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Serodiagnosis of Toxoplasmosis by Using a Recombinant Form of the 54-Kilodalton Rhoptry Antigen Expressed in *Escherichia coli*

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A 330-residue carboxy-terminal antigenic fragment of the *Toxoplasma gondii* 54-kDa rhoptry protein (ROP2) was expressed in *Escherichia coli* as a fusion polypeptide containing a 48-amino-acid sequence derived from phage lambda protein Cro and *E. coli* protein LacI followed by six consecutive histidyl residues. Metal chelate affinity chromatography provided an easy way to isolate the recombinant product in a highly purified form (>95%). When this material was used to develop an immunoglobulin G (IgG) enzyme-linked immunosorbent assay for diagnosis of toxoplasmosis, the test reached a sensitivity of 89%. The sensitivity of the assay was similar whether the sera contained *T. gondii*-specific IgM or were devoid of such IgM. It was also found that ROP2 is a conserved antigen since antibodies against the recombinant antigen could be detected in mice experimentally infected with 11 independent *T. gondii* isolates originating from infected human tissues tested. Thus, the 54-kDa rhoptry antigen could advantageously complement other previously described *T. gondii* antigens for the development of more-sensitive and more-informative recombinant antigen-based tests for toxoplasmosis diagnosis.

Toxoplasma gondii is an obligate intracellular parasite belonging to the coccidian family, common throughout the world, and able to infect all warm-blooded animals. In the human species, most infections with *T. gondii* are asymptomatic. However, there are several circumstances in which toxoplasmosis is a very severe and sometimes life-threatening disease. One of the risk groups consists of immunocompromised patients (e.g., AIDS patients and transplant recipients, etc.) in whom infection (acute or, more frequently, recrudescent) can become fatal. Another group is made up of pregnant women in whom primary infection is often transmitted to the fetus, causing abortion, neonatal malformations, or severe sequelae appearing in later life (including psychomotor defects, mental retardation, and blindness) (for a review, see reference 18).

The tests presently used for toxoplasmosis diagnosis are mostly serological assays. Although they give satisfying results, accurate differentiation between recently acquired and chronic toxoplasmosis still remains problematic and time-consuming. Moreover, the production of constant-quality *T. gondii* antigens necessary for the tests is an expensive and laborious technique because the organism must be grown in host cells (for a review, see reference 7). The use of recombinant antigen could avoid these drawbacks and permit development of improved diagnostic tests.

We have recently reported the cloning of a cDNA coding for a 54-kDa antigen of *T. gondii* (19). This antigen was recently shown to be a component of the rhoptries, specialized organelles located in the apical part of the sporozoan zoites and involved in the host cell invasion process (5). This rhoptry protein had previously been termed ROP2 (11, 20). Because the cDNA clone (Tg34) was originally selected from an expression library by screening with a pool of immune human sera and because immunoblot experiments showed that the corresponding native antigen was one of the major bands recognized by a pool of human immune immunoglobulin G (IgG) (19), this antigen could be useful as a serodiagnostic tool. Here we describe the expression in *Escherichia coli* of a recombinant truncated form of this antigen, its purification, and its evaluation as a tool for the serological diagnosis of toxoplasmosis.

MATERIALS AND METHODS

Reagents. Reagents were of analytical grade and obtained from E. Merck AG (Darmstadt, Germany), Sigma Chemical Co. (St. Louis, Mo.), or Bio-Rad Laboratories (Richmond, Calif.). Restriction enzymes and DNA-modifying enzymes were from Boehringer-Mannheim (Brussels, Belgium). Guanidine HCl (ultrapure) was obtained from ICN Biomedical, Inc. (Cleveland, Ohio). Culture media components were from Difco Laboratories (Detroit, Mich.).

Sera. The serum samples used in this study were referred to the clinical biology laboratory for routine screening or diagnosis of toxoplasmosis. They were tested in the modified Sabin-Feldman dye test (10) and in a commercial double-sandwich IgM enzyme-linked immunosorbent assay (ELISA) (Toxonostika IgM; Organon Teknica, Turnhout, Belgium). To be used as probes in Western blot experiments, a pool of 15 serum samples displaying a dye test titer of ≥1/2,000 and a pool of 15 serum samples displaying a dye test titer of <1/10 were constituted. Mouse sera were collected from outbred Swiss mice chronically infected with 11 independent *T. gondii* isolates originating from infected human tissues (placenta and cord blood).

