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Identification of a Virulence-Associated Antigen of *Toxoplasma gondii* by Use of a Mouse Monoclonal Antibody†

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A monoclonal antibody generated against the mouse-lethal RH strain of *Toxoplasma gondii* was developed. Tachyzoites of virulent and avirulent *T. gondii* isolates grown in permanent macrophage cell cultures were examined for differences in reactivity with this antibody. Virulence of these *Toxoplasma* isolates was quantified by injecting different numbers of tachyzoites into NMRI mice and observing the animals for signs of infection or death. The monoclonal antibody identified a 23-kDa antigen expressed by the mouse-lethal strains BK and RH, whereas this antigen was not detected in low-mouse-virulent strains, which were all clinical isolates from Europe. Using Western blot (immunoblot), immunofluorescence, and immunoelectron microscopy, we localized the 23-kDa antigen to the membrane compartment. From these results, we suggest that this 23-kDa antigen is a marker of strain virulence upon which a virulence classification of *T. gondii* may be based.

Toxoplasma gondii is an obligate intracellular protozoan parasite. So far, only one species of the genus *Toxoplasma* has been shown to exist, although marked differences in mouse virulence between different isolates of this parasite have been observed. It is commonly accepted to divide *T. gondii* into mouse-lethal and non-mouse-lethal isolates (6, 7, 15, 25, 30, 34). Interestingly, those isolates maintained for more than 40 years by passage in mice are able to kill mice in less than 10 days after intraperitoneal inoculation of at least 100 pathogens. These isolates do not form cysts in the brains of infected mice. In contrast, fresh clinical human isolates of *T. gondii* are usually non-mouse lethal but produce a general infection by developing cysts mainly located in the brains of infected mice. It was argued that multiple passages in mice might increase the virulence of *T. gondii* because of the lack of sexual development in the life cycle of this parasite or different mechanisms of parasite multiplication (6, 7, 10, 23). At present, only a few virulence factors of *T. gondii* have been identified. Among them, the best-analyzed virulence determinant is the so-called penetrating-enhancing factor (16, 18). In addition to variation in virulence for mice, *T. gondii* can be differentiated by growth rate in cell cultures (14, 15), protein pattern generated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (2), isoenzyme profiles (1, 5), and serological methods (28, 29, 33).

Using a panel of several monoclonal antibodies (MAbs) generated against tachyzoites of the virulent RH strain, we serologically typed several clinical isolates of *T. gondii* to determine whether antigenic differences existed which correlated with virulence. This report describes an MAb that reacts exclusively with the most common virulent laboratory strains of *T. gondii*, RH and BK.

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† Cordially and respectfully dedicated to J. Morenz on the occasion of his 60th birthday.

MATERIALS AND METHODS

Bradyzoites and tachyzoites. Female NMRI mice (20 to 24 g) were injected intraperitoneally with the *T. gondii* isolates NTE, 561, 177, ALT, DX, GAIL, and RKR (Table 1). At 6 weeks postinfection, brains were removed and cysts were isolated as described elsewhere (4). To obtain tachyzoites of these isolates, mice pretreated with cortisone were injected with a cyst-containing brain suspension of mice formerly injected with the respective isolates. At 5 days postinfection, a peritoneal lavage was done and the resulting tachyzoite-containing lavage suspensions were used to infect cell cultures of P388 macrophages (20). The medium consisted of RPMI 1640 (Biochrom, Berlin, Germany) supplemented with 5% fetal calf serum, 300 mg of L-glutamine per liter, and antibiotics and was changed every second day. Tachyzoites were separated from macrophages by filtering the supernatant through a 3.0-μm-pore-size polycarbonate filter membrane (Nuclepore, Bromma, Sweden). After two washing steps with phosphate-buffered saline (PBS) (150 mM NaCl, 20 mM sodium phosphate, pH 7.2), tachyzoites were resuspended in PBS at a concentration of 10^6/μl (for Western blot [immunoblot] analysis) or 10^5/μl (for immunofluorescence study). *T. gondii* isolates RH and BK (Table 1) were maintained as tachyzoites in P388 macrophage cell culture and harvested as described above.

