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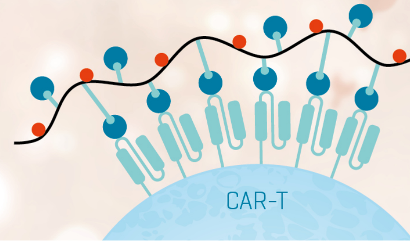

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DETECTION AND CHARACTERIZATION OF MEMBRANE ANTIGENS OF *TOXOPLASMA GONDII*[1]

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Toxoplasma gondii **tachyzoites were surface radioiodinated by the lactoperoxidase technique, and the solubilized membrane proteins were analyzed by two-dimensional polyacrylamide gel electrophoresis. Four major labeled proteins with apparent m.w. of 43,000, 35,000, 27,000, and 14,000 were detected. None of the radioiodinated proteins bound to concanavalin A-Sepharose. When a panel of eight different fluorescein-conjugated lectins was used in an attempt to characterize further the nature of the cell membrane, none of the lectins bound to intact tachyzoites.**

Two-dimensional polyacrylamide gel electrophoresis did not reveal any significant differences among three different strains of Toxoplasma. Each of the radioiodinated surface proteins was precipitable by sera from mice chronically infected with the same strain as well as by a series of sera from mice infected with other strains. Sera from humans with acute Toxoplasma infection showed more variability in that some precipitated all labeled proteins whereas others precipitated only two or three of them.

Monoclonal antibodies (2G11 and 3E6) prepared by hybridization of spleen cells from Toxoplasma-immune mice with myeloma cells consistently precipitated both the solubilized 35,000 and 14,000 dalton proteins, whereas 1E3 precipitated the 43,000-dalton protein and 1E11 the 27,000-dalton protein.

Toxoplasma gondii is a ubiquitous intracellular protozoan parasite that is capable of infecting all species of mammals (1). Serologic studies in humans indicate that from 20 to 90% of adult populations have been infected with Toxoplasma (2-4), and serious disease due to this parasite occurs in congenitally infected children and in immunosuppressed patients. In veterinary medicine, it is a significant cause of ovine abortion in England and Australia (1). Characterization and isolation of Toxoplasma antigens would be valuable for the study of the immune response to the parasite and for the development of newer diagnostic methods and vaccines. We report here the first characterization of membrane antigens of Toxoplasma.

MATERIALS AND METHODS

Mice. Swiss Webster female mice (Simonsen Laboratories, Gilroy, Calif.) were 8 weeks old at the beginning of the experiments.

Preparation of Toxoplasma. Toxoplasma isolates from humans and animals were routinely maintained in mice by yearly passage of brain tissue containing cysts. Infection was confirmed by demonstration of Toxoplasma antibodies in the Sabin-Feldman dye test (5) and by demonstration of Toxoplasma cysts in brains of the mice. The strains of Toxoplasma used for preparation of antigens were RH, C37, and C56. Strain RH tachyzoites from the peritoneal fluid of 2-day-infected mice were collected in phosphate-buffered saline (PBS), pH 7.4, containing 10 units/ml of heparin (Abbott Laboratories, Chicago, Ill.). Debris and host cells were removed by filtration through a Millipore polycarbonate membrane (pore size 3 μ; Nucleopore Corp., Pleasanton, Calif.).

On day 2 of infection, free tachyzoites showed IgM Toxoplasma antibodies on their surface as detected by immunofluorescence using a fluorescein-conjugated rabbit antiserum specific for mouse IgM (E. Handman, unpublished observation). For the preparation of strain C37 tachyzoites, homogenized brain tissue containing cysts was injected i.p. into mice that had received 200 mg/ml cyclophosphamide (Cytoxan; Mead-Johnson, Evansville, Ind.) 2 days before infection. Four days after infection, tachyzoites were collected as described above. The cyclophosphamide treatment reduced the problem of antibody coating of free tachyzoites in the peritoneal fluid but did not completely eliminate it.

Toxoplasma antisera. Antisera to numerous strains of Toxoplasma were obtained from a bank of mice in our laboratory that were infected with the various strains. In these experiments, groups of mice chronically infected with the following isolates were used: Ariadne, CML, Conley, DH, Edeza, Hill, RJ, Sterling, and Vales (from humans); Ligon (from a field mouse); and C37 and C56 (from chickens). Sera collected from patients 2 to 6 months after the clinical onset of acute Toxoplasma infection were obtained from a bank of such sera stored in our laboratory. All sera were stored in aliquots at −70°C until used.