Protein assay. The protein concentration was determined by the Bradford method as modified by Read and Northcote

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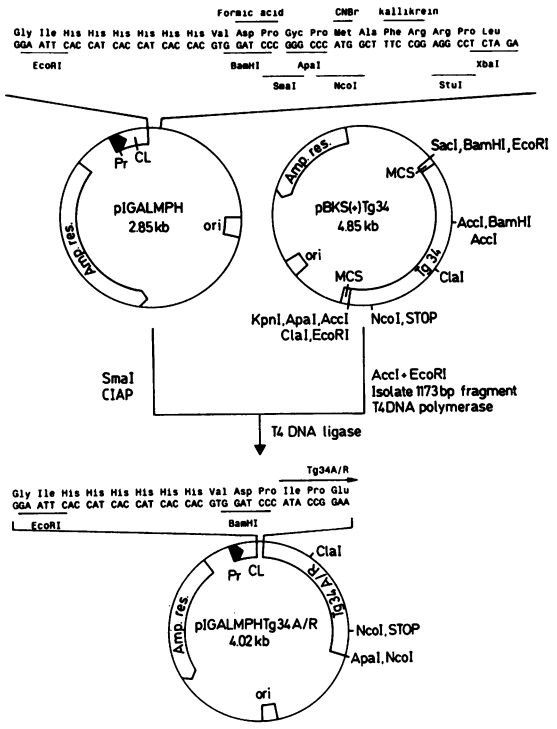

FIG. 1. Construction of expression plasmid pIGALMPHTg 34A/R. Abbreviations: P_r, rightward promoter of phage lambda; CL, Cro-LacI gene fusion; ori, origin of replication [ColE1 in pBKS(+) Tg34, pMB1 in pIGALMPH); Amp. res., ampicillin resistance gene; MCS, multiple cloning site; Tg34, *T. gondii* cDNA clone Tg34; CIAP, calf intestinal alkaline phosphatase.

(16), using bovine serum albumin as the standard. All determinations were done in the presence of 300 mM guanidine HCl.

Plasmid construction. (i) pBKS(+)Tg34. The construction of a *T. gondii* cDNA library in the lambda gt11 expression vector and the isolation of the Tg34 clone by immunoscreening with a pool of immune human serum have been described before (19). The 1.8-kbp *Eco*RI insert of this clone was subcloned into the *Eco*RI site of plasmid pBluescript KS(+) (Stratagene, La Jolla, Calif.), resulting in plasmid pBKS(+) Tg34 (Fig. 1).

(ii) pIGALMPH. The expression vector pIGALMPH is a derivative of the vector pIGAL (21) (Fig. 1). An *Eco*RI-*Xba*I oligonucleotide containing a multiple cloning site and coding for an amino acid sequence containing a stretch of six histidyl residues followed by several specific proteolysis sites was cloned in the *Eco*RI- and *Xba*I-digested pIGAL vector. The resulting expression vector, pIGALMPH, contains the p_R promoter of phage lambda, the coding information for a 48-amino-acid fusion of phage lambda protein Cro and *E. coli* protein LacI, the multipurpose oligonucleotide described above, and the translation stop signal and the tandem 103-bp terminator fragment of phage fd. The vector further contains an ampicillin resistance gene and the pMB1 origin of replication.

(iii) pIGALMPHTg34A/R. The 1,173-bp fragment resulting from the digestion of pBKS(+)Tg34 by *Acc*I and *Eco*RI was isolated by agarose gel electrophoresis, blunted by treatment with T4 DNA polymerase, and ligated to the *Sma*I-digested and calf intestine alkaline phosphatase-dephosphorylated pIGALMPH vector (Fig. 1). All enzymatic reactions described were performed by using standard techniques (22). *E. coli* DH1 (ATCC 33849) made lysogenic for phage lambda was used for transformation with this construct. Transformants were selected on ampicillin-containing Luria-Bertani plates, and the plasmids they contained were characterized by restriction enzyme digestion as previously described (22).

Induction of recombinant antigen expression. *E. coli* MC1061 (ATCC 53338) containing plasmid pcI857 (17) was transformed with either plasmid pIGALMPH or plasmid pIGALMPHTg34A/R. Transformants were grown at 28°C in Luria-Bertani medium containing 50 mg of ampicillin per liter with good aeration until the optical density at 600 nm (OD_{600}) reached 0.4. The temperature of the culture was then rapidly increased to 42°C by adding an equal volume of fresh medium equilibrated at 60°C. The incubation was then continued for 5 h at 42°C with good aeration.

