Parasitol Res (2000) 86: 775–780

SHORT COMMUNICATION

V. Rivera-Aguilar · D. Hernández-Martínez
S. Rojas-Hernández · G. Oliver-Aguillón · V. Tsutsumi
N. Herrera-González · R. Campos-Rodríguez

Immunoblot analysis of IgA antibodies to *Naegleria fowleri* in human saliva and serum

Received: 20 September 1999 / Accepted: 17 March 2000

Abstract The objective of this study was to evaluate the secretory IgA (SIgA) antibody response to *Naegleria fowleri* (Nf) in individuals living in a parasite endemic area. Saliva and serum samples were obtained from both healthy subjects and patients suffering from a respiratory illness (chronic bronchitis or rhinitis) and were analyzed by immunoblot assay. SIgA from the patients' samples recognized more intensely a greater number of Nf proteins than did SIgA from the healthy control group. The proteins more frequently recognized were those with a molecular weight of 171, 107, 102, 62, 50, 46, and 10 kDa. Some IgA antibodies recognized proteins from Nf and *Entamoeba histolytica* (Eh) of similar molecular weight. These results suggest that some of those antibodies could have been elicited by a previous intestinal infection with Eh. Through the common mucosal immune system the IgA B-cells activated by Eh antigens can be disseminated to all the mucosae, including the nasal mucosa. SIgA antibodies recognizing Nf proteins, induced either by specific immunization or by cross-reaction, could participate in the resistance to the infection, probably by inhibiting the adherence of Nf trophozoites to the nasal mucosa.

N. Herrera-González · R. Campos-Rodríguez (✉)
Department of Biochemistry, School of Medicine,
National Polytechnic Institute,
Plan de San Luis y Díaz Mirón, Mexico,
D.F. CP 11340, Mexico
Fax: +1-57-501314

V. Rivera-Aguilar · D. Hernández-Martínez
S. Rojas-Hernández · G. Oliver-Aguillón
Project of Conservation and Improvement of Environment,
ENEP-Iztacala, UNAM, Avenida de los Barrios s/n,
Tlalnepantla Edo. de Mexico, CP 54090, Mexico

V. Tsutsumi
Department of Experimental Pathology, CINVESTAV,
Avenida IPN No. 2508, Mexico, D.F. CP 07360, Mexico

Introduction

Free-living amebas belonging to the genus *Naegleria* are abundant in the environment and have been isolated from the noses and throats of patients suffering from respiratory illness (Visvesvara and Stehr-Green 1990), but they cause very few clinical infections (Cursons et al. 1979). It has been speculated that host factors may be capable of preventing infection and tissue invasion by *Naegleria*. One host factor could be represented by the presence of secretory IgA (SIgA) antibodies against this parasite in the nasal mucosa.

Antibodies in secretions, particularly SIgA, protect humans and animals against viral and bacterial infections, mainly by preventing adhesion to the mucosae (Kilian and Russell 1994). Susceptibility to infection with free-living amebas could be due to a deficient production of secretory antibodies (Cursons et al. 1979). Therefore, the aim of this study was to analyze whether serum and salivary SIgA from individuals living in Nf-endemic and nonendemic areas recognized antigens of this parasite.

Materials and methods

Approximately 5 ml of saliva collected from 25 adults suffering from a respiratory illness (chronic bronchitis or rhinitis), from 100 healthy adults residing in a parasite-endemic area (Ciudad Valles, San Luis Potosí, México; Rodríguez et al. 1993), and from 85 healthy adults living in a parasite-nonendemic area (Mexico City) were collected in sterile flasks (after its production had been stimulated by mastication on a piece of waxed paper). Serum from 5 ml of venous blood was obtained by centrifugation at 1500 *g* for 10 min; the samples were stored at −20 °C until used.