[^{35}S]-methionine labeling of Toxoplasma tachyzoites. Monolayers of mouse peritoneal macrophages were prepared and infected with the RH strain of Toxoplasma, as previously

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described (6). Briefly, the cells were allowed to adhere to Petri dishes (Falcon Plastics, Los Angeles, Calif.) for 60 min at 37°C in an atmosphere of 5% CO_2. The monolayers were washed with warm RPMI 1640 medium (Flow Laboratories, Rockville, Md.) and infected with Toxoplasma tachyzoites obtained from the peritoneal fluid of mice infected with the RH strain of Toxoplasma. An initial ratio of four parasites per adherent cell resulted in infection of 50% to 60% of the cells. The infected monolayers were washed with methionine-free RPMI 1640 medium and then labeled with 500 μCi [^{35}S]-methionine (800 Ci/mmole; Amersham Searle Corp., Arlington Heights, Ill.) for 15 hr in methionine-free RPMI 1640 medium containing 10% dialyzed fetal calf serum. The monolayers were then washed in RPMI 1640 and lysed for 30 min at 4°C in 0.5% Nonidet P-40 (Particle Data, Inc., Elmhurst, Ill.) in PBS, pH 7.4. The lysates were centrifuged at 5000 × G for 20 min to remove nuclei and debris. Lysates were "precleared" with 50 μl of a 10% v/v suspension of heat killed and formalin-fixed *Staphylococcus aureus* of the Cowan I strain (New England Enzyme Centre, Boston, Mass.) in order to remove any material that bound to staphylococci alone (7). Extracts were then treated for 1 hr at 4°C with 50 μl of the appropriate mouse antiserum, and complexes were isolated by binding to *S. aureus* as described by Kessler (8). The wash buffer, pH 8.3, consisted of 0.05 M Tris, 0.5 M NaCl, and 0.5% Nonidet P-40. Sodium dodecyl sulfate (SDS)[5] gel electrophoresis (10% acrylamide) was performed as described by Jones (9).

[^{125}I]-labeling of Toxoplasma membrane proteins. Intact tachyzoites of the RH, C37, or C56 strain of Toxoplasma were harvested from the peritoneal cavities of mice, as described above. Debris and host cells were removed by centrifugation and filtration through a Millipore polycarbonate membrane, as described above. Viability was >95% as assessed by staining with acridine orange and ethidium bromide (10), and there was <0.1% contamination with host cells.

Tachyzoites (5×10^7 in 200 μl PBS, pH 7.4) were radioiodinated by the lactoperoxidase technique (11), using 1 mCi [^{125}I] (sodium iodide; Amersham Searle Corp., Arlington Heights, Ill.) and 10-μl pulses of H_2O_2 (0.3 mM, 1 mM, 3 mM, and 9 mM) at 1-min intervals at 20°C. (The radioiodination procedure did not result in detectable loss of viability.) Tachyzoites were then washed twice and lysed in 0.5% Nonidet P-40 in PBS, pH 7.4, and antigens were precipitated as described above. Before precipitation, extracts were "precleared" with staphylococci as described above.

Two-dimensional gel electrophoresis was performed as described by O'Farrell *et al.* (12), with 13% acrylamide in the second dimension.

Polyacrylamide gel electrophoresis. In some experiments, samples were run on one-dimensional slab gels by utilizing the discontinuous SDS gel electrophoresis system described by Laemmli (13) in 13% or 10% acrylamide as specified in *Results*. Molecular weight standards (bovine serum albumin, ovalbumin, lactate dehydrogenase, immunoglobulin light chains, and lysozyme) were run on each slab gel, and the resulting Coomassie Blue-stained bands were used to determine the approximate m.w. of bands on the autoradiograms. In other experiments, radiolabeled proteins were electrophoresed in two dimensions by the method developed by O'Farrell *et al.* (12). Briefly, for separation in the first dimension, samples were run in a nonequilibrium pH gradient in thin glass tubes (9). The second dimension electrophoresis was done, as described above, in 13% acrylamide slab gels. Gels were stained in 0.1% Coomassie Blue in 50% trichloroacetic acid, destained in 7% acetic acid, and dried. Gels of the [^{125}I]-labeled proteins were autoradiographed with Kodak X-Omat R film and Dupont Cronex Lightning Plus intensifying screens (14). Exposure time was typically 24 hr. Gels of the [^{35}S]-labeled proteins were autoradiographed with Kodak NS-2T no-screen x-ray film. Exposure time was 4 days.