Determination of mouse virulence. The different *T. gondii* isolates were inoculated intraperitoneally into four NMRI mice at doses of 1, 10, 100, or 1,000 tachyzoites per test group. All tachyzoites were derived from cell cultures. The infected mice were observed for 21 days. Virulence in mice was classified according to the following categories: asymptomatic, symptomatic, and lethal upon injection of 100 tachyzoites. The cumulative percent mortality and mean time of death were calculated as previously described (10, 19, 24).

Generation of monoclonal antibody 5B10. BALB/c mice were immunized with preparations of *T. gondii* isolate RH as previously described (21). Briefly, 100 to 500 μg of *Toxoplasma* lysate antigen was injected intraperitoneally on days 0, 28, and 42 into 10 female BALB/c mice, which were then injected with 10^6 to 10^7 formalin-treated tachyzoites on days

TABLE 1. Characterization of *T. gondii* isolates

Isolate	Origin	Diagnosis	Year of isolation	Reference
RH	Human cerebrospinal fluid	Connatal toxoplasmosis	1941	Sabin, U.S.A. (26)
BK	Human cerebrospinal fluid	Connatal toxoplasmosis	1948	Winser, Holland (35)
177	Human cerebrospinal fluid	Connatal toxoplasmosis	1987	Janitschke, Berlin, Germany
ALT	Human	Toxoplasmosis	1965	Werner, Berlin, Germany
561	Human lung biopsy	AIDS	1987	Janitschke, Berlin, Germany
NTE	Human brain biopsy	AIDS	1990	Gross, Würzburg, Germany
RKR	Human lymph node	Toxoplasmosis	1990	Gross, Würzburg, Germany
GAIL	Human	Toxoplasmosis	?	Saathoff, Bonn, Germany
DX	Pig	?	?	Saathoff, Bonn, Germany

160 and 174. On day 177, spleen cells of the three mice with the highest antibody titers were fused with the myeloma cell line P3X63Ag8.653 (HGPRT$^-$) (8). Hybridoma cells were maintained in RPMI 1640 medium supplemented with 10% fetal calf serum and subcloned by Poisson limited dilution. Beginning after 10 to 14 days, culture supernatants were analyzed for the presence of *T. gondii*-specific antibodies by immunofluorescence and enzyme immunoassay (22).

SDS-PAGE and Western blot. After resuspension in a buffer consisting of 60 mM Tris (pH 6.8), 2% SDS, 10% glycerol, 5% mercaptoethanol, and 0.001% bromphenol blue and boiling for 5 min, total cell lysate of 7.5×10^6 tachyzoites was analyzed on an SDS-13% polyacrylamide gel (17), and the separated peptides were electrophoretically transferred onto 0.45-μm-pore-size nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany) (32). After blocking with 1.5% bovine serum albumin, the nitrocellulose membrane was incubated for 8 h either with MAbs diluted 1:10,000 in PBS or with polyclonal rabbit antiserum generated against the RH isolate and then incubated for 1 h with either alkaline phosphatase-conjugated goat anti-mouse immunoglobulin G (IgG) or alkaline phosphatase-conjugated goat anti-rabbit IgG diluted 1:1,000 (Sigma Chemical Co., Deisenhofen, Germany). Using 5-bromo-4-chloro-3-indolylphosphate incorporating nitroblue tetrazolium as a substrate, reactive bands were visualized.

Isolation of soluble and insoluble protein fractions. Tachyzoites of the RH isolate grown in P388 macrophage cell culture were harvested, filtered, and washed three times in PBS. They were then resuspended in 1 mmol EDTA—10 mmol Tris (pH 6.8) at a concentration of 10^6 tachyzoites per μl and incubated overnight at −80°C. Following three freeze/thaw cycles, the suspension was centrifuged and the supernatant and pellet were further processed in the following way. (i) The supernatant was centrifuged for 30 min at 100,000 × *g* to separate soluble cytosol proteins from insoluble cytosol proteins. (ii) The pellet was resuspended in a buffer consisting of 50 mmol Tris (pH 8.0) and 1% Nonidet P-40 supplemented with the protease inhibitors iodoacetamide, aprotinin, and phenylmethylsulfonyl fluoride and incubated for 12 h at 4°C. Finally, the suspension was centrifuged at 100,000 × *g* for 30 min, and the supernatant, containing solubilized membrane proteins (9), was stored at −20°C. The three protein fractions were analyzed by SDS-PAGE and the Western blot technique as described above.