Naegleria fowleri ATCC 30808 (a strain originally isolated in Europe from the cerebrospinal fluid of a human) was grown axenically at 37 °C in 2% bactocasitone medium enriched with 20% fetal bovine serum. Early stationary-growth-phase amebas were harvested during the logarithmic growth phase by centrifugation (1500 *g*, 10 min, 20 °C) and washed three times in 0.15 *M* NaCl. The cellular pellet was suspended in 0.15 *M* NaCl containing

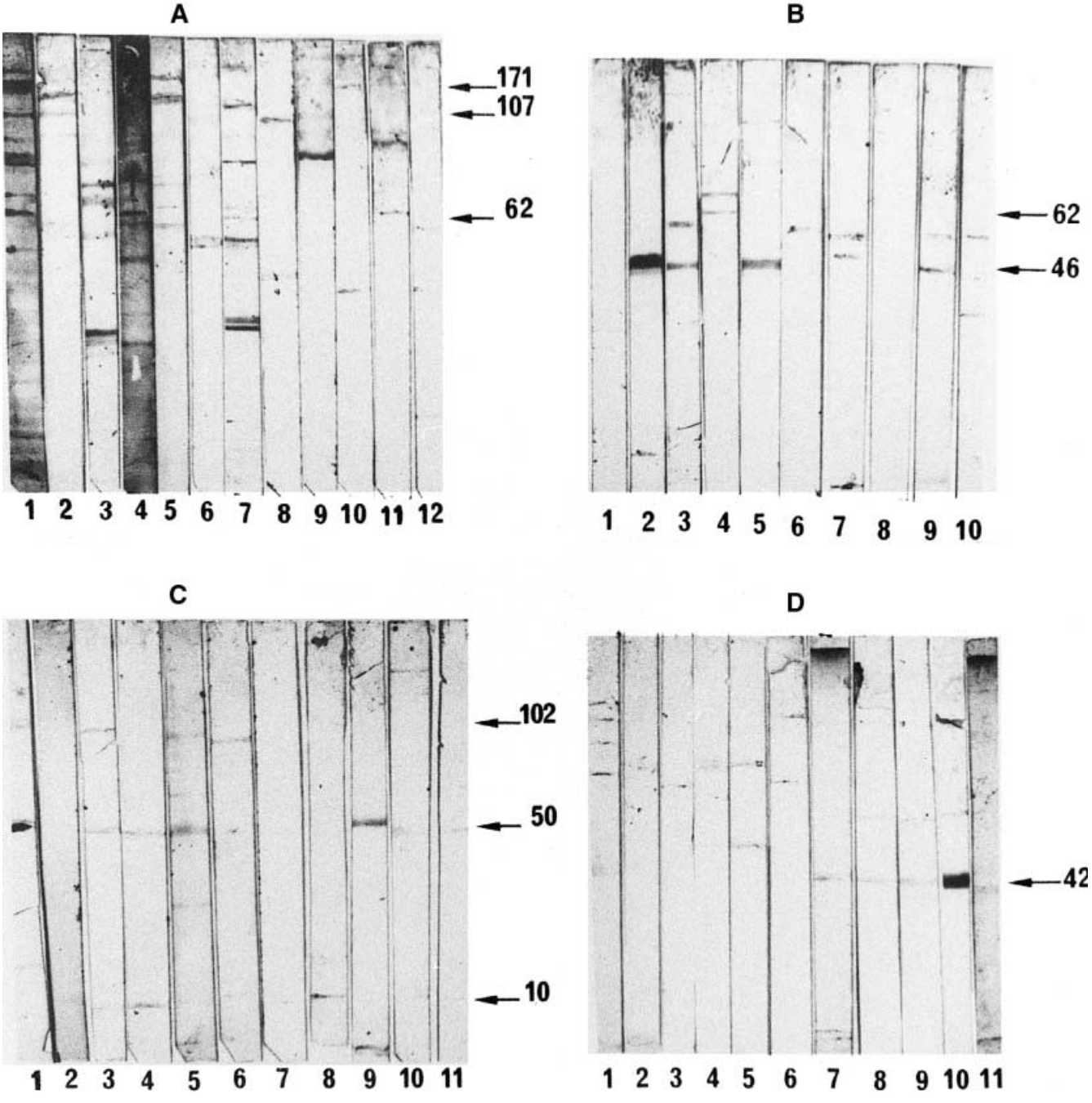

Fig. 1A–D Immunoblots of *Naegleria fowleri* proteins and IgA antibodies from human saliva and serum. SDS-PAGE was performed using a 7.5% acrylamide separating gel and the proteins were transferred to nitrocellulose paper. The strips of nitrocellulose paper were incubated with either **A** saliva or **B** serum samples from the group of patients or with **C** saliva or **D** serum samples from healthy individuals from the same endemic area. Afterward, goat IgG anti-human IgA antibodies labeled with peroxidase were added. *Numbers on the right* represent molecular weights in kDa, and *arrows* indicate the main components

5 m*M* *p*-hydroxymercuribenzoate. The cell suspension was lysed, separated into aliquots of 200 µl, and frozen at −70 °C. *Entamoeba histolytica* trophozoites of strain HM1:IMSS were cultivated in axenic medium (Diamond et al. 1978), collected during the logarithmic growth phase, washed six times with 0.15 *M* NaCl (pH 6.5) at 40 °C, and resuspended at a concentration of 5×10^7 cells/µl in 0.15 *M* NaCl containing 5 m*M* *p*-chloromercuribenzoate. The cell suspension was frozen at −70 °C for 24 h and then thawed at room temperature to lyse the amebas. The protein concentration of the lysates was estimated by the dye-binding method (Bradford 1976) using serum albumin as the standard.

For sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblot assay, 100 µl of the amebic cell lysate containing 100 µg of protein was mixed with an equal volume of sample buffer and then boiled for 5 min. SDS-PAGE was performed according to Laemmli (1970). In brief, loading of the samples into a 7.5% separating gel using a blind comb was followed by electrotransfer to a nitrocellulose membrane (Towbin et al. 1979). Strips were cut and blocked with 10% skim milk in