Lectin binding to Toxoplasma membranes. Gel columns containing 0.5 ml concanavalin A-Sepharose (Pharmacia, Piscataway, N. J.) were prepared in tuberculin syringes and washed with either Tris-buffered saline containing 10 mM Ca^{++} and Mg^{++} or 0.2 M α-methyl-mannoside (Sigma Chemical Co., St. Louis, Mo.) in the same buffer. A volume of 500 μl of radioiodinated detergent extract of Toxoplasma was passed over these columns before electrophoresis.

Studies on lectin binding by intact living tachyzoites were done by using eight different fluorescein-conjugated lectins (E. Y. Laboratories, San Mateo, Calif.). Tachyzoites were collected, washed in PBS, pH 7.4, containing 10 mM Ca^{++} and Mg^{++}, and adjusted to 1×10^8/ml; 50 μl of the suspension were dispensed in each well of round bottom microtiter trays. The parasites were incubated in triplicate wells for 60 min at 4°C with 5 μg of each of the fluorescein-conjugated lectins. As a control for lectin activity, mouse spleen cells and *Trypanosoma cruzi* parasites were used. Normal mouse spleens were forced through an 80-mesh stainless steel screen, and the cell suspension was washed in PBS, pH 7.4, containing 10 mM Ca^{++} and Mg^{++}. A volume of 50 μl containing 5×10^6 cells was dispensed in each well and incubated with the fluorescein-conjugated lectins, as described above. The three morphologic forms of *T. cruzi*--epimastigotes, trypomastigotes, and amastigotes--were also used as controls for lectin activity (F. G. Araujo *et al.*, submitted for publication).

All cells were incubated for 60 min at 4°C, washed three times in PBS, pH 7.4, and examined with a Zeiss fluoresence microscope equipped with vertical illumination.

Production of monoclonal antibodies to membrane antigens of Toxoplasma. Monoclonal antibodies, produced by the technique of Köhler and Milstein (15) as modified by Oi *et al.* (16), were used to characterize Toxoplasma antigens. Chronically infected mice were boosted once i.p. with 5×10^6 formalin-fixed tachyzoites, and their spleen cells were hybridized with the NS-1 variant of the P-3 (MOPC-21) myeloma cell line 3 days later. Supernatants were screened by radioimmunoassay on intact tachyzoites, and positive cultures were cloned by limiting dilution (16; E. Handman *et al.*, manuscript submitted for publication).

RESULTS

Biosynthetically labeled Toxoplasma antigens. In initial studies, we attempted to define Toxoplasma antigens by biosynthetic labeling with [^{35}S]-methionine. Monolayers of mouse peritoneal macrophages infected with the RH strain of Toxoplasma were cultured in medium containing [^{35}S]-methionine. Control cultures consisted of noninfected macrophages. Cells were lysed in Nonidet P-40, and the lysate was incubated with the antiserum to Toxoplasma collected from mice chronically infected with the RH strain of the organism. Immune complexes were collected by the addition of protein A bearing *S. aureus*, and the resulting precipitates were dissociated and analyzed by gel electrophoresis. As can be seen in Figure 1, the antigenic structure of Toxoplasma recognized by antisera from mice infected with viable tachyzoites is obviously complex, with more

[5] Abbreviations used in this paper: SDS, sodium dodecyl sulfate.

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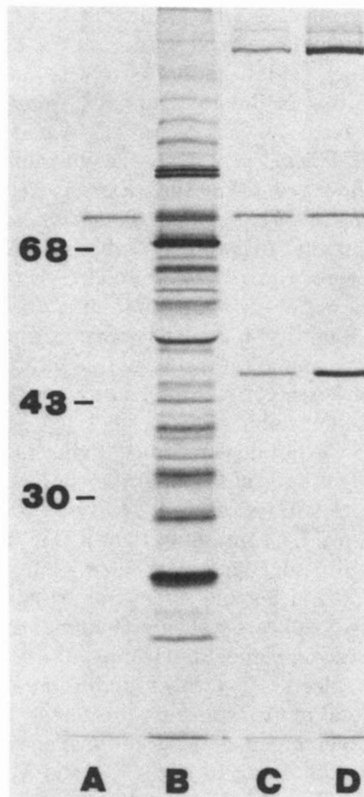