Extraction of recombinant antigen. Bacteria from a 6-liter induced culture were harvested by centrifugation at 17,700 × *g* for 10 min, resuspended in 170 ml (five-pellet volume) of lysis buffer (100 mM KCl, 10 mM Tris-HCl [pH 6.8], 5 mM EDTA, 20 mM ε-amino-caproic acid, 1 mM dithiothreitol), and kept frozen at −80°C until use. After defreezing, the cells were lysed in a French press (three cycles at 16,000 lb/in^2). The resulting lysate was centrifuged at 10,000 × *g* for 20 min at 4°C. The pellet was extracted with 90 ml of 7 M guanidine HCl–50 mM sodium phosphate (pH 7.2) buffer in a Dounce homogenizer (10 strokes), and the extract was centrifuged at 27,000 × *g* for 20 min at 4°C. The supernatant containing the recombinant antigen was stored at −20°C until further purification.

Recombinant antigen purification by metal chelate affinity chromatography. Twenty milliliters of chelating Sepharose fast flow (Pharmacia, Uppsala, Sweden) was poured into a column (16 by 100 mm) and charged by applying 60 ml of a 5-mg/ml $NiCl_2 \cdot 6H_2O$ solution. The column was then successively equilibrated with 6 M guanidine HCl–50 mM sodium phosphate (pH 7.2) buffer, loaded with recombinant extract (60 mg of total protein), washed with 6 M guanidine HCl–50 mM sodium phosphate (pH 7.2) buffer, and eluted with a step gradient of increasing imidazole concentrations (15, 25, 35, and 100 mM) in 6 M guanidine HCl–50 mM sodium phosphate (pH 7.2) buffer. The purified material was stored at −20°C.

***E. coli* lysate.** *E. coli* MC1061 carrying plasmid pcI857 was grown at 28°C until the OD_{600} reached 0.2. The culture was then incubated for 3 h at 42°C. Cells were collected by centrifugation, resuspended in lysis buffer (100 mM KCl, 5 mM EDTA, 10 mM Tris-HCl [pH 6.8]), and lysed by passage through a French press. The volume of the lysate was then adjusted to 1/30 of the original culture volume. This lysate contained 6 mg of protein per ml.

Gel electrophoresis and Western blotting (immunoblotting). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out in the discontinuous system described by Laemmli (9). Proteins of guanidine-containing samples were recovered by overnight precipitation at −20°C in the presence of 75% (vol/vol) methanol and centrifugation (14,000 × *g*, 15 min). Prior to loading, samples were incubated for 5 min at 100°C in sample buffer containing 2.5% SDS, 5% (vol/vol) β-mercaptoethanol, 62.5 mM Tris-HCl (pH 6.8), 10% glycerol, and 0.005% bromophenol blue. The electrophoretic transfer to a nitrocellulose membrane (Hybond C; Amersham, Brussels, Belgium) was carried out as described by Towbin et al. (24) with 25 mM Tris–192 mM glycine–20% (vol/vol) methanol buffer and by using a

Transphor electrophoresis unit (Hoefer Scientific Instruments, San Francisco, Calif.) at 100 V for 2 h with cooling to 4°C. Blocking of the membrane was achieved by incubation in washing buffer (10 mM Tris-HCl, 150 mM NaCl, 0.05% [vol/vol] Tween 20 [pH 8.0]) containing 5% skimmed milk and 1% bovine serum albumin for 2 h at room temperature (RT). The blot was then incubated overnight at 4°C with pooled human serum (1/2,000 dilution) or with monoclonal antibody (MAb)-containing ascitic fluid (1/2,000 dilution); bound antibodies were detected with alkaline phosphatase-labeled rabbit anti-human IgG (gamma chain; Dako, Glostrup, Denmark; 1/2,000 dilution) or rabbit anti-mouse immunoglobulin (Dako, 1/2,000 dilution) incubated for 2 h at RT. All sera and labeled antibody dilutions were prepared in washing buffer containing 1% bovine serum albumin. The alkaline phosphatase activity was revealed by using the chromogenic substrate nitroblue tetrazolium–5-bromo-4-chloro-3-indolyl-phosphate in 50 mM Tris-HCl (pH 9.5)–150 mM NaCl–5 mM Mg^{2+} buffer (1). Molecular mass standards were myosin (H-chain, 200 kDa), phosphorylase *b* (97 kDa), bovine serum albumin (68 kDa), ovalbumin (43 kDa), carbonic anhydrase (29 kDa), β-lactoglobulin (18.4 kDa), and α-lactalbumin (14.3 kDa), all supplied by GIBCO-BRL (Ghent, Belgium).