Immunofluorescence assay. A 30-μl suspension of 10^5 tachyzoites per μl of all isolates analyzed in this study was dropped on a glass slide and air dried. After fixation in ice-cold methanol for 10 min, the slides were incubated in turn with (i) MAb 5B10 diluted 1:10,000 for 1 h and (ii) fluorescein-conjugated goat anti-mouse IgG diluted 1:50 for 45 min. In parallel, slides were incubated with (i) a polyclonal rabbit antiserum generated against the RH isolate of *T. gondii* and (ii) fluorescein-conjugated goat anti-rabbit IgG. An irrelevant murine MAb of the IgG class was used as a negative control. Dilutions of antibodies and conditions of the positive and negative control reactions were as described above. Fluorescein-conjugated antibodies were obtained from Sigma (Deisenhofen, Germany). Antibody binding was analyzed with a fluorescence microscope (Leitz, Wetzlar, Germany).

Immunoelectron microscopy. Pelleted *T. gondii* tachyzoites of the RH strain were fixed with 2% paraformaldehyde and 0.1% glutaraldehyde, washed with PBS, dehydrated, and embedded in LR white, medium grade (The London Resin Company, Woking, England). Sections were incubated either with 1:10 diluted MAb 5B10 or with an irrelevant murine MAb of the IgG class and then incubated with gold-labeled antimouse conjugate (Bioclinical Service, Cardiff, United Kingdom). The specimens were stained with uranyl acetate and lead citrate and were examined with a Zeiss EM 10 electron microscope.

RESULTS

MAbs. The fusion resulted in the generation of 10 stable MAbs. Six MAbs were positive by immunofluorescence, three were positive by enzyme immunoassay, and one was positive by either method. Seven of these MAbs were of the IgG class, two were of the IgM class, and one was not determined (21). MAb 5B10 had the isotype IgG2a.

Determination of mouse virulence. Virulence of the different *T. gondii* isolates was determined by infecting NMRI mice with different doses of tachyzoites and calculating the cumulative percent mortality and the mean time of death for each group. It was shown that *T. gondii* isolates could be divided into three groups according to their virulence potential for mice (Table 2). Strains RH and BK were mouse lethal upon injection of at least 100 tachyzoites. Strain 177 expressed an intermediate potential for mouse virulence: upon injection of 1,000 tachyzoites, about 50% of the mice died, whereas all mice challenged with lower doses survived. Clinical signs of infection, like a rough coat, were observed in all animals which were infected with at least 100 tachyzoites. All other *T. gondii* strains analyzed in this study were asymptomatic at an infection dose of 1,000 tachyzoites or less (Table 2). Tachyzoites were demonstrated in the peritoneal cavities of all mice that died upon infection of strains RH, BK, and 177.

Protein analysis. To analyze whether *T. gondii* exhibits strain-specific antigens, we compared protein samples of the studied isolates by their reactivity with the MAbs. Polypep-

TABLE 2. Comparison of mouse virulence of *T. gondii* isolates

T. gondii isolate	No. of parasites inoculated	Cumulative % mortality	Average day of death
RH	1	40	10.00
	10	83	9.00
	100	100	8.50
	1,000	100	7.50
BK	1	60	9.00
	10	85	9.33
	100	100	8.75
	1,000	100	7.75
177	1	0	
	10	0	
	100	0	
	1,000	50	9.00
ALT	1	0	
	10	0	
	100	0	
	1,000	0	
GAIL	1	0	
	10	0	
	100	0	
	1,000	0	
RKR	1	0	
	10	0	
	100	0	
	1,000	0	
NTE	1	0	
	10	0	
	100	0	
	1,000	0	
561	1	0	
	10	0	
	100	0	
	1,000	0	
DX	1	0	
	10	0	
	100	0	
	1,000	0	

tides of a total cell lysate of 5×10^6 tachyzoites of the isolates RH, BK, 177, ALT, 561, NTE, DX, GAIL, and RKR grown in P388 macrophage cell culture were separated by SDS-PAGE and were then electrophoretically transferred onto nitrocellulose membrane. Analysis by SDS-PAGE alone did not reveal major differences (Fig. 1), whereas by immunoblotting with MAb 5B10, a protein of approximately 23 kDa could be detected (Fig. 2). Surprisingly, this reactivity was observed exclusively with mouse-lethal strains RH and BK. Besides additional proteins, the 23-kDa antigen was also shown to be recognized by a polyclonal immune serum generated against the RH isolate (data not shown). Using other *T. gondii*-specific MAbs, no strain-specific differences were detected.