Figure 1. One-dimensional polyacrylamide gel electrophoresis patterns of [^{35}S]-labeled Toxoplasma antigens bound by antibodies from mice chronically infected with the RH strain of Toxoplasma. *A*, proteins from infected macrophages that had bound to staphylococci (no antiserum); *B*, proteins from infected macrophages bound by mouse anti-Toxoplasma antibodies; *C*, proteins from uninfected macrophages that had bound to staphylococci (no antiserum); *D*, proteins from uninfected macrophages precipitated by mouse anti-Toxoplasma antibodies. Numbers represent m.w. standards: bovine serum albumin (68,000), ovalbumin (43,000), and lactate dehydrogenase (30,000).

than 32 bands identified on one-dimensional gels. Approximately 70 spots were seen on autoradiographs of two-dimensional gels of immune precipitates, whereas over 1000 spots were seen on autoradiographs of two-dimensional gels of the whole extract in the absence of antiserum (data not shown).

Surface radioiodinated antigens from three different strains of Toxoplasma. In view of the complexity of the antigenic pattern obtained with metabolic labeling, we chose to label the surface of parasites with [^{125}I] using the lactoperoxidase technique. In contrast to the complex pattern shown in Figure 1, gels of the radioiodinated proteins revealed a simple pattern. Only five major bands and some additional minor spots were identified on two-dimensional gels (Fig. 2*A*). The major radioiodinated species were relatively acidic, although the nonequilibrium pH gradient electrophoresis used does not allow assignment of isoelectric points (12).

Four of the major radioiodinated proteins seen when the whole extract was run on a two-dimensional gel (Fig. 2*A*) were consistently detected in all experiments and were immunoprecipitated by antibodies from chronically infected mice (Fig. 2*B* and 2*C*) and acutely infected humans (Fig. 4). We suggest that these bands be designated according to their apparent m.w. as p43,000, p35,000, p27,000, and p14,000. A protein with a m.w. of approximately 40,000 daltons, apparent in Fig. 2*A* and immunoprecipitated by antibodies from chronically infected mice (Fig. 2*C*), was detected occasionally in some experiments. It was not apparent when the immunoprecipitates were analyzed on a two-dimensional gel (Fig. 2*B*). This polypeptide may be a proteolytic breakdown product of p43,000 and may lack some antigenic determinants.

A two-dimensional gel analysis of the extracts of the C37 strain (Fig. 3*A*) and the C56 strain (data not shown) revealed the same pattern of radioiodinated proteins as did the RH strain. Also, each of the major radioiodinated proteins seen in the whole extract was recognized by antibody from mice chronically infected with the C37 strain (Fig. 3*A* and *B*) or the C56 strain (data not shown). When the RH strain was used as an antigen, antisera from mice chronically infected with the RH, C56, or C37 strain precipitated identical bands on two-dimensional gels (data not shown).

Lectin binding by Toxoplasma membranes. Passage of detergent lysates of radioiodinated parasites over concanavalin A-Sepharose before electrophoresis did not result in depletion of any of the major antigens. Moreover, none of a panel of eight different fluorescein-conjugated lectins bound to intact tachyzoites. The lectins used and their sugar specificity were from *Canavalia ensiformis* (mannose), *Limulus polyphemus* (sialic acid), *Ulex europaeus* (α-L-fucose), *Triticum vulgaris* (*N*-acetyl-β [1 > 4]-D-glucosamine) 2 (sialic acid), *Ricinus communis* (β-D-galactose), *Glycine max* (*N*-acetyl-α-D-galactosamine), *Dolichos biflorus* (*N*-acetyl-α-D-galactosamine) and *Arachis hypogaea* (D-gal-β [1 > 3]-gal-*N*-acetyl). The lectins were functionally active, as shown by their specific binding to *T. cruzi* parasites and to spleen cells.

Toxoplasma antigens detected by mouse and human antibodies. We considered it of interest to compare the membrane antigens of Toxoplasma detected by sera obtained from humans who had a recent history of acute Toxoplasma infection with those detected by sera obtained from mice that were chronically infected with various strains of Toxoplasma. A detergent lysate preparation of radiolabeled tachyzoites of the RH strain was immunoprecipitated with the human or mouse sera and analyzed by one-dimensional gel electrophoresis.