50 m*M* TRIS-HCl/200 m*M* NaCl (pH 7.4). The blocking reagent was removed, and human serum (1:100) or saliva (1:2) diluted in blocking buffer was added for 3 h of incubation at 37 °C. After a washing step, peroxidase-conjugated goat anti-human IgA (alpha-chain-specific) diluted 1:1000 in blocking buffer was added and developed with the substrate solution (H_2O_2, 3.6 m*M* 4-chloro-1-naphthol).

Results

Salivary IgA antibodies from 12 (48%) of the 25 sick persons recognized a heterogeneous group of proteins present in the amebic lysate (Fig. 1A). The most frequently recognized antigens were those with a molecular weight of 62 kDa (54%), 107 kDa (54%), and 171 kDa (45%). The antigen-recognition patterns differed among individuals. In the serum ($n = 10$) the IgA antibodies recognized a smaller number of proteins, but in a similarly heterogeneous manner (Fig. 1B); proteins with a mass of 62 kDa (60%) and 46 kDa (70%) were those most frequently recognized.

Altogether, 11 (11%) subjects among the 100 donors from the endemic area (Fig. 1C) had salivary IgA antibodies that recognized proteins of 50 kDa (58%), 10 kDa (50%), and 102 kDa (33%). However, the recognition pattern was very similar among all these individuals. The serum IgA antibodies recognized a smaller number of proteins, and a molecule of 42 kDa (43%) was the protein most frequently recognized in this group (Fig. 1D).

With regard to the antigen-recognition patterns observed for IgA antibodies in the serum versus the saliva of the same subject from the group of patients (Fig. 2A) or the healthy group from the endemic area (Fig. 2B), it is noteworthy that there was no clear correlation between them; i.e., the same protein was not recognized by the antibodies isolated from saliva as opposed to serum. However, exceptions did exist; for example, the proteins of 62, 50, and 46 kDa were recognized in both serum and saliva. A similar antigen-recognition pattern was observed when we compared the reactivity of serum IgG versus IgA from the same individual using the same antigen preparation; however, proteins of 62 and 46 kDa were recognized both in serum IgG and in serum IgA from two patients (Fig. 3, subjects 1 and 2).