ELISA. ELISA plates (Immuno Plate Maxisorp F96; Nunc, Roskilde, Denmark) were coated by overnight incubation at 4°C with a recombinant antigen solution in 0.2 M glycine-HCl (pH 4) buffer (6 μg/ml, 100 μl per well). Blocking of the solid phase was carried out by incubation with phosphate-buffered saline (PBS) containing 0.1% casein, 0.25 M glycine, and 0.01% merthiolate for 2 h at RT (300 μl per well). After three washes with PBS containing 0.05% Tween 20 (washing buffer), human or mouse sera were added at a 1/20 dilution in 10 mM phosphate (pH 7.4)–0.35 M NaCl–0.1% caseine–0.2% Triton X-705–0.01% merthiolate–10% (vol/vol) *E. coli* lysate and incubated for 2 h at RT (100 μl per well). The wells were then washed four times and incubated with horseradish peroxidase-labeled goat anti-human IgG (Fc) (Bethesda Research Laboratories, Gaithersburg, Md.) at a 1/10,000 dilution or with peroxidase-labeled goat anti-mouse IgG (Fc) (Jackson ImmunoResearch, West Grove, Pa.) at a 1/20,000 dilution in PBS containing 0.1% caseine, 0.0033% $K_3Fe(CN)_6$, and 0.01% merthiolate (100 μl per well, 2 h, at RT). After four washes, the peroxidase activity was detected with H_2O_2 and 3,3',5,5'-tetramethylbenzidine (TMB substrate kit, Bio-Rad; 100 μl per well). The reaction was stopped after 15 min by adding 100 μl of 1 N H_2SO_4, and the OD_{450} was read.

RESULTS

Expression of a truncated form of the 54-kDa *T. gondii* antigen in *E. coli*. The attempt at expressing in *E. coli* the whole polypeptide encoded by the cDNA clone Tg34 as a fusion protein by using the pIGALMPH vector was confronted with problems of low expression level and rapid intracellular degradation of the expressed product (data not shown). The use of *E. coli* strains with reduced proteolytic activity (UT5600 [$OmpT^-$] and SG4044 [Lon^-]) failed to circumvent this problem. We thus looked for clone Tg34 fragments coding for polypeptides that could be expressed at a high level and with minimal degradation in *E. coli* and that would retain immunoreactivity with sera of *T. gondii*-infected individuals. We found that the *Acc*I-*Eco*RI fragment of clone Tg34 containing the coding information for the 330 carboxy-terminal residues fulfilled these requirements. This fragment was cloned in the pIGALMPH expression vector, resulting in plasmid pIGALMPHTg34A/R (Fig. 1), which was introduced in *E. coli* strain MC1061 containing the compatible plasmid p*c*I857. In the expression plasmid, transcription is under control of the lambda rightward promoter and is regulated by the *c*I857 repressor delivered from the compatible plasmid. The *c*I857 thermosensitive mutant repressor can be inactivated by shifting the culture from 28 to 42°C. Culture samples of expression plasmid-transformed bacteria drawn at various time intervals after raising the temperature were analyzed by SDS-PAGE and Western blot. Coomassie brilliant blue staining (Fig. 2A) revealed a 43-kDa protein present in induced cultures of *E. coli* containing the expression plasmid pIGALMPHTg34A/R but absent in noninduced cultures of *E. coli* containing the same plasmid, as well as in induced and noninduced cultures of *E. coli* containing the vector pIGALMPH. The apparent molecular mass of the expressed product is in agreement with the one expected for the fusion polypeptide encoded by pIGALMPHTg34A/R (7 kDa for the Cro-LacI leader peptide and 38 kDa for the 330 residues encoded by the Tg34 *Acc*I-*Eco*RI fragment). This kinetic experiment also showed that the fusion polypeptide continued to accumulate in the bacteria for up to 5 h. Western blot and immunological analysis with *T. gondii*-immune human serum showed the presence of a major immunoreactive product with the same molecular mass (43 kDa) in the induced culture of *E. coli* containing pIGALMPHTg34A/R only. In addition to this band, a higher-molecular-mass component (≈50 kDa) and a minor degradation product (≈40 kDa) were also detected as faint bands (Fig. 2B). When the Western blot was probed with anti-Cro-LacI mouse MAb, a main 43-kDa band was also stained specifically. The high sensitivity achieved with this MAb also led to the detection of some additional immunoreactive components of higher and mainly lower molecular weights. The former components could result from a low-frequency process of reading through the *T. gondii* gene stop translation signal by the *E. coli* ribosomes while the latter ones presumably correspond to degradation products (Fig. 2C). An analysis of samples drawn at different time points showed that degradation had already occurred after 1 h of induction and that the relative amount of degradation products with respect to intact product did not increase as the induction proceeded further (data not shown).