We observed a wider variability in the relative intensity of the antigen bands detected by antibodies from acutely infected humans (Fig. 4*A*) than in those detected by antibodies from chronically infected mice (Fig. 4*B*). For example, all bands are apparent in lanes a–d, whereas p43,000 and p27,000 are not seen in lane e (Fig. 4*A*). Using antibody from sequential bleedings of the same patient over a period of one year, we observed the same inconsistent variability in the pattern of antigen bands detected on one-dimensional gels (data not shown). Antibodies from mice infected with one or five different strains of Toxoplasma isolated from humans detected the same antigen bands on one-dimensional gel electrophoresis (Fig. 4*B*). These antigens are identical to the ones immunoprecipitated by antisera from mice infected with the RH strain (Fig. 2) or the C37 strain (Fig. 3) and represent all of the radioiodinated membrane proteins of Toxoplasma.

Toxoplasma antigens detected by monoclonal antibodies. Monoclonal antibodies produced against membrane antigens of Toxoplasma tachyzoites were also used for the immunochemical analysis of membrane antigens.

Four clones were used for these studies (Fig. 5). Clone 1E3 (IgG2) precipitated p43,000, whereas 1E11 (IgG3) precipitated p27,000. In each case, a single band was noted, and the m.w. of unreduced proteins were similar to those obtained after reduction with mercaptoethanol. On the other hand, the monoclonal antibodies from two independently arising clones, 3E6 (IgG2) and 2G11 (IgG2) (data not shown), consistently precipitated

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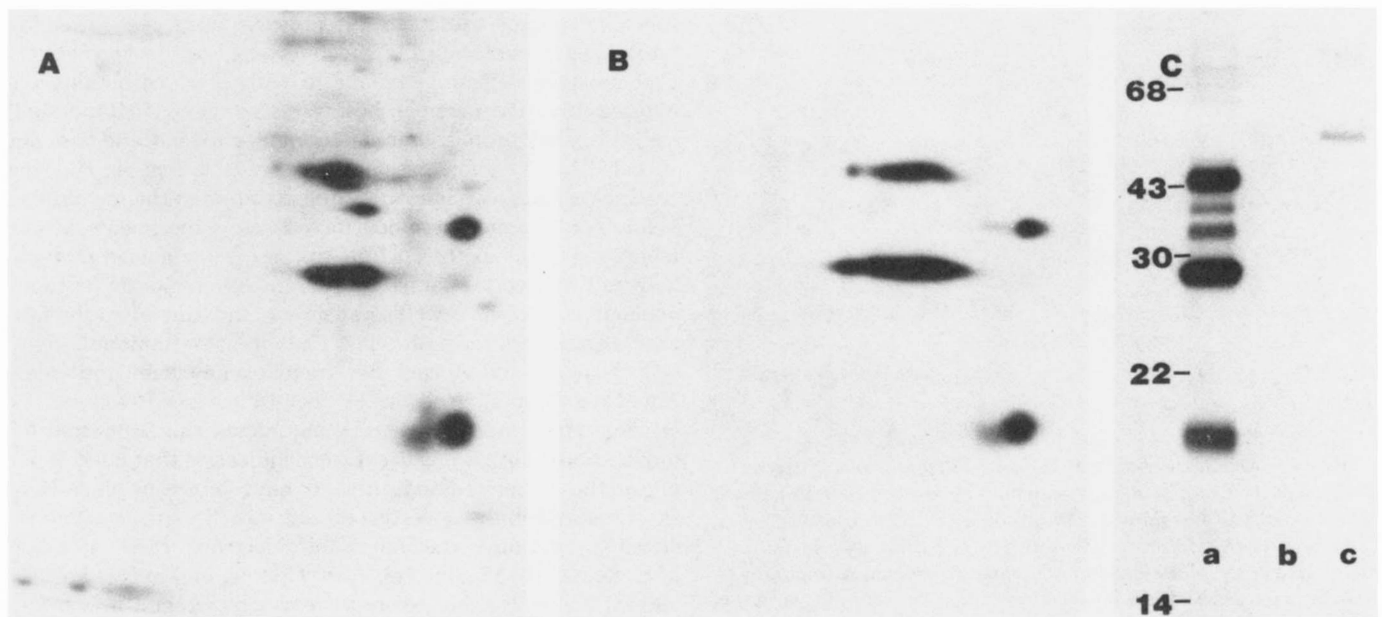

Figure 2. Two-dimensional polyacrylamide (*A* and *B*) and one-dimensional SDS (*C*) gel electrophoresis patterns of [^{125}I]-labeled Toxoplasma antigens bound by antibodies from mice chronically infected with the RH strain of Toxoplasma. *A,* proteins in whole lysate; *B,* proteins precipitated by mouse anti-Toxoplasma antibodies; *C.a,* proteins precipitated by mouse anti-Toxoplasma antibodies; *C.b,* proteins that had bound to staphylococci (no antiserum); *C.c,* proteins precipitated by normal mouse serum. Bands in lane *C.c* have the expected mobilities of μ-chains, γ-chains, and light chains and presumably represent traces of host antibody bound to the parasite membrane before radioiodination. Numbers represent m.w. standards: bovine serum albumin (68,000), ovalbumin (43,000), lactate dehydrogenase (30,000), immunoglobulin light chains (22,000), and lysozyme (14,000).