For the determination of possible cross-reactivity between Nf and Eh antigens the antigen-recognition patterns observed for both amebas were analyzed. Different Eh and Nf proteins were recognized in the lysates by IgA antibodies in the saliva and serum samples obtained from the group of patients (Fig. 4A, B) as opposed to those obtained from the healthy subjects living in the endemic area (Fig. 4C, D). However, some samples contained antibodies that recognized proteins of similar molecular weight. The proteins recognized in both Nf and Eh lysates were molecules of 170 and 105 kDa in the saliva of patients 1 and 4; proteins of 62, 50, and 46 kDa in serum samples from patients 2, 4, and 5; and a molecule of 10 kDa in the saliva of donor 3.

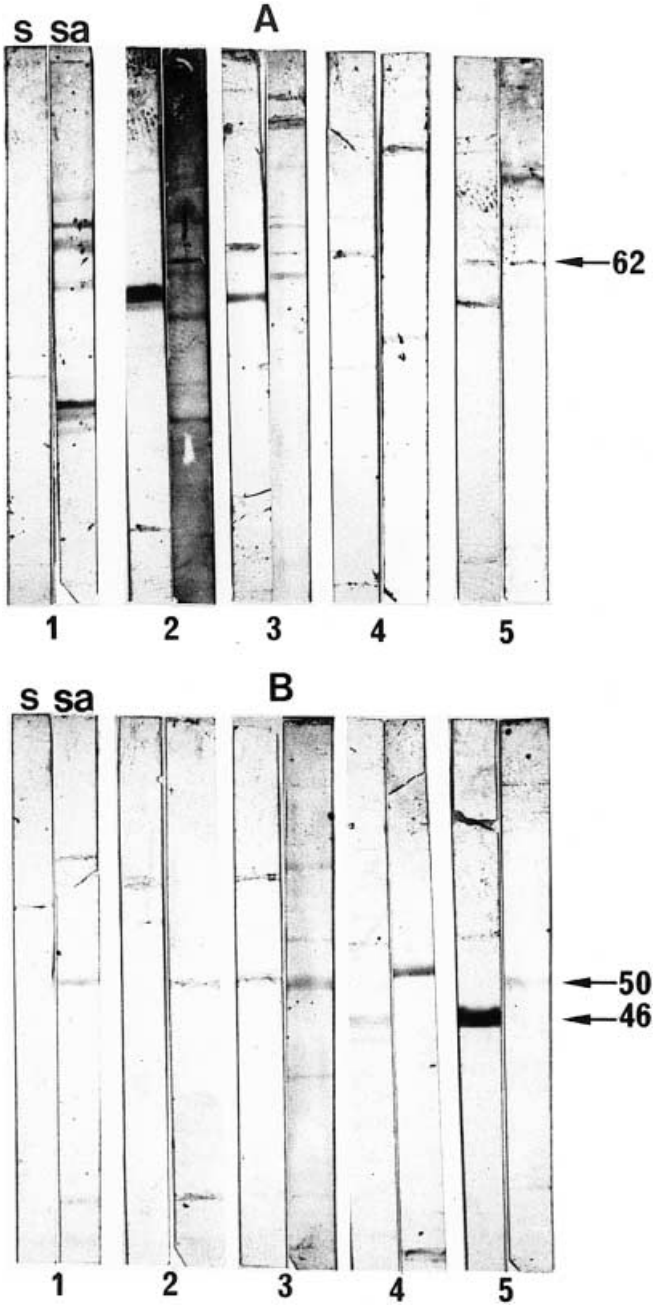

Fig. 2A, B Patterns of antigen recognition of *N. fowleri* proteins by IgA antibodies in serum and saliva from the same individual. Nitrocellulose paper strips were incubated with serum (*s*) or saliva (*sa*) obtained from the same individual from **A** the group of patients or **B** the healthy group. Afterward, goat IgG anti-human IgA antibodies labeled with peroxidase were added. The positions of the main amebic proteins are shown *on the right*. Note the prominent recognition of the 62- and 50-kDa proteins

