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Monoclonal Rat Antibodies Directed against *Toxoplasma gondii* Suitable for Studying Tachyzoite-Bradyzoite Interconversion In Vivo

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We previously reported the in vitro analysis of stage differentiation of *Toxoplasma gondii* in murine bone marrow-derived macrophages. The purpose of this study was to generate monoclonal rat antibodies that might be suitable for investigating tachyzoite-bradyzoite interconversion in vivo with the murine model. Immunization of Fischer rats with cysts of *T. gondii* NTE resulted in the generation of seven monoclonal antibodies of the immunoglobulin G2a, G2b, or M isotype, which were further characterized by the immunoblot technique, immunofluorescence assay, immunohistology, and immunoelectron microscopy. Immunoblots demonstrated specific reactivity of five monoclonal antibodies with proteins with molecular masses of 40, 52, 55, 60, 64, 65, and 115 kDa. One antibody (CC2) appeared to recognize a differently expressed antigen depending on the parasite stage, reacting with a 40-kDa molecule in tachyzoites and a 115-kDa antigen in bradyzoites and oocysts. Several other monoclonal antibodies were shown to be stage specific and to react in immunofluorescence assays or in immunoblots with either tachyzoites or bradyzoites. Kinetics of stage conversion in vitro could be monitored by immunofluorescence with two of these monoclonal antibodies. Preliminary immunohistological investigations of tissue sections from infected mice demonstrated the possible usefulness of these monoclonal antibodies for future in vivo studies on stage differentiation of *T. gondii* in the murine system.

An acute infection with the protozoan parasite *Toxoplasma gondii* usually is overcome by the development of bradyzoite-harboring cysts that persist lifelong and generally cause no clinical symptoms in immunocompetent individuals. In conditions causing T-cell deficiency, such as infection with human immunodeficiency virus, the latent infection may reactivate. It is generally believed that reactivation correlates with *T. gondii* reverting from dormant bradyzoites into rapidly dividing tachyzoites, resulting predominantly in toxoplasmic encephalitis (16, 20). By using the murine model, it has been previously shown that gamma interferon is a major mediator of resistance against toxoplasmosis and that simultaneous depletion of $CD4^+$ and $CD8^+$ T lymphocytes is required to reactivate chronic infection with *T. gondii* (8, 17, 23). However, acute or reactivated toxoplasmosis in these studies was not determined by identification of the tachyzoite stage but by measuring parasite replication as a marker of disease activity.

Until recently, the analysis of bradyzoite-tachyzoite interconversion of *T. gondii* in vitro was not possible. One prerequisite for these studies was the availability of stage-specific markers such as bradyzoite- and tachyzoite-specific monoclonal antibodies (MAbs) (2, 22, 25). These antibodies have been used to study various factors affecting stage conversion in various cell culture systems (2, 10, 22). We have previously demonstrated that gamma interferon-mediated induction of nitric oxide in murine bone marrow-derived macrophages seems to be important for triggering differentiation from tachyzoites to bradyzoites (3).

Since these observations were derived from experiments that had been performed in cell culture, we were interested in confirming the results in an in vivo animal model. The mouse system seems to be an appropriate animal model, because murine immunomodulators can easily be manipulated by, for example, depletion of cytokines. Therefore, this animal model is suitable for studying stage conversion and determining stage-influencing factors. Since the use of MAbs generated in the mouse might carry certain disadvantages in this host, such as background reactivity with murine tissue, we decided to use another animal species to generate MAbs for subsequent analysis of stage conversion. This report describes the characterization of heterologous stage-specific MAbs generated in the rat. Since the antibody response between mice and rats has been shown to be different (9), one might expect to generate MAbs that recognize different epitopes from those recognized by murine MAbs.

MATERIALS AND METHODS

Parasites. *T. gondii* NTE was used for generating rat MAbs. This strain was isolated in Germany 1990 from the brain of a patient who had died of AIDS. It has been used previously to study bradyzoite-tachyzoite interconversion in vitro (2, 3). Eleven additional *T. gondii* strains were used to investigate possible strain-specific expression of antigens recognized by the MAbs. These parasites consisted of (i) oocysts of *T. gondii* GTP1 and KSP1, which were originally isolated from pigs in Hannover, Germany; (ii) bradyzoites of *T. gondii* NTE, ALT, KSU, and ME49, which have been described previously (11, 18), as well as bradyzoites of *T. gondii* LMS, which was originally isolated from a pig in Hannover; and (iii) tachyzoites of *T. gondii* NTE, ALT, RKR, 177, GAIL, DX, LMS, RH, and BK (11, 18).

Isolation of *T. gondii* cysts, oocysts, and tachyzoites. *T. gondii* cysts were injected intraperitoneally into 7-week-old CBA/J mice (10 to 20 cysts per mouse). At 3 to 4 weeks postinfection (p.i.) the animals were killed with CO_2, the brains were removed, and the cysts were isolated as described previously (5). Oocysts were isolated from infected cats by the "sucrose floating technique" as previously described (13). Tachyzoites were maintained in cell cultures of P388D1 macrophages (11). The medium consisted of Dulbecco's modified Eagle's medium

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(Biochrom, Berlin, Germany) supplemented with 10% fetal calf serum, 2% *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), 1% nonessential amino acids, 300 mg of L-glutamine per liter, 1% sodium pyruvate, 25‰ β-mercaptoethanol, and antibiotics and was changed every second day. Host cell rupture occurred between days 4 and 8 depending on the strain of *T. gondii*. Liberated tachyzoites were separated from intact host cells by filtering the supernatant through a 3.0-μg-pore-size polycarbonate filter membrane (Nuclepore, Bromma, Sweden) and washed twice in phosphate-buffered saline (PBS).