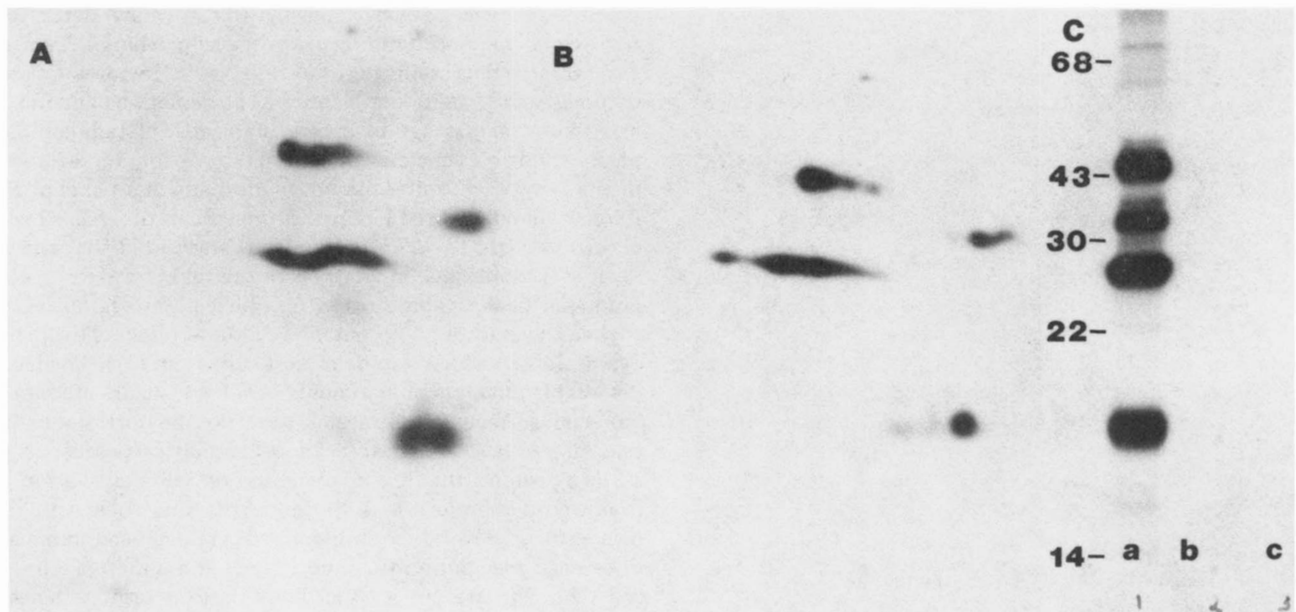

Figure 3. Two-dimensional polyacrylamide (*A* and *B*) and one-dimensional SDS (*C*) gel electrophoresis patterns of [^{125}I]-labeled Toxoplasma antigens bound by antibodies from mice chronically infected with the C37 strain of Toxoplasma. *A,* proteins in whole lysate; *B,* proteins precipitated by mouse anti-Toxoplasma antibodies; *C.a,* proteins precipitated by mouse anti-Toxoplasma antibodies; *C.b,* proteins that had bound to staphylococci (no antiserum); *C.c,* proteins precipitated by normal mouse serum. Numbers represent m.w. standards (see legend to Figure 2).

both p35,000 and p14,000. There was some variability in the relative intensities of the p35,000 and p14,000 bands, suggesting that p14,000 may represent a proteolytic breakdown product of p35,000, which still possesses the relevant antigenic determinant. However, incorporation of the protease inhibitors, phenylmethylsulfonyl-fluoride and iodoacetamide, into the cell lysis buffer had no effect on the ratio of intensity of these two bands. Proteins p35,000 and p14,000 are not attached to each other by disulfide bonds since analysis of precipitates reduced with mercaptoethanol resulted in electrophoretic patterns identical to those of nonreduced precipitates.