Immunofluorescence assay. To verify the observed strain specificity of MAb 5B10, we analyzed tachyzoites by indirect immunofluorescence. After fixation with methanol, tachyzoites were incubated either with MAb 5B10 or with a polyclonal control antiserum generated against *T. gondii* isolate RH. Both antisera were used at a dilution of 1:10,000. Tachyzoites of all isolates showed a diffuse immunofluorescence staining pattern with the polyclonal rabbit antiserum, whereas a similar staining was only observed with isolates RH and BK when the MAb was used (Fig. 3). The diffuse staining pattern indicated that these antibodies were probably reactive with membrane components. No reactivity was observed with an irrelevant murine MAb (data not shown).

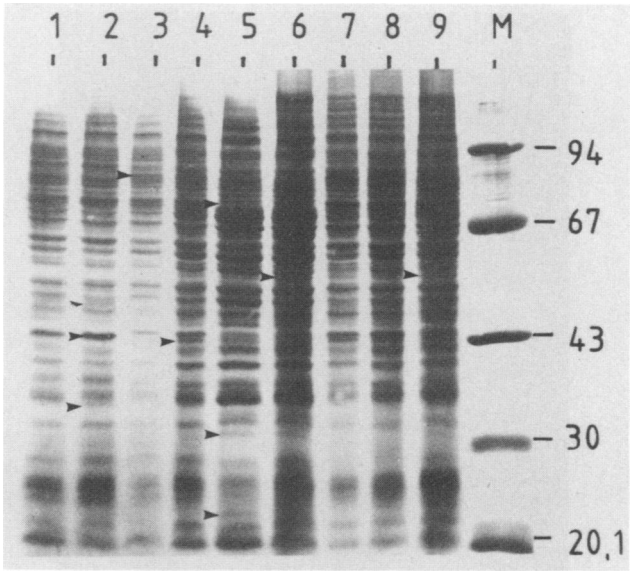

FIG. 1. SDS-PAGE analysis of total cell lysates of nine *T. gondii* strains. Lanes: M, molecular mass markers in kilodaltons; 1 and 2, virulent strains RH and BK; 3, intermediate virulent strain 177; 4 to 9, avirulent strains ALT, GAIL, RKR, NTE, 561, and DX, respectively. Arrowheads indicate uncommon proteins.

Localization of the reactive epitope. To further characterize the location of the reactive 23-kDa protein in the virulent isolates, proteins of the RH strain were separated into soluble cytoplasmic fractions, membrane fractions, and insoluble components. By immunoblotting, the 5B10-reactive antigen was most abundant in the membrane fraction (Fig. 4). In addition, immunoelectron microscopy revealed that MAb 5B10 recognized epitopes predominantly located on the entire surface of tachyzoites of the virulent strain RH. No prevalence for a distinctive surface area was detectable. This reactivity was not observed when an irrelevant murine MAb of the IgG class or PBS as a negative control was used (Fig. 5).

DISCUSSION

Even though *T. gondii* has been isolated in countries all over the world, no serological typing of different isolates is available so far. Studies were undertaken to differentiate *T. gondii* isolates by performing protein analysis by using SDS-PAGE (2), isoenzyme analysis (1, 5), and immunolog-

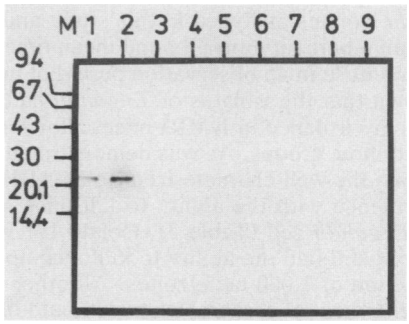

FIG. 2. Western blot analysis of reactivity of MAb 5B10 with nine *T. gondii* strains. For order of strains, see the legend to Fig. 1.