Generation of rat MAbs. Fisher or Lewis rats (6 to 8 weeks old) were fed brain homogenates of mice chronically infected with the *T. gondii* NTE. Each rat was fed three times at intervals of 5 weeks and received a total of approximately 12,000 to 15,000 tissue cysts. Antisera were collected 2 weeks after each infection. The last antiserum sample was collected 4 days after the final challenge with NTE tissue cysts. The antibody response of each animal was monitored by immunoblots with both tachyzoites or bradyzoites. The Fisher rat with the most prominent bradyzoite-specific immune response was killed 4 days after the last infection, and the spleen cells from this animal were fused with the murine cell line P3-X63-Ag8.653 (1). On day 14 postfusion, hybridoma supernatants were assayed for reactivity with bradyzoites and tachyzoites by the immunoblot technique and the immunofluorescence assay (IFA). Positive hybridoma supernatants were expanded and rescreened. Those still positive were expanded in 24-well plates, cloned twice by Poisson limiting dilution, and frozen in liquid nitrogen.

Isotyping of MAbs. A 30-μl sample of 1:20-diluted hybridoma supernatant was mixed with 30 μl of isotype-specific antibodies coupled to sheep erythrocytes (Serotec, Oxford, United Kingdom), and the mixture was incubated for 1 h at 22°C. Agglutination demonstrated isotype-specific reactivity of hybridoma supernatant.

SDS-PAGE and immunoblot. Samples containing 7.5×10^6 tachyzoites, 1,500 tissue cysts, or 4×10^6 oocysts were resuspended in a buffer consisting of 60 mM Tris (pH 6.8), 2% sodium dodecyl sulfate (SDS), 10% glycerol, 5% mercaptoethanol, and 0.01% bromophenol blue. After being boiled for 5 min, each of these lysates was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE; 11% polyacrylamide) (15) and the separated peptides were electrophoretically transferred onto 0.45-μg-pore-size nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany) (26). Following blocking with 1% bovine serum albumin, the nitrocellulose membrane was incubated for 8 h with immune rat serum diluted 1:1,000 in PBS or with undiluted rat hybridoma supernatant and then for 1 h with alkaline phosphatase-conjugated goat anti-rat immunoglobulin G or M (IgG or IgM; Jackson ImmunoResearch Lab., West Grove, Pa.) or alkaline phosphatase-conjugated sheep anti-rat IgA (Serotec), each diluted 1:1,000. Reactive bands were visualized by using 5-bromo-4-chloro-3-indolylphosphate incorporating nitroblue tetrazolium as a substrate.

IFA. Individual 10-μl suspensions of 10^4 tachyzoites, 15 mechanically ruptured tissue cysts, or 50 oocysts were dropped onto a glass slide and air dried. After fixation in acetone, methanol, or 4% paraformaldehyde, the slides were incubated in turn with undiluted rat MAb for 1 h and then fluorescein isothiocyanate-conjugated goat anti-rat Ig (Jackson ImmunoResearch Lab.) diluted 1:50 in PBS, for 30 min. An unrelated *Yersinia*-specific MAb and polyclonal anti-*T. gondii* rat serum served as negative and positive controls, respectively. Antibody binding was analyzed with a fluorescence microscope (Leitz, Wetzlar, Germany). To determine whether MAbs are reactive with components of the surface or cytosol, reactions were as well carried out with unfixed parasites at 4°C.

In vitro induction of stage conversion from tachyzoites to bradyzoites. L929 murine fibroblasts were infected with tachyzoites of *T. gondii* NTE. Stage conversion from the tachyzoite to the bradyzoite stage was induced by increasing the pH of the culture medium (pH 8.0) as previously described (21) and by keeping it under these alkaline conditions. To determine the ratio of parasites expressing stage-specific antigens within the entire *T. gondii* population, the differentiation process from tachyzoites to bradyzoites was monitored daily by an indirect double-immunofluorescence assay with the entire parasite population being labelled with tetramethylrhodamine isothiocyanate (TRITC) and the parasite fraction expressing stage-specific antigens being labelled with fluorescein isothiocyanate. Labelling was performed by the following four-step protocol: (i) either tachyzoite-specific MAb DC2 or bradyzoite-specific MAb DC11, (ii) fluorescein isothiocyanate-conjugated goat anti-rat IgG, (iii) murine polyclonal antiserum generated against *T. gondii* NTE, and (iv) TRITC-conjugated goat anti-mouse IgG. The ratio of parasites reacting with stage-specific MAbs was determined by counting 50 visual fields under a fluorescence microscope.