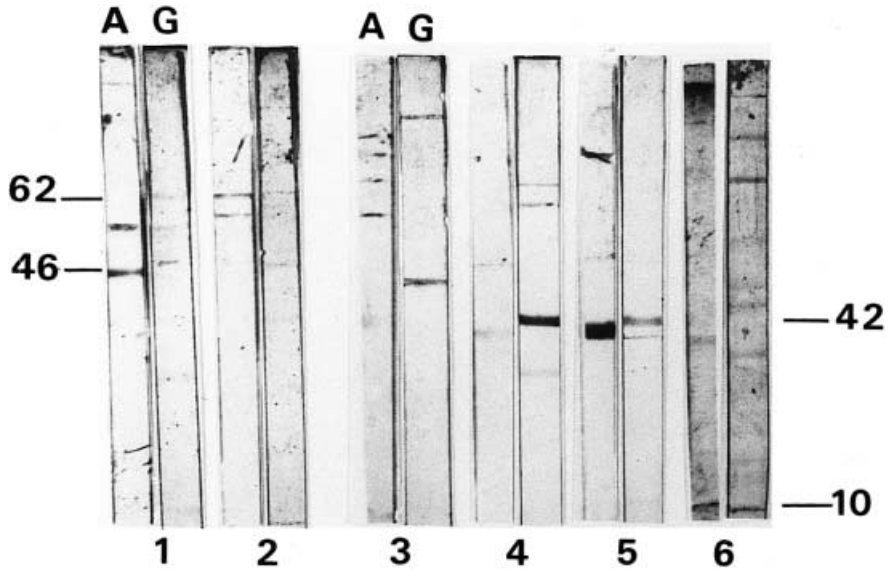

Fig. 3 Patterns of antigen recognition of *N. fowleri* proteins by IgG and IgA antibodies in serum from the same individual. Nitrocellulose paper strips were incubated with serum obtained from the same individual from the group of patients (*1, 2*) or the healthy group (*3–6*). Afterward, goat IgG anti-human IgA antibodies labeled with peroxidase (*A*) or goat IgG anti-human IgG antibodies labeled with peroxidase (*G*) were added

Discussion

The objective of this study was to evaluate the secretory IgA (SIgA) antibody response to *Naegleria fowleri* (Nf) in individuals living in either a parasite-endemic area or a nonendemic area.

The most relevant aspect of these results is that they demonstrate the existence of IgA antibodies that recognized antigens of Nf in the saliva and serum of patients with upper respiratory disease (rhinitis or bronchitis) and in healthy individuals who had no history of amebiasis but lived in an Nf-endemic area. IgA antibodies in many patients recognized greater numbers of Nf antigens more intensely than did those in healthy individuals from the same endemic area. The antigen-recognition pattern was more diverse in the group of patients than in the control group. The group of patients presented a stronger degree of antigen recognition by IgA antibodies and an increased diversity in the pattern of recognition that could be explained by the observation that Nf can more readily cross an inflamed mucosa. The discrepancy in recognition patterns could be attributable to previous infections with different strains or species of nonpathogenic free-living amebas or could depend on genetic differences among the individuals that are manifested as individual responses.

In general, the serum and secretory IgA antibodies obtained from the same individual recognized different proteins of Nf. It is difficult to explain the cause of these differences; but it might be that some proteins of Nf elicit a stronger immune response at the systemic level, whereas others are more immunogenic for the mucosa. However, it should be pointed out that in some individuals, saliva and serum IgA antibodies recognized the same proteins. The IgA antibodies present in saliva could have been produced locally by the plasma cells located in the salivary glands or, less probably, could have resulted from transudation from the blood.