Purification. The purification strategy of the recombinant fusion polypeptide was based on the observation that it accumulated in *E. coli* in insoluble inclusion bodies (data not shown) and on the fact that it has been engineered to contain a stretch of six consecutive histidyl residues endowing it with a high affinity for metal ions (Fig. 1) (6). Inclusion bodies were recovered by centrifugation at low speed and solubilized in 6 M guanidine HCl. The soluble fraction of this extract was loaded on a nickel chelate affinity chromatography column. The resulting chromatogram is shown in Fig. 3A. Besides the nonbinding protein fraction in the flow-through, the majority of the proteins eluted at an imidazole concentration of 15 mM, with a minor peak at 25 mM. As could be expected from its sequence which contains two pairs of adjacent histidyl residues apart from the engineered hexa-histidyl sequence, the recombinant fusion polypeptide displayed a very high affinity towards the chelated Ni^{2+} matrix and was eluted at an imidazole concentration of 100 mM. Indeed, Western blot analysis of the fractions with the anti-Cro-LacI MAb indicated the presence of immunoreactive material in the 100 mM imidazole fraction only (data not

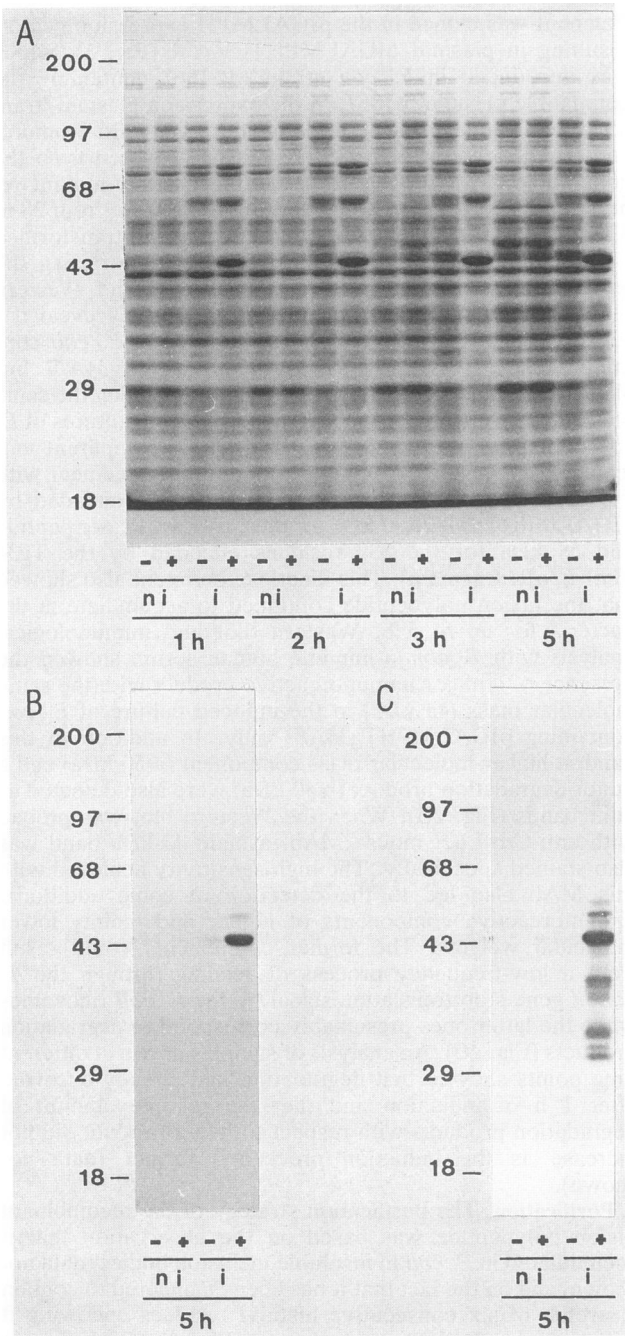

FIG. 2. Expression of the *Acc*I-*Eco*RI fragment of clone Tg34 as a Cro-LacI fusion polypeptide in *E. coli*. *E. coli* MC1061 pcI857 transformed with plasmid pIGALMPHTg34A/R (+) or with vector pIGALMPH as the control (−) was cultured at 28°C until the OD_{600} reached 0.2. The cultures were then split into two equal fractions which were incubated at either 28°C (ni) or 42°C (i). One-milliliter aliquots of each culture were drawn after 1, 2, 3, and 5 h, and the OD_{600} was recorded. The cells were collected by centrifugation (12,000 × g, 30 s), resuspended in SDS-sample buffer (200 μl/ml of culture × OD_{600} unit), incubated for 5 min in a boiling water bath, and chilled. Aliquots were loaded onto an SDS–10% polyacrylamide gel (10 [A] or 2 [B, C] μl per lane). After electrophoresis, the gel was stained with Coomassie brilliant blue (A) or Western blotted (B and C). The blotted material was probed by using either pooled *T. gondii*-immune human sera and alkaline phosphatase-conjugated rabbit anti-human IgG (B) or anti-Cro-LacI MAb and alkaline phosphatase-conjugated rabbit anti-mouse immunoglobulin (C). MAb and serum dilutions were prepared in buffer containing 1% (vol/vol) *E. coli* lysate. The alkaline phosphatase activity was detected by using the chromogenic substrate. The positions and molecular masses (in kilodaltons) of protein standards are also shown.

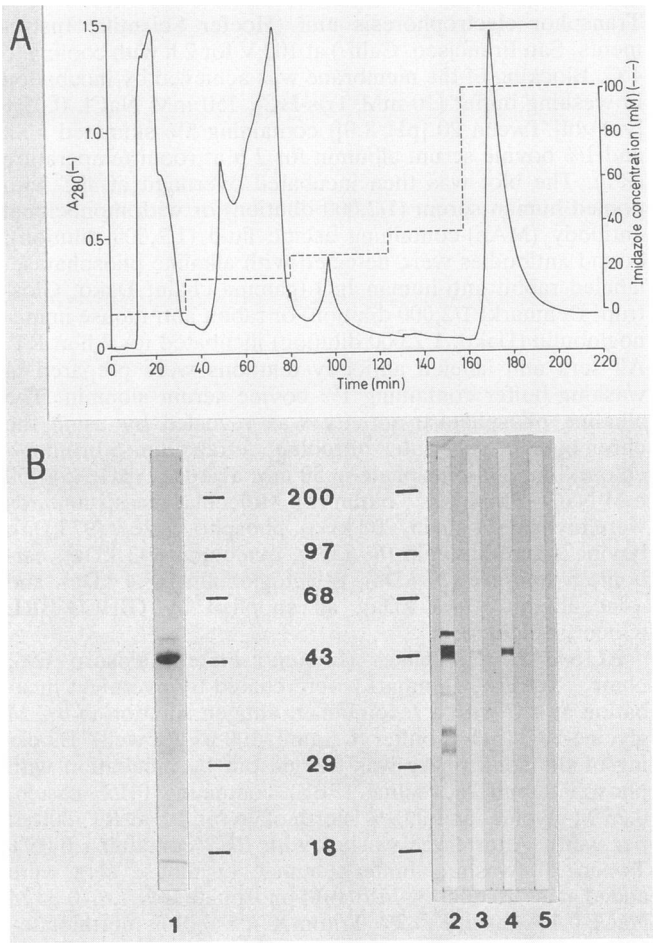

FIG. 3. Purification of recombinant fusion polypeptide by metal chelate affinity chromatography. (A) An Ni^{2+}-charged chelating Sepharose column was loaded with a 7 M guanidine extract of *E. coli* expressing the recombinant antigen. Elution was achieved by increasing the imidazole concentration (dotted line), and the A_{280} of the eluate was recorded. The flow rate was 1.7 ml/min. The fractions containing the material eluted at a 100 mM imidazole concentration were pooled as indicated by the horizontal bar (see Materials and Methods for details). (B) The purified material (100 mM imidazole peak) was electrophoresed in an SDS–10% polyacrylamide gel (5 [lane 1] or 0.3 [lanes 2 to 5] μg per lane) and either stained with Coomassie brilliant blue (lane 1) or Western blotted and probed with anti-Cro-LacI MAb (lane 2), an unrelated control MAb (lane 3), a pool of immune human sera (lane 4), or a pool of nonimmune human sera (lane 5). Bound antibodies were detected as described in Fig. 2. The positions and molecular masses (in kilodaltons) of protein standards are also shown.