Immunohistology. Female NMRI mice (20 to 24 g) were infected intraperitoneally with either BK or NTE tachyzoites. BK-infected mice were killed with CO_2 2 days after infection, and their lungs were removed. The lung tissue was frozen in Tissue-Tek (Miles Inc., Elkhart, Ind.) embedding medium, and 8-μm sections were prepared with a Frigocut E cryomicrotome (Reichert-Jung, Nussloch, Germany). NTE-infected mice were killed 6 weeks p.i., and frozen sections of their brains were prepared as described above. Following fixation with either acetone or 4% paraformaldehyde, the slides were stained by the peroxidase-antiperoxidase method essentially as described previously (4). Briefly, after being blocked with PBS–20% normal sheep serum, the sections were incubated in turn with (i) a rat MAb diluted 1:2 in PBS–20% normal sheep serum for 45 min, (ii) a 1:50 dilution of peroxidase-conjugated goat anti-rat Ig for 45 min, and (iii) a 1:50 dilution of mouse peroxidase-antiperoxidase for 45 min. Following the final wash, the peroxidase was visualized with 3,3′-diaminobenzidine tetrahydrochloride–hydrogen peroxide–Tris buffer. A 100-ml volume of this buffer consisted of 0.05 M Tris-hydrochloric acid (pH 7.6), 25 mg of diaminobenzidine, and 10 μl of a solution of 30% hydrogen peroxide. After being developed and washed, the slides were counterstained for 1 min with hematoxylin, dehydrated with graded ethyl alcohol followed by xylene, and mounted with Entellan (Merck, Darmstadt, Germany). The counterstain was used for visualization of host cells without infering with the *T. gondii*-specific immunological staining.

Immunoelectron microscopy. Samples of peritoneal exudate containing tachyzoites were obtained from mice infected 2 days previously with the mouse-virulent *T. gondii* BK. In addition, samples of mouse brains containing tissue cysts were obtained from mice infected 3 months previously with cysts of the RRA strain. The samples were fixed overnight in 2% paraformaldehyde in 0.1 M phosphate buffer, dehydrated rapidly, and embedded in LR white resin. For immunostaining, a three-stage protocol was used. Thin sections containing tachyzoites or tissue cysts were placed on nickel grids. The grids were floated initially on 1% BSA in Tris buffer to block nonspecific staining followed by rat MAb CC2 (diluted 1:2). Sections were washed and floated on rabbit anti-rat Ig (1:50). The final stage was either goat anti-rabbit Ig conjugated to 10-nm colloid gold or protein A conjugated to 5-nm gold. Controls included omission of the primary antibody or use of an irrelevant antibody (anti-amyloid). Sections were stained with uranyl acetate prior to examination in a JEOL 1200 EX electron microscope.

RESULTS

Kinetics of antibody responses in cysts-infected rats. None of the preimmune sera were reactive with lysates of either tachyzoites or bradyzoites. An antigen of 40 kDa was the first component of cysts that reacted with antiserum from infected rats and appeared 2 weeks p.i. At 5 weeks p.i., several additional antigens, with molecular masses of 32, 43, 52, 72, 78, 85, and 115 kDa, were recognized by rat serum. A high-molecular-mass reactive component of 160 kDa was the last band recognized by immune rat serum and appeared at 12 weeks p.i. (data not shown). Although no significant difference in humoral immune responses between Fisher and Lewis rats was detected, we decided to use one of the Fisher rats for generating MAbs because of its overall enhanced reactivity with bradyzoites in comparison with tachyzoites.

Isotypes of rat MAbs. The fusion of immune spleen cells of the infected Fisher rat with the murine cell line P3-X63-Ag8.653 resulted in the generation of seven independent hybridoma clones. By using sheep erythrocytes coupled with isotype-specific antibodies, one of the rat MAbs was determined to belong to isotype IgG2a (DC2), three MAbs were of the IgG2b isotype (CC2, DC11, and EE5), and the final three MAbs belonged to the IgM isotype (AD11, BB7, and DD4). None of the MAbs were of the IgG1 or IgA isotype.

Reactivity of MAbs in IFA and immunoblots. Indirect IFA was performed after acetone, methanol, or paraformaldehyde fixation of tachyzoites or bradyzoites. None of the seven MAbs showed reactivity with brain tissue of uninfected mice. The method of fixation had no significant effect on the reactivity of the MAbs. IFA indicated that three MAbs DC2, DC11, and EE5 reacting with *T. gondii* parasites showed predominant surface labelling. These MAbs also showed positive labelling with unfixed parasites at 4°C, confirming their reactivity with a surface component of *T. gondii*. Whereas MAb DC11 reacted exclusively with bradyzoites by immunofluorescence, MAb EE5 recognized both bradyzoites and tachyzoites. MAb DC2 showed a tachyzoite-specific reactivity in immunofluorescence (Table 1). Controls with an unrelated *Yersinia*-specific MAb or controls lacking either primary MAb or secondary antibody were negative.