The origin of the IgA antibodies recognizing *Naegleria fowleri* proteins is unknown. Exposure to nonpathogenic strains of free-living amebas that are antigenically related to the pathogenic strains may induce protection against more virulent amebas (Cursons et al. 1979; Haggerty and John 1982; Marciano-Cabral et al. 1987; Powell et al. 1992). Another explanation might be that previous infections with *Entamoeba histolytica*/*E. dispar* can induce antibodies that recognize antigens of *N. fowler*i via cross-reactions. Our results show that in most of the individuals evaluated the antigen-recognition patterns observed for these two species differ; however, some samples recognized similar proteins in Nf and Eh lysates.

Whatever the origin and mechanism of induction of the IgA antibodies that recognize proteins of *N. fowleri* (specific immunization or cross-reaction), these antibodies can participate in the resistance against the amebic invasion, probably by inhibiting the adherence of the trophozoites to the nasal mucosa. As a consequence, a deficiency in IgA secretion could be responsible for the susceptibility to infection with pathogenic free-living amebas (Cursons et al. 1979). The importance of IgA in protection of the host against invasion by *N. fowleri* remains to be established.

Acknowledgements This work was supported by the Dirección General de Asuntos del Personal Académico (Universidad Nacional Autónoma de México DGAPA) IN214296 and DEDICT-COFAA, IPN. We also thank Dr. Fernando Toranzo for his technical assistance.