DISCUSSION

The results presented above characterize certain of the properties of cell membrane antigens of Toxoplasma. In the initial

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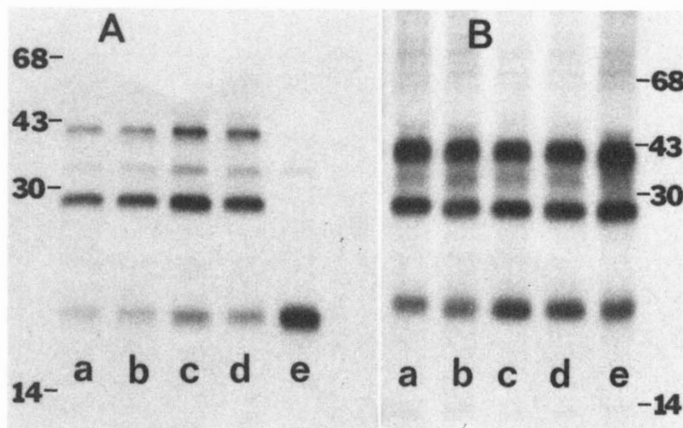

Figure 4. One-dimensional SDS gel electrophoresis patterns of [^{125}I]-labeled Toxoplasma antigens bound by antibodies from humans acutely infected and mice chronically infected with Toxoplasma. *A*, proteins precipitated by sera from five humans acutely infected with Toxoplasma (see *Materials and Methods*). *B*, proteins precipitated by sera from mice chronically infected with one of the following strains of Toxoplasma: *a*, CML; *b*, RJ; *c*, Sterling; *d*, DH; or *e*, RH; of these five human isolates, two (CML and RH) were from congenitally infected infants. Numbers represent m.w. standards (see legend to Figure 2).

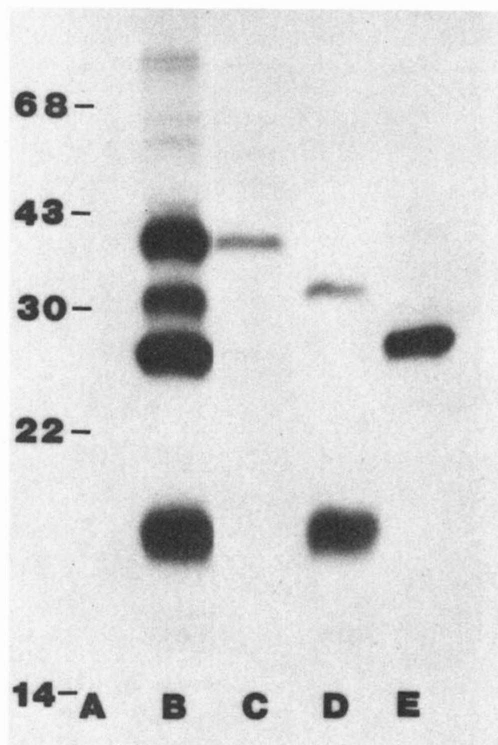

Figure 5. One-dimensional SDS gel electrophoresis patterns of [^{125}I]-labeled Toxoplasma antigens bound by monoclonal antibodies. *A*, proteins that had bound to staphylococci (no antiserum); *B*, proteins precipitated by mouse anti-Toxoplasma antibodies; *C, D,* and *E*, proteins precipitated by antibodies from clone 1E3, clone 3E6, and clone 1E11, respectively. Numbers represent m.w. standards (see legend to Figure 2).

studies, in which we attempted to define Toxoplasma antigens by biosynthetic labeling with [^{35}S]-methionine, a complex pattern of proteins was noted and only a few of them were recognized by antibodies from chronically infected mice. In contrast, gels of radioiodinated membrane proteins of Toxoplasma tachyzoites showed a remarkably simple pattern of labeled proteins and all of these labeled proteins were recognized by antibodies from chronically infected mice. We consider it likely that most of the antigens labeled with [^{35}S]-methionine are cytoplasmic. It is possible, however, that some [^{35}S]-metabolically labeled proteins are membrane antigens and are not revealed by [^{125}I]-labeling because they lack exposed tyrosine residues are not available for radioiodination in the membrane.