Immunoblotting under reducing conditions with parasite lysates was performed with each hybridoma supernatant. Five of the MAbs reacted with bands with molecular masses between 40 and 115 kDa (Table 1). MAbs EE5 and AD11 each recognized antigens of 52 and 55 kDa (Fig. 1). While MAb EE5 was

TABLE 1. Characterization of rat MAbs

MAb	Bradyzoites		Tachyzoites		Oocysts		B/T/O[b]
	Reactivity in IFA[a]	Band (kDa) in blot	Reactivity in IFA[a]	Band (kDa) in blot	Reactivity in IFA[a]	Band (kDa) in blot	
DC11	+[c]		−		−		B
DD4	−	60 + 64/65	−		−		B
BB7	−	64/65	−		−		B
CC2	+	115	−	40	+	115	B/T/O
EE5	+[c]		+[c]	52/55	−		B/T
DC2	−		+[c]		−		T
AD11	−		−	52/55	−		T

[a] IFA results obtained on fixed samples. The kind of fixation (methanol, acetone, or 4% paraformaldehyde) had no significant influence on reactivity with MAbs.
[b] B, bradyzoite-specific reactivity; T, tachyzoite-specific reactivity; O, oocyst-specific reactivity.
[c] This MAb was also reactive against native antigen.

also positive in immunofluorescence with both tachyzoites and bradyzoites, AD11 reacted only with tachyzoites. The bradyzoite-specific MAbs DD4 and BB7 both reacted with similar antigens with molecular masses of 64 and 65 kDa. In addition to these antigens, MAb DD4 recognized a 60-kDa antigen. The previously described murine MAb 7E5, which served as a control for the antigen preparation, recognized a bradyzoite-specific antigen with a molecular mass of 30 kDa (2). None of these MAbs except CC2 reacted with total-cell lysates of oocysts isolated from cat feces (Fig. 1).

Identification of an antigen that seems to be differently expressed in tachyzoites compared with oocysts and bradyzoites. By IFA, MAb CC2 reacted with bradyzoites and oocysts but not with tachyzoites (Table 1). Immunoblotting revealed reactivity with a 115-kDa antigen of bradyzoites and oocysts and a 40-kDa antigen of tachyzoites (Fig. 1). Immunoelectron microscopy was used to identify the MAb CC2-reactive component at the ultrastructural level. Within the tissue cysts in mouse brain (Fig. 2A), there was intense labelling of the cyst wall but not the adjacent ground substance (Fig. 2C). However, in a few cysts, clumps of material within the ground substance with similar electron density to the cyst wall were also stained (Fig. 2B). Within the bradyzoites, there appeared to be labelling of a proportion of dense granules (Fig. 2B). It is difficult to differentiate between cross sections through the electron dense bulbous ends of the rhoptries and dense granules, but in suitably orientated sections where the rhoptries could be identified, they appeared negative, as were the micronemes (Fig. 2B). A few gold particles were observed associated with the conoid and the pellicle, more often located at the inner membrane (Fig. 2B).

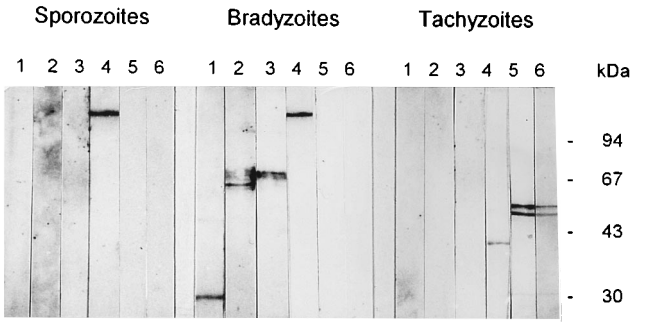

FIG. 1. Immunoblot demonstrating MAb-reactive antigens of sporozoites, bradyzoites, and tachyzoites. The previously described murine MAb 7E5 (2) served as a control for bradyzoites (lanes 1). Lanes: 2, MAb DD2; 3, MAb BB7; 4, MAb CC2; 5, MAb EE5; 6, MAb AD11. Molecular size markers are indicated on the right.

Examination of tachyzoites stained with CC2 showed low-level labelling that appeared to be localized to a proportion of the dense granules and electron-lucent vacuoles present anterior to the nucleus (Fig. 2D and E). Within the vacuoles, the gold particles were concentrated over the more electron-dense material to the periphery. As with the bradyzoites, a few gold particles were observed associated with the pellicle and the conoid. The rhoptries appeared to be negative.

Determination of stage- or strain-specific antigens with rat MAbs. Since rat MAbs were generated for studying stage conversion of *T. gondii* in vivo in the mouse model, it was important to determine whether these MAbs reacted with stage-specific antigens. Five of the MAbs showed reactivity with bradyzoites in the IFA and/or by the immunoblot technique. One of these MAbs, DC11, reacted in IFA only with bradyzoites but not with tachyzoites. Two other MAbs, DD4 and BB7, were bradyzoite specific, recognizing antigens of 60, 64, and 65 kDa in immunoblots (Fig. 1). These bradyzoite-specific MAbs reacted with neither oocysts nor tachyzoites in immunoblots. The other two bradyzoite-reacting MAbs were not stage specific, since they also showed reactivity with tachyzoites (Table 1). In addition, MAb CC2 recognized antigens in all three stages (Fig. 1). Since rats were infected with viable parasites, it is assumed that most of them converted to the tachyzoite stage before the final reconversion to persistent bradyzoite-harboring cysts. Therefore, not only bradyzoite- but also tachyzoite-specific MAbs were generated. One of these, MAb DC2, showed reactivity in IFA only with tachyzoites, whereas MAb AD11 recognized two antigens with molecular masses of 52 and 55 kDa in the immunoblot (Fig. 1).

Recently, it was shown that *T. gondii* isolates can be classified into three groups (18). To investigate whether the MAb-reactive antigens might be strain specific, parasites of 12 different isolates belonging to at least two of these three *Toxoplasma* groups were analyzed in IFA and immunoblots. When bradyzoites, oocysts, or tachyzoites of these different *T. gondii* strains were used, no strain-specific reactivity could be detected by any of the seven rat MAbs (data not shown).