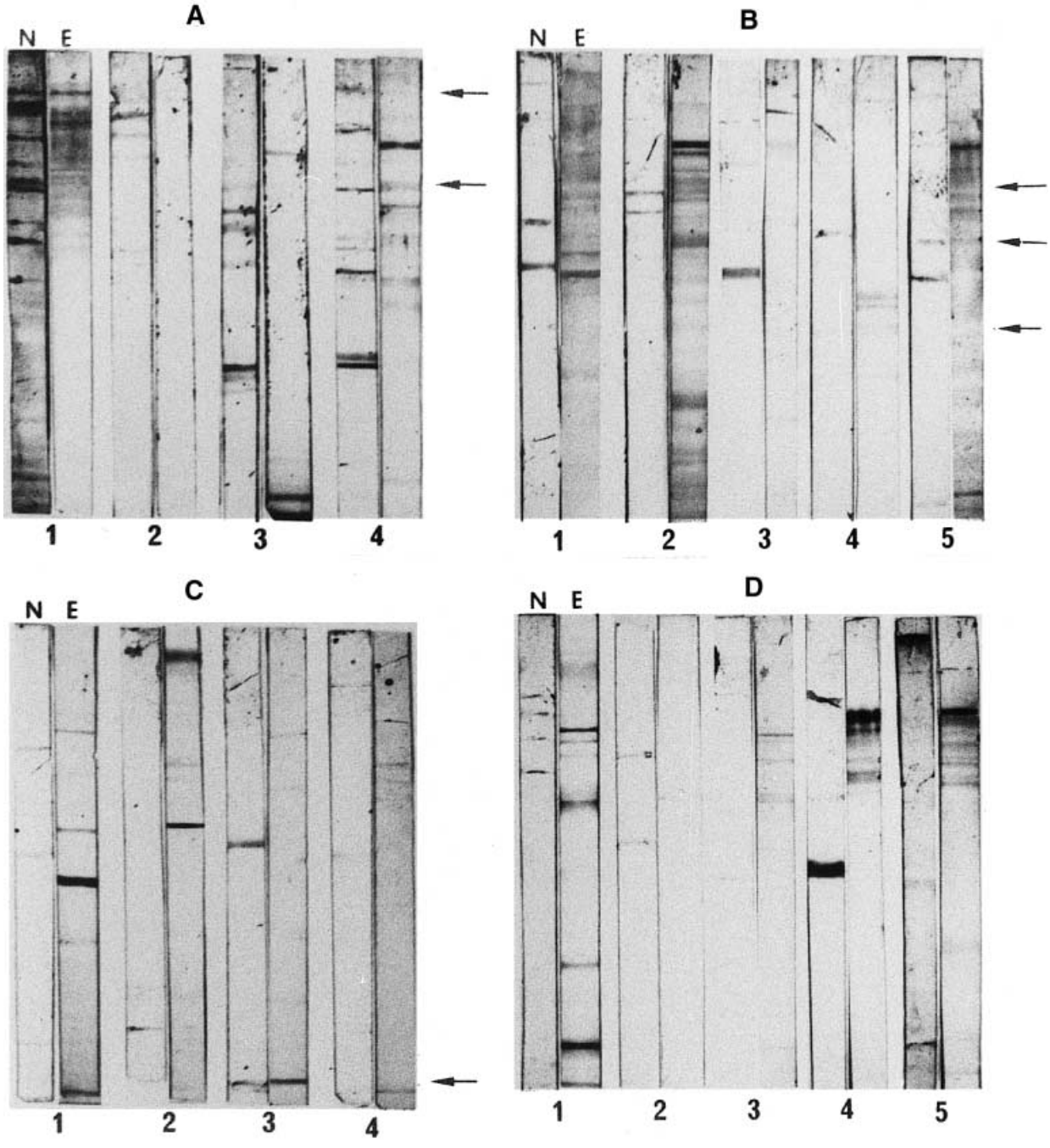

Fig. 4A–D *Entamoeba histolytica* and *N. fowleri* proteins recognized by IgA antibodies from the same individual. *N. fowleri* (*N*) and *E. histolytica* (*E*) proteins were simultaneously separated in SDS-PAGE and transferred to nitrocellulose paper. Afterward, the strips of nitrocellulose paper were incubated with **A** saliva or **B** serum samples from the group of patients or with **C** saliva or **D** serum samples from healthy individuals from the same endemic area. Goat IgG anti-human IgA antibodies labeled with peroxidase were added. *Arrows* indicate the positions of the main proteins recognized in Nf and Eh lysates. The antigen-recognition patterns clearly differ

References

Bradford M (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 116: 53–64

Cursons RTM, Keys EA, Brown TJ, Learmonth J, Campbell C, Metcalf P (1979) IgA and primary amoebic meningoencephalitis. Lancet ii: 223–224

Diamond LS, Harlow DR, Cunnick CC (1978) A new medium for the axenic cultivation of *Entamoeba histolytica* and other *Entamoeba*. Trans R Soc Trop Med Hyg 72: 431–432

Haggerty RM, John DT (1982) Serum agglutination and immunoglobulin levels of mice infected with *Naegleria fowleri*. J Protozool 29: 117–122

Hudson L, Hay FC (1989) Practical immunology, 3rd edn. Blackwell, Oxford, UK

Kilian M, Russell MW (1994) Handbook of mucosal immunology. Academic Press, San Diego

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680–685

Marciano-Cabral F, Cline ML, Bradley SG (1987) Specificity of antibodies from human sera for *Naegleria fowleri* species. J Clin Microbiol 25: 692–697

Powell E, Newsome A, Allen S, Knudson G (1992) Identification of antigens of pathogenic free living amoebae by protein immunoblotting with rabbit immune and human sera. Clin Diagn Lab Immun 1: 493–499

Rodríguez S, Rivera F, Bonilla P, Ramírez E, Gallegos E, Calderón A, Ortiz R, Hernández D (1993) Amoebological study of the atmosphere of San Luis Potosí, SLP, México. J Exp Anal Environ Epidemiol [Suppl] 1: 229–241

Towbin H, Staehelin T, Gordon J (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci USA 76: 4350–4354

Visvesvara GS, Stehr-Green JK (1990) Epidemiology of free-living ameba infections. J Protozool 37: 25S–33S