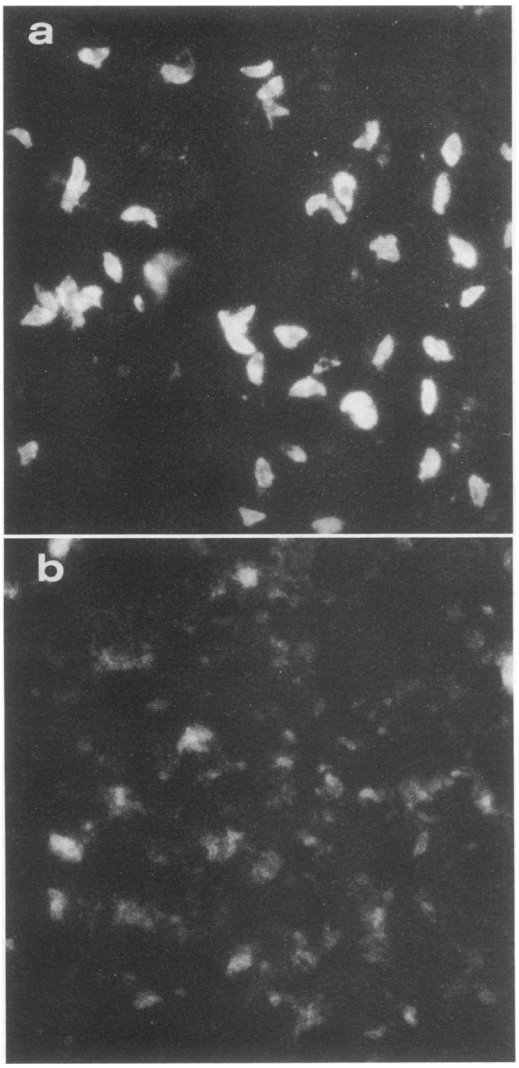

FIG. 3. Representative immunofluorescence assay with MAb 5B10 and tachyzoites of virulent strain BK (a) and avirulent strain NTE (b).

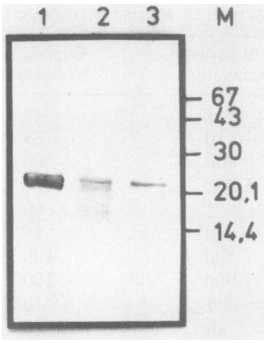

FIG. 4. Western blot demonstrating reactivity of MAb 5B10 with protein fractions of *T. gondii* RH. Lanes: 1, membrane fraction; 2, insoluble cytosol fraction; 3, soluble cytosol fraction. M, molecular mass markers in kilodaltons.

ical techniques (28, 29), by comparing their behavior in cell cultures (14, 15), or by examining their virulence in mice (6, 7, 15, 25, 30, 34).

So far, *T. gondii* isolates are classified in only two groups according to their virulence in mice. To determine the grade of virulence, we infected mice with 1 to 1,000 tachyzoites of all *T. gondii* isolates analyzed in this study and determined the cumulative percent mortality and mean time of death for this infection dose in an observation period of up to 21 days. It was shown that the isolates of *T. gondii* had quantifiable differences in virulence in NMRI mice, allowing them to be divided into three groups. As was demonstrated by previous investigators, the well-characterized isolates RH and BK are of high virulence with the ability to kill mice injected with only one *T. gondii* cell (Table 2). Isolate 177 was of lower virulence but still had the ability to kill mice upon intraperitoneal injection of 1,000 tachyzoites. All other isolates lead to the development of cerebral cysts without killing the mice with an inoculum of 1,000 tachyzoites or less.

Moreover, we were able to show that the mouse lethality phenotype of *T. gondii* correlates closely with the ability to damage cell cultures. The RH and BK strains destroyed a monolayer of P388 macrophages much faster than did the other isolates described in this study (7a). A similar behavior was observed for isolate RH with epithelial cell lines (14).

Even though strains RH and BK expressed a higher virulence potential than other isolates, SDS-PAGE analysis of total cell lysates did not reveal significant differences in the protein profiles. This result is in agreement with a recent investigation of three *T. gondii* strains (2).