Immunohistology. Since the generation of rat MAbs was intended for studying stage conversion of *T. gondii* in vivo, the use of these MAbs for immunohistological investigations had to be determined. In this preliminary study, frozen sections of infected lungs and brains of mice were used. It could be demonstrated that tachyzoites were recognized by MAb DC2 but not by MAb DC11 in the lungs of acutely infected mice. This

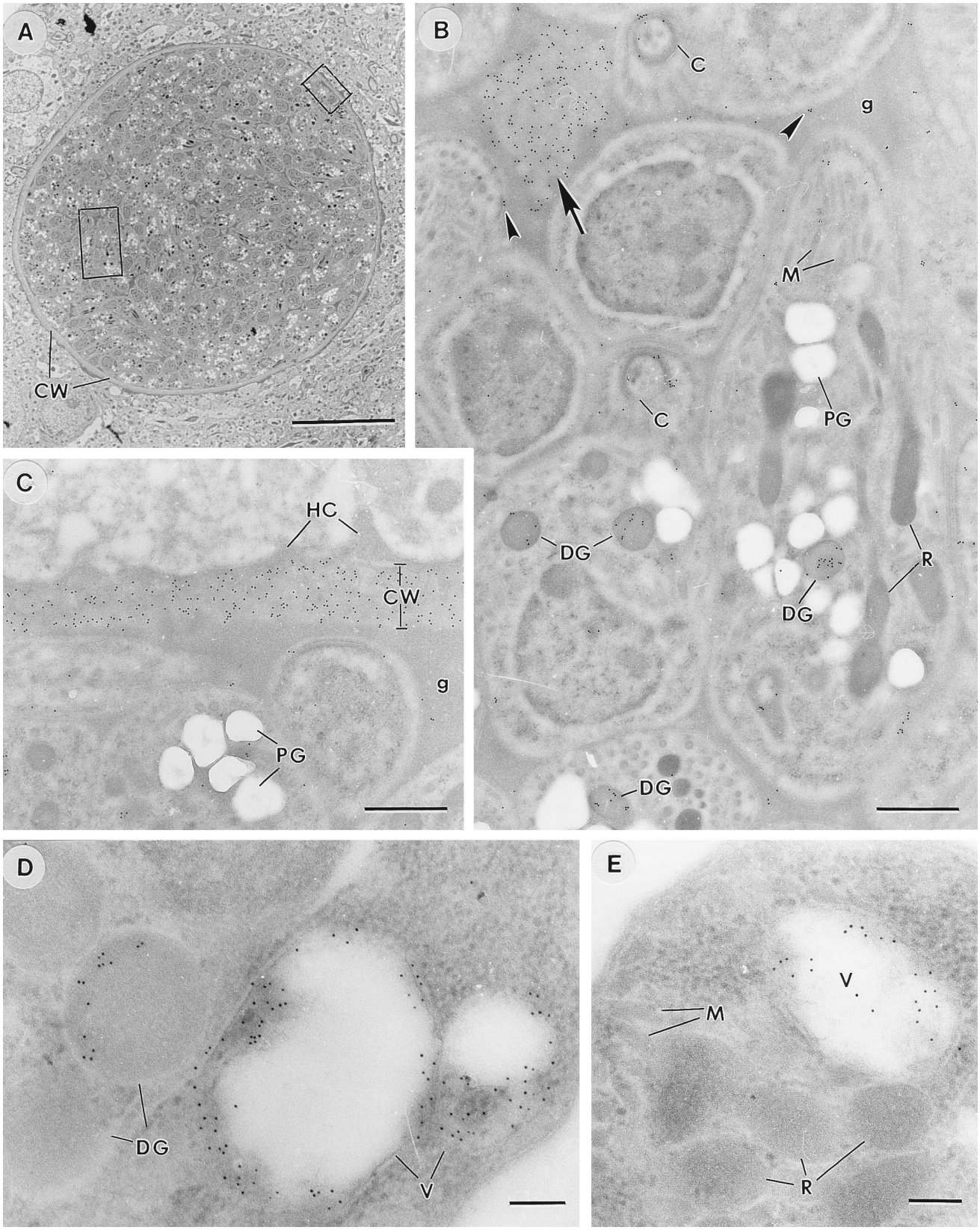

FIG. 2. (A) Low-power electron micrograph through a tissue cyst in mouse brain. CW, cyst wall. Bar, 10 μm. (B) Enlargement of the interior of the tissue cyst (enclosed area on the left in panel A) immunostained with MAb CC2 showing labelling of the dense granules (DG) plus the conoid (C) and pellicle (arrowheads) of the bradyzoites. A clump of material with similar electron density to the cyst wall (see panel C) is also positively stained. The ground substance (g), rhoptries (R), micronemes (M), polysaccharide granules (PG), and nuclei are unlabelled. Gold particles (diameter, 10 nm) were used. Bar, 0.5 μm. (C) Enlargement through the periphery of the tissue cyst (enclosed area at the top right of panel A) immunostained with MAb CC2 showing strong labelling of the cyst wall (CW). The host cell (HC), ground substance (g), and polysaccharide granules (PG) are unlabelled. Gold particles (diameter, 10 nm) were used. Bar, 0.5 μm. (D and E) Details of part of the anterior cytoplasm of tachyzoites immunostained with MAb CC2 showing labelling of the periphery of electron-lucent vacuoles (V) and certain dense granules (DG). The rhoptries (R) and micronemes (M) are unlabelled. Gold particles (diameter, 5 nm) were used. Bars, 0.1 μm.