shown). Figure 3B shows the results of the characterization of the purified product by SDS-PAGE and Western blot. The 43-kDa band observed in the Coomassie brilliant blue-stained gel represented >95% of the stainable material. The faint extra bands detected by Coomassie staining were also

TABLE 1. Amino acid composition of the purified recombinant antigen[a]

Amino acid	No. of residues	
	Experimental	Theoretical
D	35.8	31
E	44.9	41
S	21.5	23
G	17.9	20
H	18.6	19
R	34.3	36
T	21.8	21
A	24.2	26
P	22.5	25
Y	10.3	9
V	19.5	23
M	8.8	7
I	20.9	19
L	46.5	50
F	11.8	14
K	14.8	13

[a] Amino acid analysis was carried out automatically by using a model 420 A derivatizer and a model 130 A separation system from Applied Biosystems (Foster City, Calif.). Experimental values are the means of two independent analyses. Asparagine was determined as aspartic acid; glutamine was determined as glutamic acid. Cysteine and tryptophan were not determined.

recognized specifically by the anti-Cro-LacI MAb and thus correspond to degradation products or modified forms of the fusion polypeptide which are copurified by the Ni chelate affinity chromatography. The good agreement between the experimentally determined amino acid composition of the purified product and the predicted composition (Table 1) further supports the identity and the purity of the recombinant fusion polypeptide. The yield of purified product was 18 mg/liter of culture.

ELISA. We developed an ELISA by using the recombinant fusion polypeptide as the coating antigen to detect IgG antibodies in human serum and evaluated its performance for the serodiagnosis of toxoplasmosis. Since it appeared that the recombinant polypeptide still contained traces of *E. coli* components, the interference of anti-*E. coli* antibodies present in the sample sera was reduced by preparing the serum dilution in buffer containing *E. coli* lysate. The human sera assayed in the recombinant antigen-based ELISA had previously been tested by the modified Sabin-Feldman dye test, which is considered to be the reference test for toxoplasmosis diagnosis, and by the double-sandwich IgM ELISA. Figure 4 shows the results of the analysis of 98 dye-test-positive (IgM^+ and IgM^-) and 23 dye-test-negative samples. The cutoff was set at the mean + 2 standard deviations of A_{450} values of the negative samples. The ELISA test had an overall sensitivity of 89%. The sensitivity of the assay was similar whether the sera contained *T. gondii*-specific IgM, considered to be an indicator of a more-or-less-recent infection, or were devoid of such IgM (91 and 87%, respectively). On the other hand, as shown in Table 2, the ELISA sensitivity increased as the dye test titer increased.

The ELISA was also adapted for detecting antibodies present in the sera of experimentally infected mice. We found that mice chronically infected with 11 independent *T. gondii* isolates produced highly significant titers of antibody against the recombinant antigen (data not shown).

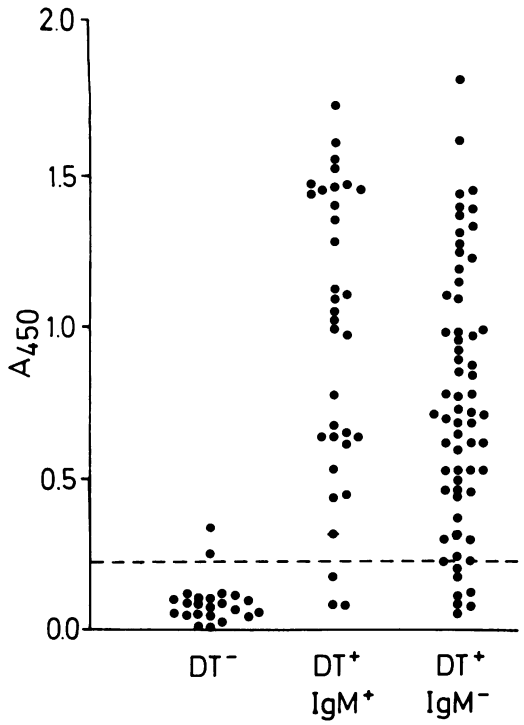

FIG. 4. Detection of IgG antibodies against the recombinant antigen in serum of *T. gondii*-infected individuals. Human serum samples were incubated in the wells of ELISA plates coated with the recombinant fusion polypeptide. Bound antibodies were revealed by using horseradish peroxidase-labeled goat anti-human IgG (Fc) and the chromogenic substrate. The panel of human sera tested included 23 samples with titers of <1/10 in the modified Sabin-Feldman dye test (DT^-), 34 samples with dye test titers of ≥1/10 and IgM titers of ≥1/100 (DT^+, IgM^+), and 64 samples with dye test titers of ≥1/10 and IgM titers of <1/100 (DT^+, IgM^-). A_{450} values are means of duplicates. The cutoff level (mean + 2 standard deviations of A_{450} values of negative samples) is also indicated (broken line).