Western blot studies were performed with a murine MAb to enhance the sensitivity of protein analysis. This MAb, 5B10, recognized a 23-kDa antigen present exclusively in the highly virulent RH and BK strains. The MAb, therefore, was able to differentiate between mouse-lethal and non-mouse-lethal *T. gondii* isolates. The 23-kDa antigen may be considered a virulence-associated antigen. Our observations in Western blot were confirmed by results of immunofluorescence, in which it was shown that only the virulent isolates were reactive with MAb 5B10.

Various experiments were performed to determine the location of the 23-kDa antigen. Western blot studies with several protein fractions of *T. gondii*, immunofluorescence, and immunoelectron microscopy revealed that MAb 5B10 recognized an epitope predominantly located on the entire surface of the parasite. The nature of this antigen still is unclear. It seemed not to be related to the penetrating-enhancing factor, the only definite virulence factor of *T. gondii* known so far (18). While penetrating-enhancing factor mainly is located in the anterior part of the parasite and also seemed to be a secreted antigen with a molecular mass of 66 kDa (16), the 23-kDa antigen described in our study covers the entire surface of virulent *T. gondii* isolates. Using a polyclonal immune serum, it was shown that the 23-kDa antigen was recognized as part of the overall repertoire of high-titer antisera against the RH isolate.

Several investigators identified four major antigens on the cell membrane surface of *T. gondii*. Among them is a protein with a molecular mass of approximately 22 kDa (11–13). In another study, this protein was shown to be reactive with sera from *T. gondii*-infected patients (28). However, the previously described 22-kDa major antigen is probably not identical with the 23-kDa antigen reported in our study because in contrast to our results, the 22-kDa protein was present in several isolates of *T. gondii* including cyst-forming isolates of low virulence (13, 33). Interestingly, the 22-kDa

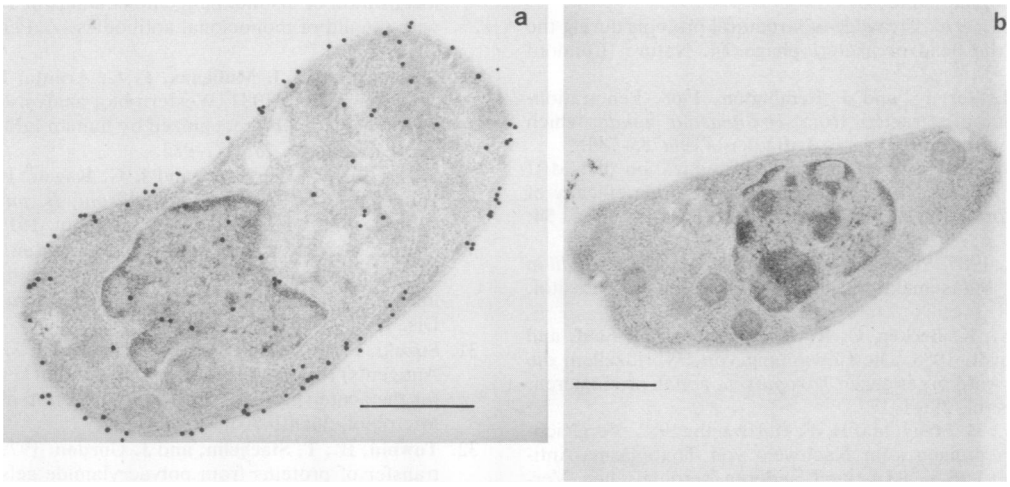

FIG. 5. Immunoelectron microscopy identifying the location of the MAb 5B10-reactive epitopes on tachyzoites of the RH strain (a) and a negative control with PBS buffer (b).

protein was shared among isolate RH and the cyst-forming isolates and was quantitatively much less reactive with RH antiserum than was the protein from cyst-forming strains (33). In a recent study, a 23-kDa antigen secreted by bradyzoites and tachyzoites of mouse-lethal and non-mouse-lethal *T. gondii* strains was identified (3). However, it is unlikely that the secreted protein is identical with the antigen described by our study because immunogold staining detected the secreted protein in the dense granules of both stages, whereas our protein was located predominantly at the surface. Suzuki et al. (31) demonstrated that a 24-kDa protein was present in tachyzoites but absent in bradyzoites. Because Suzuki et al. (31) only used tachyzoites of the virulent RH strain without comparing their results with tachyzoites of avirulent isolates, it is uncertain whether this stage-specific antigen is related to the 23-kDa antigen identified by our study. Finally, a 21-kDa antigen identified by an MAb and the Western blot technique was described by Sethi et al. (27). Using immunofluorescence, this protein was identified in four isolates of different virulence.