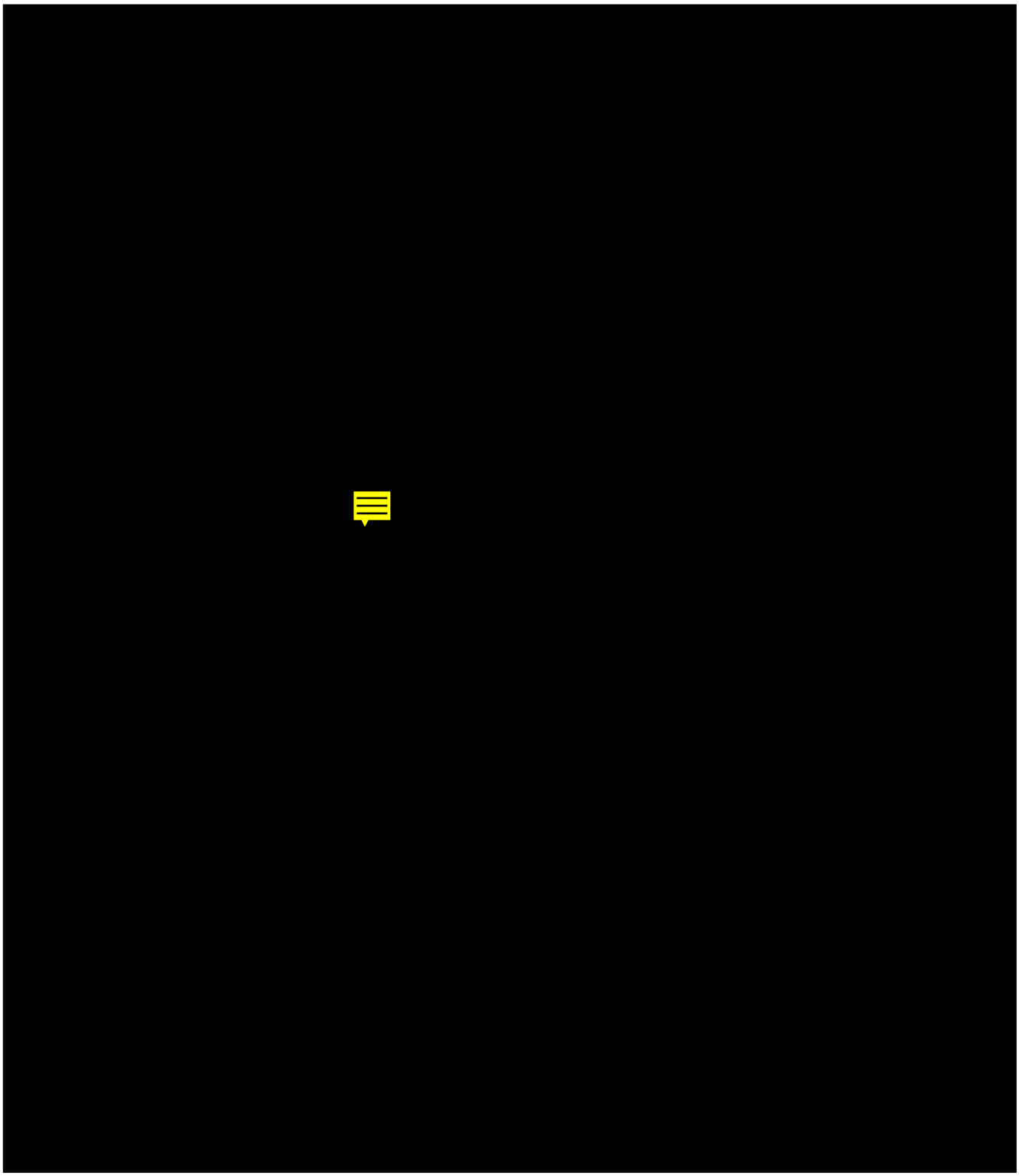

FIG. 3. Immunohistological investigations demonstrating the stage-specific reactivity of either MAb DC2 with tachyzoites within the lung (A) or MAb DC11 with cysts of *T. gondii* within the brain (B) of infected mice.

reactivity was observed only in acetone-fixed slides. As was already demonstrated by the IFA, the MAb-reacting antigen seems to be associated with the surface of tachyzoites (Fig. 3A). In the brains of chronically infected mice, MAb DC11 recognized only bradyzoites within tissue cysts, which were not stained when MAb DC2 was used (Fig. 3B). No disturbing background reactivity with murine tissue was detectable in any of these tests. Therefore, these two antibodies seem to be stage