Further evidence obtained in separate experiments in our laboratory leads us to conclude that the radiolabeled proteins detected by electrophoresis and immunoprecipitated by monoclonal antibodies, as well as by mouse and human antibodies, are integral membrane proteins. Thus, indirect immunofluorescence experiments showed that the four monoclonal antibodies were capable of binding to viable tachyzoites (see Reference 17) (E. Handman and J. S. Remington, manuscript submitted for publication). Also, we have evidence indicating that most, if not all, of the radioiodinated antigens bind detergent since they show marked shifts in electrophoretic mobility in a two-dimensional adaptation of the charge shift electrophoresis technique of Helenius and Simons (18; J. W. Goding, unpublished observation). They are thus likely to represent integral membrane proteins. In addition, when extracts were prepared from tachyzoites that were heat killed (at 60°C for 10 min) before radioiodination, the pattern obtained on two-dimensional gel electrophoresis was complex (data not shown) and differed considerably from the simple five-band pattern obtained when extracts were prepared from organisms that were intact before radioiodination.

Unlike many membrane antigens of eukaryotic cells (19), the membrane antigens of Toxoplasma that we have detected do not exhibit marked heterogeneity in size or charge. This suggests that glycoproteins may not represent a major component of the parasite membrane. There was no depletion of membrane antigens after passage of detergent lysates of radioiodinated parasites over a concanavalin A-Sepharose column. Moreover, intact living tachyzoites did not bind to any of a panel of eight fluorescein-conjugated lectins. Of interest in this regard is the report by Sethi *et al.* (20) in which it was noted that the cyst form of Toxoplasma, in contrast to the tachyzoite form, could bind specifically concanavalin A, wheat germ agglutinin, and soybean agglutinin. The outer membrane of the Toxoplasma cyst wall is considered to be of host cell origin (21). The lack of detectable amounts of commonly occurring sugars of mammalian cell surface glycoproteins may not be fortuitous. It is tempting to postulate that Toxoplasma may possess lectin-like surface proteins that are involved in host cell penetration and that would therefore lack the corresponding sugars on their own surface. Several lectin-like proteins have been implicated in cell-cell recognition (22) and in bacterial adherence to host cells (23). We are presently attempting to determine whether any of the major radioiodinated antigens have lectin-like activity.

Comparison of the two-dimensional gel analyses of three strains of Toxoplasma revealed almost identical patterns of labeled membrane proteins. The virulent RH strain (isolated from a human subject) and the moderately virulent C56 and avirulent C37 strains (isolated from chickens) had identical patterns of membrane antigens, as detected by specific antisera from mice. We were unable to detect any antigenic difference among these strains, despite their wide variability in virulence. In the case of Toxoplasma, virulence appears to correlate with generation time (24, 25) but does not seem to correlate with the antigen pattern of the parasite membrane.

When radioiodinated lysate preparations of Toxoplasma were

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immunoprecipitated with sera from mice chronically infected with Toxoplasma strains isolated from humans, it was evident that all sera detected the same major membrane antigens. In contrast, when these preparations were immunoprecipitated with a series of sera from humans acutely infected with Toxoplasma, there was some variability in the membrane antigens detected. Although these latter sera were obtained from 2 to 6 months after onset of clinical illness, some sera did and some did not detect all labeled membrane antigens. From separate studies of the time course of development of Toxoplasma antibodies in mice, it appears that all the labeled membrane antigens are detected simultaneously in an all-or-none fashion (E. Handman *et al.*, manuscript submitted for publication).

A more detailed analysis of Toxoplasma membrane antigens was made possible by the use of monoclonal antibodies directed against membrane antigens. Of the four major radioiodinated polypeptides, two (p43,000 and p27,000) were independently precipitable. The remaining two polypeptides (p35,000 and p14,000) were both consistently precipitated by antibodies from two independently arising clones. It is possible that p35,000 is a dimer that consists of two p14,000 subunits and that is not completely disrupted by boiling in SDS. Alternatively, p14,000 may be a proteolytic breakdown product of p35,000, although inclusion of protease inhibitors or prolonged incubation without protease inhibitors did not change their relative intensities. These peptides, p35,000 and p14,000, are not linked to each other by disulfide bonds since patterns of precipitates reduced with mercaptoethanol were identical to those of nonreduced precipitates. We have not ruled out the possibility that p35,000 and p14,000 are held together by noncovalent forces.

The ability to separate and identify Toxoplasma antigens should allow determination of those antigens that stimulate a protective immune response, and purification of these antigens on a preparative scale should prove to be of value for diagnostic purposes and for formulation of a vaccine. The fact that antisera from infected humans also reacted with the antigens that we have studied suggests that these antigens may be useful for detecting infection and disease in humans. The monoclonal antibodies have already been used by us to detect antigens in sera and body fluids of infected adults and infants (F. G. Araujo *et al.*, manuscript in preparation).

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