We conclude that the described 23-kDa component is a diffuse surface antigen of *T. gondii* that seems to be strain specific and closely associated with virulence. Although several other antigens of similar molecular mass have been described, the relationship between the 23-kDa antigen and these other antigens is uncertain. Genetic and cell biological studies may help to clarify the function and relevance of this antigen, particularly in regards to its role in virulence as well as its relationship to previously described *T. gondii* antigens. Experiments are now in progress to identify a 5B10-reactive clone in a cDNA library made from the virulent BK strain to determine its DNA sequence and hence better understand the role of this virulence-associated antigen.

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REFERENCES

1. **Barnert, G., A. Hassl, and H. Aspöck.** 1988. Isoenzyme studies on *Toxoplasma gondii* isolates using isoelectric focusing. Zentralbl. Bakteriol. Mikrobiol. Hyg. A **268**:476–481.
2. **Bloomfield, M. M., and J. S. Remington.** 1970. Comparison of three strains of *Toxoplasma gondii* by polyacrylamide-gel electrophoresis. Trop. Geogr. Med. **22**:367–370.
3. **Cesbron-Delauw, M. F., B. Guy, G. Torpier, R. J. Pierce, G. Lenzen, J. Y. Cesbron, H. Charif, P. Lepage, F. Darcy, J. P. Lecocq, and A. Capron.** 1989. Molecular characterization of a 23-kilodalton major antigen secreted by *Toxoplasma gondii*. Proc. Natl. Acad. Sci. USA **86**:7537–7541.
4. **Cornelissen, A. W. C. A., J. P. Overdulve, and J. M. Hoenderboom.** 1981. Separation of *Isospora* (*Toxoplasma gondii*) cysts and cystozoites from mouse brain tissue by continuous density-gradient centrifugation. Parasitology **83**:103–108.
5. **Darde, M. L., B. Bouteille, and M. Pestre-Alexandre.** 1988. Isoenzymic characterization of seven strains of *Toxoplasma gondii* by isoelectrofocusing in polyacrylamide gels. Am. J. Trop. Med. Hyg. **39**:551–558.
6. **De Roever-Bonnet, H.** 1966. Virulence of *Toxoplasma*. Trop. Geogr. Med. **18**:143–146.
7. **Ferguson, D. J. P., and W. M. Hutchinson.** 1981. Comparison of the development of avirulent and virulent strains of *Toxoplasma gondii* in the peritoneal exudate of mice. Ann. Trop. Med. Parasitol. **75**:539–546.

7a. **Gross, U.** Unpublished data.

8. **Haemmerling, G. J., U. Haemmerling, and J. F. Kearney (ed.).** 1981. Monoclonal antibodies and T-cell hybridomas. Elsevier Biomedical Press, New York.
9. **Harlow, E., and D. Lane.** 1988. Antibodies: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
10. **Jacobs, L., and M. L. Melton.** 1954. Modifications in virulence of a strain of *Toxoplasma gondii* by passage in various hosts. Am. J. Trop. Med. Hyg. **3**:447–457.
11. **Johnson, A. M.** 1985. The antigenic structure of *Toxoplasma gondii*: a review. Pathology **17**:9–19.
12. **Kasper, L. H.** 1989. Identification of stage-specific antigens of *Toxoplasma gondii*. Infect. Immun. **57**:668–672.
13. **Kasper, L. H., and P. L. Ware.** 1985. Recognition and characterization of stage-specific oocyst/sporozoite antigens of *Toxoplasma gondii* by human antisera. J. Clin. Invest. **75**:1570–1577.
14. **Kaufman, H. E., M. L. Melton, J. S. Remington, and L. Jacobs.** 1959. Strain differences of *Toxoplasma gondii*. J. Parasitol. **45**:189–190.
15. **Kaufman, H. E., J. S. Remington, and L. Jacobs.** 1958. Toxoplasmosis: the nature of virulence. Am. J. Ophthalmol. **46**:255–260.
16. **Kimata, I., and K. Tanabe.** 1987. Secretion by *Toxoplasma gondii* of an antigen that appears to become associated with the parasitophorous vacuole membrane upon invasion of the host