DISCUSSION

The 330-residue carboxy-terminal antigenic fragment of the *T. gondii* 54-kDa rhoptry protein (ROP2) was expressed in *E. coli* as a fusion polypeptide containing a hexa-histidyl sequence. Metal chelate affinity chromatography provided an easy way to recover highly purified (>95%) recombinant product with a good yield (18 mg/liter of culture). When this material was used to develop an IgG ELISA for diagnosis of toxoplasmosis, the test reached a sensitivity of 89%.

Several factors could explain why our recombinant antigen-based ELISA was unable to detect antibodies in about 10% of infected individuals. It could be argued that the sera of our panel were collected at different time intervals after infection, and antibodies against this antigen are only

TABLE 2. Relationship between dye test titer and ELISA sensitivity

Dye test titer	No. of serum samples tested	ELISA sensitivity (%)
1/10	5	20
1/100	35	89
1/1,000	30	90
≥1/2,000	28	100

present at a certain stage of infection (acute or chronic phase). It was previously shown by Western blot analysis of follow-up sera obtained from the same patient at different time intervals after infection that a 35-kDa antigen is the first antigen eliciting a clear IgG response soon after infection. Antibodies against a 53- to 54-kDa band which could correspond to the ROP2 antigen appeared somewhat later (14, 25). It is therefore possible that in the very early stage of infection, a time during which antibodies against surface components (measurable by the dye test) are already present, antibodies against the 54-kDa ROP2 antigen are not yet detectable. This could explain some of the false-negative results obtained in the series of dye-test-positive, IgM^+ (acute) sera but not the false negatives observed in the chronic sera series.

The hypothesis that this antigen could be specific for a defined stage of the parasite life cycle may also be rejected since this 54-kDa rhoptry antigen has been shown to be present in tachyzoites (proliferative form), bradyzoites (encysted latent form), and sporozoites (20). It could also be possible that this antigen is not present in all *T. gondii* strains or is polymorphic. Strain-specific antigens have indeed been observed in *T. gondii* by Ware and Kasper (26), Weiss et al. (27), and Gross et al. (4). However, this hypothesis seems improbable since mice infected with 11 independent *T. gondii* isolates (cyst-forming strains) had antibody in their sera reacting with our recombinant antigen, the gene of which was isolated from the virulent Wiktor strain.

The most probable explanation could involve the heterogeneity of the immune response in the infected human population; i.e., some infected individuals may not produce an antibody response against the epitopes present on this recombinant antigen. Thus, improvement of the ELISA sensitivity will probably require the use of one or several other parasite antigens used together.

In this respect, Tenter and Johnson (23) recently described the expression of two recombinant truncated fragments of *T. gondii* antigens (gene H4 coding for a 25-kDa antigen and gene H11 coding for a 41-kDa antigen) as fusion proteins with glutathione *S*-transferase in *E. coli*. When used together in an ELISA for toxoplasmosis serodiagnosis, these two recombinant antigens detected 68% of patients with acute toxoplasmosis but only 14% of patients with chronic infection. Parmley et al. (13) also very recently reported the expression of the surface antigen P22 of *T. gondii* as a glutathione *S*-transferase fusion protein in *E. coli* and evaluated the serological reactivity of this recombinant antigen. They found that IgG antibodies in serum samples from 31 acutely infected patients in general reacted more strongly to the fusion protein than did those in serum samples from 31 patients with chronic infection. By using a cutoff set at the mean + 1 standard deviation of the absorbance of negative sera, these authors observed that eight (26%) of the serum samples from chronically infected patients did not display significant reactivity with the recombinant P22 antigen. Thus, the 54-kDa rhoptry antigen appears as a more-sensitive marker for detection of chronic infection than the previously used recombinant antigens.

In the future, it will be interesting to examine whether the combined use of the above-mentioned and other recombinant antigens of *T. gondii* which are now available (2, 3, 8, 12, 15) will allow the development of more-reliable, -sensitive, -informative, and -inexpensive diagnostic tests.

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