the North Carolina subjects had titers of 1:16 or greater for at least one species. Absorption studies indicate that *N. fowleri* and *N. gruberi* do not share surface antigens, nor do *N. australiensis* and *N. lovaniensis*. Absorption of serum 22 with *N. fowleri* removed agglutinating activity for *N. lovaniensis*. Absorption of sera 22 and 28 with *N. lovaniensis* lowered agglutinating activity for *N. fowleri*, *N. gruberi*, and *N. lovaniensis* but not for *N. australiensis*. The surface antigens of *N. lovaniensis* may be designated as *l*, *f*, and *g*; those of *N. fowleri* may be designated as *f* and *l*. These results confirm earlier conclusions that *N. fowleri* and *N. lovaniensis* are antigenically related (5). Absorption of serum 23 with *N. australiensis* lowered agglutinating activity for both *N. australiensis* and *N. gruberi*. The surface antigens of *N. australiensis* may be designated as *a* and *g*, whereas the

TABLE 5. Absorption of IgM binding activity of human serum sample DEF specific for *Naegleria* species

Serum sample absorbed with:	Dot blot titer for[a]:			
	N. australiensis	*N. fowleri*	*N. gruberi*	*N. lovaniensis*
	0.32	0.04	0.08	0.08
N. australiensis	>5.0	0.08	0.16	0.08
N. fowleri	1.25	>5.0	0.04	2.5
N. gruberi	5.0	0.04	>5.0	0.04
N. lovaniensis	0.63	>5.0	0.08	>5.0

[a] Dot blot titers are expressed as the least amount of antigen (micrograms of protein per dot) that gave an observable peroxidase reaction.

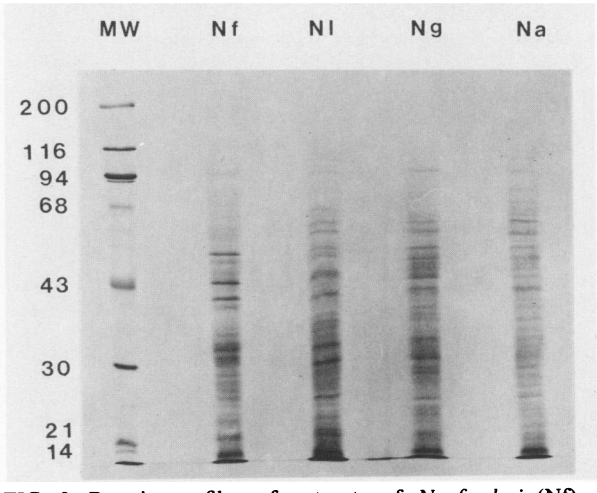

FIG. 2. Protein profiles of extracts of *N. fowleri* (Nf), *N. lovaniensis* (Nl), *N. gruberi* (Ng), and *N. australiensis* (Na) were subjected to SDS-PAGE and stained with Coomassie blue. Molecular size markers (MW) were run simultaneously.

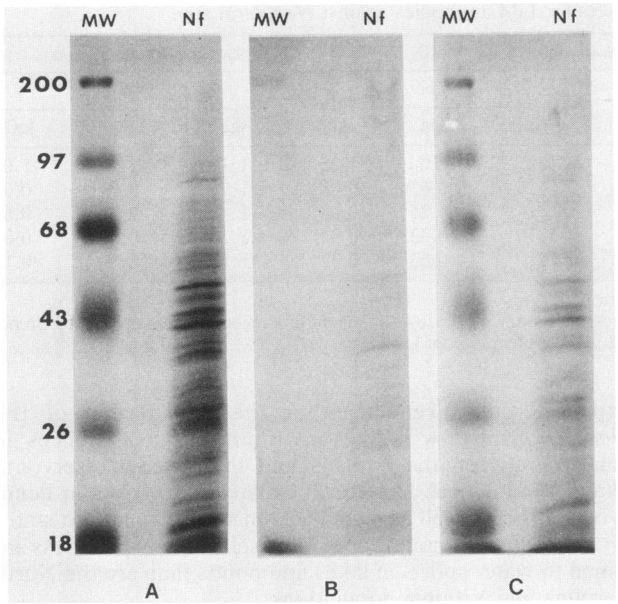

FIG. 3. Protein profiles of *N. fowleri* (Nf) in polyacrylamide gel were stained with Coomassie blue before (A) and after (B) blotting to nitrocellulose, and those on nitrocellulose were stained with amido black (C). Molecular size markers (MW) are shown.

surface antigens of *N. gruberi* may be designated as *g*. These results confirm earlier conclusions that *N. gruberi* and *N. australiensis* are antigenically related.