cell. J. Cell Sci. **88**:231–239.
17. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
18. **Lycke, E., R. Norrby, and J. Remington.** 1968. Penetration-enhancing factor extracted from *Toxoplasma gondii* which increases its virulence for mice. J. Bacteriol. **96**:785–788.
19. **Masihi, K. N., and H. Werner.** 1977. Immunization of NMRI mice against virulent *Toxoplasma gondii*. Differing efficacy of eleven cyst-forming *Toxoplasma* strains. Z. Parasitenkd. **54**: 209–216.
20. **Müller, W. A.** 1985. Stammerhaltung von *Toxoplasma gondii* in permanenten Mäusemakrophagenkulturen. Angew. Parasitol. **26**:221–222.
21. **Müller, W. A., K. Becker, G. Koch, A. Schäfer, H. Lauf, and H. L. Borkhardt.** 1988. Die Gewinnung von Hybridzellen, die monoklonale Antikörper gegen *Toxoplasma gondii* produzieren. Angew. Parasitol. **29**:11–17.
22. **Müller, W. A., H. Struy, and H.-F. Holzwarth.** 1982. Vergleichende Untersuchungen zum Nachweis von Toxoplasma-Antikörpern mit ELISA, RIA, und anderen serologischen Verfahren. Z. Gesamte Hyg. **28**:375–377.
23. **Pettersen, E. K.** 1977. Experimental toxoplasmosis in mice and rabbits. Acta Pathol. Microbiol. Scand. Sect. B **85**:95–102.
24. **Reed, L. J., and H. Muench.** 1938. A simple method of estimating fifty percent endpoints. Am. J. Hyg. **27**:493–497.
25. **Reikvam, A., and A. M. Lorentzen-Styr.** 1976. Virulence of different strains of *Toxoplasma gondii* and host response in mice. Nature (London) **261**:508–509.
26. **Sabin, A. B.** 1941. Toxoplasmic encephalitis in children. JAMA **116**:801–807.
27. **Sethi, K. K., Y. Omata, and H. Brandis.** 1984. Topographical localization of distinct antigenic domains of *Toxoplasma gondii* with the aid of monoclonal antibodies. Z. Parasitenkd. **70**:699–707.
28. **Sharma, S. D., J. Mullenax, F. G. Araujo, H. A. Erlich, and J. S. Remington.** 1983. Western blot analysis of the antigens of *Toxoplasma gondii* recognized by human IgM and IgG antibodies. J. Immunol. **131**:977–983.
29. **Suggs, M., K. W. Walls, and I. G. Kagan.** 1968. Comparative antigenic study of *Besnoitia jellisoni*, *B. panamenis* and five *Toxoplasma gondii* isolates. J. Immunol. **101**:166–175.
30. **Suzuki, Y., F. K. Conley, and J. S. Remington.** 1989. Differences in virulence and development of encephalitis during chronic infection vary with the strain of *Toxoplasma gondii*. J. Infect. Dis. **159**:790–794.
31. **Suzuki, Y., P. Thulliez, G. Desmonts, and J. S. Remington.** 1988. Antigen(s) responsible for immunoglobulin G responses specific for the acute stage of *Toxoplasma* infection in humans. J. Clin. Microbiol. **26**:901–905.
32. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76**:4350–4354.
33. **Ware, P. L., and L. H. Kasper.** 1987. Strain-specific antigens of *Toxoplasma gondii*. Infect. Immun. **55**:778–783.
34. **Werner, H., and I. Egger.** 1968. Vergleichende Untersuchungen an zystenbildenden Toxoplasmen-Stämmen. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. **206**:259–266.
35. **Winser, J., J. D. Verlinde, P. H. van Thiel, J. Davel, and P. van der Elst.** 1948. Isolation of *Toxoplasma* from cerebrospinal fluid of a living infant in Holland. Proc. Soc. Exp. Biol. Med. **67**:292–294.