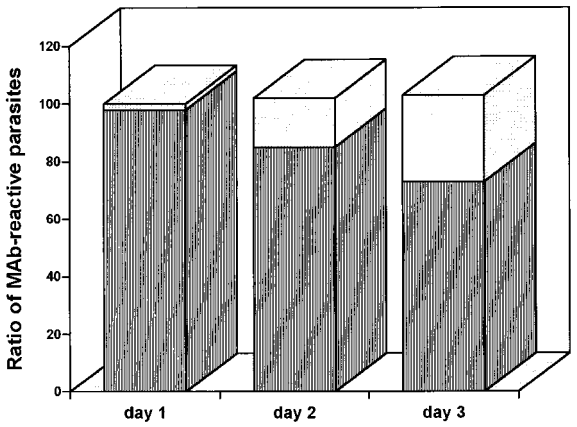

FIG. 4. In vitro induced differentiation from the tachyzoite to the bradyzoite stage analyzed from days 1 to 3 p.i. (representative experiment). The ratio of parasites that were reactive either with tachyzoite-specific MAb DC2 or bradyzoite-specific MAb DC11, respectively was determined by double immunofluorescence as described in Materials and Methods. Symbols: ▤, DC2 reactive; ■, DC11 reactive.

specific and may be useful tools for studying stage conversion in the murine model.

In vitro induced stage conversion. The differentiation process from tachyzoites to bradyzoites of strain NTE was induced in vitro with alkaline pH conditions in the cell culture medium (21). It could be demonstrated that tachyzoite-specific MAb DC2 and bradyzoite-specific MAb DC11 are suitable for monitoring this process, showing decreasing ratios of DC2-reactive parasites during the 3-day observation period. Conversely, the ratio of MAb DC11-reacting parasites increased from days 1 to 3 p.i. (Fig. 4).

DISCUSSION

Interconversion between the bradyzoite and tachyzoite stages is an important event for the pathogenesis of *T. gondii* infection. Following the ingestion of cysts, the bradyzoites convert into tachyzoites, which are associated with the acute phase of toxoplasmosis. As a consequence of the developing immune response, rapidly dividing tachyzoites reconvert into cyst-forming bradyzoites that are believed to persist for the rest of the host's life. In the presence of severe immunosuppression, reactivation of this latent infection is possible, with differentiation from the bradyzoite stage to the tachyzoite stage (12).

Since the importance of immunological factors for the regulation of acute, chronic, or reactivated toxoplasmosis has been investigated predominantly in the murine model (7, 8, 24), this animal would be a suitable candidate for studying stage conversion in vivo. Since it is possible that murine MAbs would have disadvantages for use in examining mouse tissue, we decided to generate rat MAbs as stage-specific markers for future investigations of the interconversion between tachyzoites and bradyzoites in the mouse model.

The fusion of myeloma cells with spleen cells of chronically infected Fisher rats resulted in the generation of seven independent rat hybridomas. Five of these MAbs have been shown to react with stage-specific antigens of *T. gondii* in the IFA or by the immunoblot technique. In the IFA, MAb DC11 recognized a surface antigen expressed only in bradyzoites, being negative for tachyzoites and oocysts. This reactivity was confirmed in immunohistological investigations with either lung or brain tissue of infected mice. Conversely, MAb DC2 was reactive only with a surface antigen of tachyzoites, as could be demonstrated by IFA and immunohistology. It was possible to monitor in vitro-induced differentiation from the tachyzoite to the bradyzoite stage by using these stage-specific rat MAbs. Since these MAbs have also been shown to react stage specifically with parasites in tissue sections of infected mice, these two rat MAbs may be useful candidates for studying stage conversion in vivo. However, since no reactivity of these MAbs has so far been demonstrated in immunoblots or by immunoelectron microscopy, the reactive antigen still has to be determined.

MAb EE5 reacted with tachyzoite and bradyzoite antigens detected in IFA as well as with two antigens with molecular masses of 52 and 55 kDa in immunoblots of tachyzoites. Therefore, this MAb seemed not to be stage specific. Interestingly, MAb AD11, which did not show any reactivity in IFA, also recognized antigens with identical molecular masses in immunoblots of tachyzoites. An antigen of 52 kDa has previously been detected in cysts of some *T. gondii* strains (28). However, since the EE5- and AD11-reactive antigens were present only in immunoblots derived from tachyzoite lysates, the relationship of this antigen to the cyst-specific antigen is unclear. MAbs BB7 and DD4 reacted with bradyzoite antigens of 60 to 65 kDa. Although antigens with similar molecular masses have been described, these antigens were present not only in bradyzoites but also in tachyzoites (6). However, it is possible that the previously described dominant cyst antigen with a molecular mass of 67 kDa is identical to one of the antigens recognized by our bradyzoite-specific MAbs: the 60- to 65-kDa complex and the 67-kDa antigen have been identified in all strains tested (28). Recently, Parmley et al. (19) cloned a gene encoding a cyst-matrix antigen of 65 kDa that has been designated MAG1. This antigen and the 65-kDa antigen recognized by rat MAbs DD4 and BB7 could be the same, because it was shown that MAG1 is secreted into the matrix of the cyst and does not accumulate in bradyzoites. Therefore, IFA of free bradyzoites was negative, but IFA and immunoblots of whole cysts were positive. This is consistent with the result obtained with MAbs DD4 and BB7.