Agglutinating activity in human serum is due to IgM antibodies (10). Both agglutinating activity and IgM binding in dot-blot assays reflect the surface antigens of the *Naegleria* rounded cell. As shown earlier by Tew et al. (14), normal serum contains more antibodies directed against internal *Naegleria* antigens than against surface antigens. IgG binding in dot blot assays reflects internal antigens which are more abundant and show extensive cross-reactivity among the four species. Absorption of serum DEF with *N. lovaniensis* removed IgM dot-blotting activity for both *N. fowleri* and *N. lovaniensis*, confirming the results with agglutination assays. Absorption of serum DEF with *N. gruberi* lowered dot-blotting activity for *N. australiensis*. These results indicate that *N. gruberi* contains a cross-reactivity or shared surface antigen with *N. australiensis*. The surface antigens of *N. gruberi* may therefore be designated as *g* and *a*.

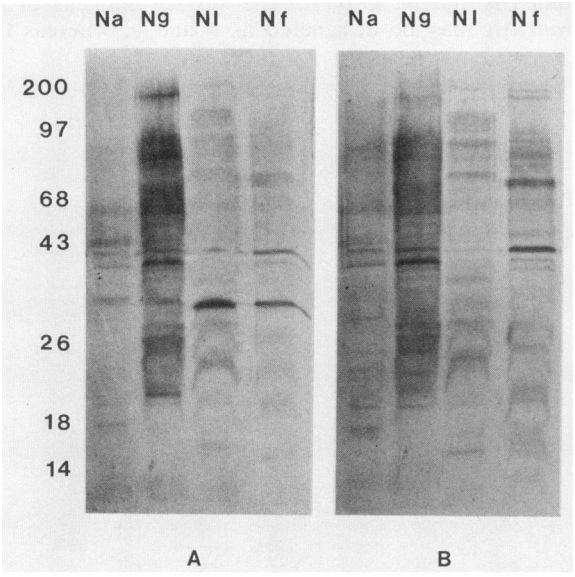

FIG. 4. Western immunoblots of antigens in extracts of *N. australiensis* (Na), *N. gruberi* (Ng), *N. lovaniensis* (Nl), and *N. fowleri* (Nf) were subjected to SDS-PAGE and reacted with anti-IgG (A)- or anti-IgM (B)-linked peroxidase.

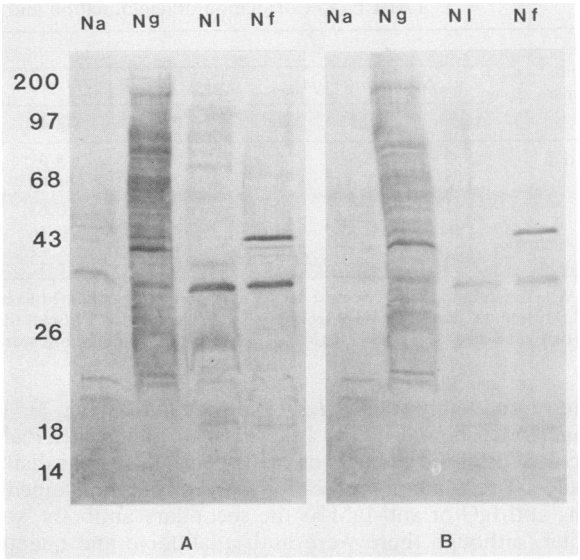

FIG. 5. Western immunoblots of antigens in extracts of *N. australiensis* (Na), *N. gruberi* (Ng), *N. lovaniensis* (Nl), and *N. fowleri* (Nf) were developed with serum sample DEF that had been absorbed with fixed whole cells of *N. australiensis* (A) or *N. lovaniensis* (B) and incubated with anti-IgG-linked peroxidase.

Previous studies have shown that the SDS-PAGE protein profiles of *N. fowleri* and *N. gruberi* are different (2), and agarose isoelectric focusing profiles for proteins of the four species are distinctive (5). Although protein profiles stained with Coomassie blue did not reveal any obvious relationships among the four species, Western immunoblots showed that *N. fowleri* and *N. lovaniensis* are immunologically related. Absorption with rounded, killed cells did not obviously remove any IgG that binds to proteins separated by SDS-PAGE. This confirms that the majority of proteins are internal proteins and that absorption of antibodies to surface proteins does not markedly alter the immunoblot staining profile. There are a few proteins (e.g., the 40,000-dalton antigen) which appear to be common to the four species. Limited cross-reactions involving a few internal proteins have been noted by others (15).

These results document that human sera contain antibodies specific for the surfaces of particular *Naegleria* species. The four species examined have characteristic surface antigens. Different human subjects appear to have been exposed to and have responded to various combinations of the four *Naegleria* species. All four species appear to be rather ubiquitous as antigens, at least in the geographic regions we studied.

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