CC2 was the only rat MAb that recognized an antigen of oocysts in IFA and immunoblots; although the CC2-reactive antigen was shown to have a molecular mass of 115 kDa in oocysts and bradyzoites, its molecular mass was 40 kDa in tachyzoites. Immunoelectron microscopy revealed that the reacting antigen seemed to be located predominantly within the cyst wall. In addition, clumps of material with similar electron density to the cyst wall were labelled, as were dense granules within the bradyzoites. In contrast, when tachyzoites were used, reactivity was observed not only with dense granules but also within electron-lucent vacuoles in the anterior cytoplasm. At present, it is not clear whether the antigens recognized by CC2 in either bradyzoites or tachyzoites are related to each other. It is possible that the electron-lucent vacuole in the tachyzoites represents some form of lysosome and that the 40-kDa antigen is related to degradation. Further studies are under way to investigate the nature of the CC2-reactive antigen(s). A bradyzoite-specific antigen of 116 kDa has previously been identified with a murine MAb that might be related to the CC2-reactive antigen (27). However, this murine MAb, in contrast to our rat MAb, did not recognize an antigen of tachyzoites. In addition, no immunoelectron microscopy was performed with the murine MAb. It is therefore not clear whether these two MAbs react with the same antigen. However, the 115-kDa antigen reacting with rat MAb CC2 also seems to be associated with the oocyst/sporozoite stage of *T. gondii*. So far, two major membrane proteins of 25 and 67 kDa

have been identified in sporozoites by using murine MAbs (13). An additional antigen of 190 kDa has been detected by analyzing sera of infected humans (14).

In conclusion, we have generated rat MAbs, which may be useful for future studies on stage differentiation of *T. gondii* in the murine host. Further investigations must be carried out to determine whether the MAb-reactive antigens are important for the pathogenesis of human toxoplasmosis and its diagnosis.

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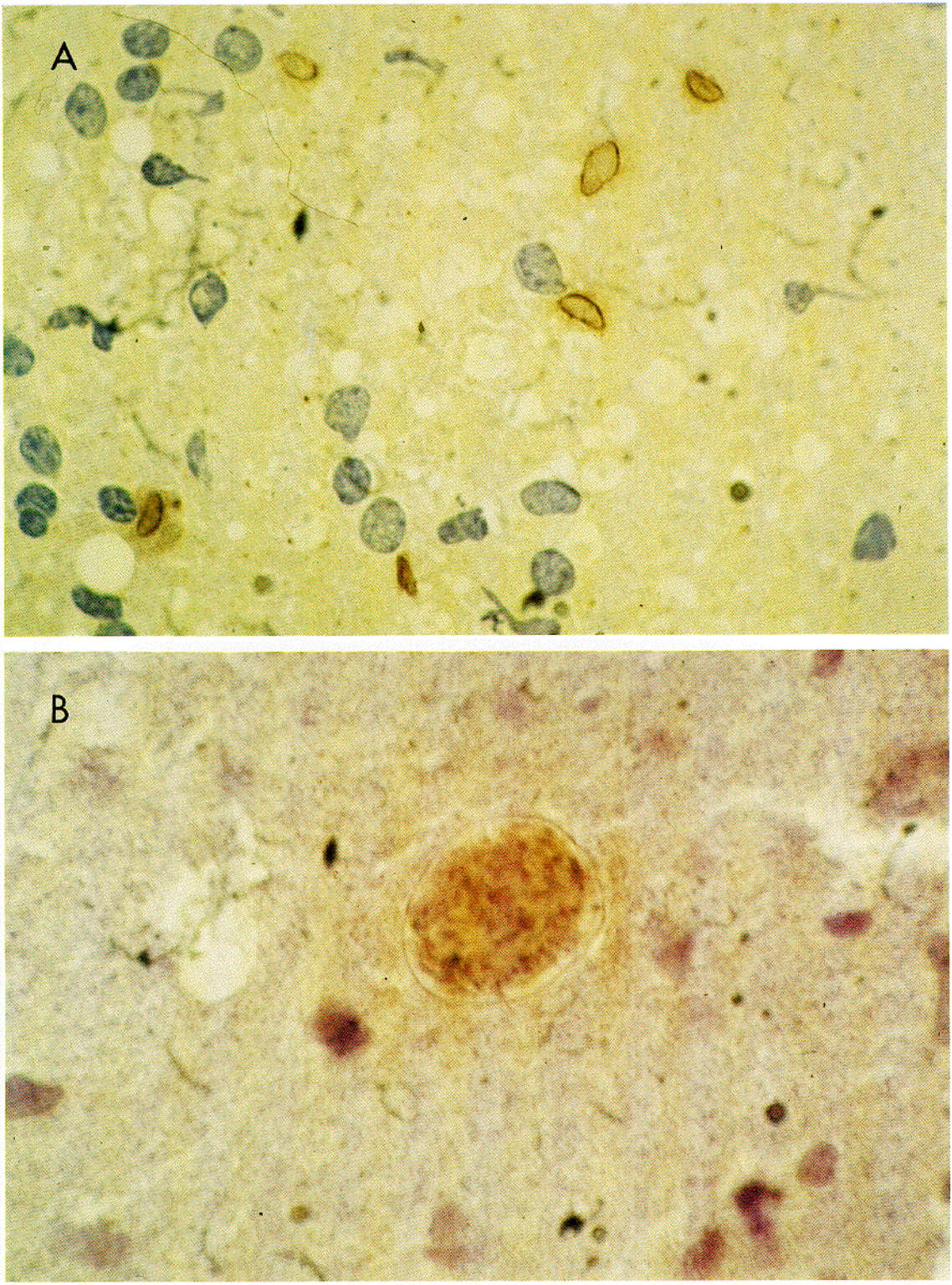
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