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Value of PCR for Evaluating Occurrence of Parasitemia in Immunocompromised Patients with Cerebral and Extracerebral Toxoplasmosis

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PCR was used to evaluate the occurrence of *Toxoplasma gondii* parasitemia by detection of the *B1* gene in blood samples in two groups of immunosuppressed patients (148 subjects) suspected of having cerebral or extracerebral infection, respectively. Group I consisted of 52 patients with AIDS with suspected cerebral toxoplasmosis. The diagnosis was clinically proven in 15 cases. Parasitemia was detected by PCR in only two of these patients (13.3%), both showing evidence of disseminated infection. Group II consisted of 96 immunocompromised patients, either with AIDS or receiving iatrogenic immunosuppressive therapy. Of these patients, 65 (34 with AIDS and 31 others) showed abnormalities only in chest radiography and were first screened for the presence of *Toxoplasma* DNA in bronchoalveolar lavage fluid. Blood was then analyzed when the parasite was detected in the bronchoalveolar lavage fluid. The remaining 31 subjects (22 with AIDS and 9 others) were suspected of having extracerebral, pulmonary, or disseminated toxoplasmosis, and blood was studied directly in these cases. Among the nine patients with clinically diagnosed extracerebral infection in group II, the parasite was detected by PCR in the blood of five patients (55.5%), all having pulmonary toxoplasmosis. If all patients with clinical manifestations of extracerebral toxoplasmosis (from both groups) who had not received antitoxoplasma therapy when the samples were collected are considered, PCR detected parasitemia in seven of the nine cases (77.8%). The present study indicates that examination of blood by PCR may be valuable in cases of extracerebral toxoplasmosis because of the disseminated nature of the disease. Since most cases of cerebral toxoplasmosis result from the local reactivation of latent brain cysts, detection of parasitemia by PCR is useful only in cases associated with severe cerebral infection or dissemination of this disease.

In recent years, *Toxoplasma gondii* has emerged as an important life-threatening opportunistic pathogen in immunocompromised patients. In the United States, toxoplasmic encephalitis has been estimated to occur in one-third of AIDS patients with latent *T. gondii* infection (29). In Europe and Africa, where a higher prevalence of latent infection is present, 25 to 50% of patients with AIDS will ultimately develop toxoplasmic encephalitis (30, 33). The lung was found to be one of the most common sites of extracerebral toxoplasmosis in immunocompromised patients (7, 39). Involvement of the central nervous system and the lung may occur alone or in association with disseminated toxoplasmosis affecting multiple organs such as the heart, liver, kidney, lymph nodes, adrenal glands, bone marrow, stomach, colon, and testes (3, 31, 39). While published data indicate that toxoplasmic encephalitis usually results from the local reactivation of latent infection (28, 29), recent reports of the isolation of the parasite from blood of patients with toxoplasmic encephalitis (17, 18, 38) suggest the systemic nature of the disease in some cases. Pulmonary toxoplasmosis may result from the local rupture of latent cysts or be due to the spread of the parasite from distant sites (31). Since most of these patients have immunoglobulin G (IgG) antitoxoplasma antibodies from latent infection and IgM antibodies are rarely found (27), for diagnosis it is necessary to detect the parasite by direct methods. These approaches are time-consuming, requiring up to 6 weeks for mouse inoculation (16) and 4 to 10 days for less sensitive tissue culture (15). Furthermore, in many cases they make invasive intervention such as brain biopsy or bronchoscopy necessary. This necessitates the consideration of new, noninvasive, rapid and sensitive techniques. One method that has been used successfully in this regard for the detection of *T. gondii* in clinical specimens is PCR (5, 17, 19–21, 24, 36).

Here, we report on 1½ years of experience in employing PCR to detect *T. gondii* DNA in samples obtained from immunocompromised AIDS patients and transplant recipients. The aim of our study was to explore further the value of PCR for detecting the parasite in blood samples in cases of suspected cerebral and extracerebral toxoplasmosis.

MATERIALS AND METHODS

Study groups. The study included a total of 186 patients composing two groups (groups I and II) with suspected cerebral and extracerebral toxoplasmosis, respectively, as shown in Table 1 (148 patients), and a separate group of 38 subjects without acute toxoplasmosis serving as controls (data not shown). Group I consisted of 52 patients with AIDS CDC stage IV, as defined by the Centers for Disease Control, who were suspected of having cerebral toxoplasmosis. Forty sam-

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TABLE 1. Clinical data, number of samples, and results of serology and mouse inoculation for the two study groups

Group and patient clinical data	No. and type(s) of samples	Results (no. positive/no. tested)[a]			
		Serology		Mouse inoculation	Clinically proven acute toxoplasmosis
		IgG	IgM		
Group I (suspected cerebral toxoplasmosis)					
AIDS CDC IV patients (*n*, 52) Age: 29–59 yr Male/female: 49/3 $CD4^+$ cell count: 0–120/μl	40, blood and CSF	23/39 (1 ND)	0/23	0/31 (9 ND)	8/40
	12, blood	10/12	1/10	1/12	7/12
Group II (screened for *T. gondii* DNA in BAL fluid [*n*, 65]; suspected extracerebral toxoplasmosis [*n*, 31])					
AIDS CDC IV patients (*n*, 56) Age: 23–53 yr Male/female: 51/5 $CD4^+$ cell count: 0–315/μl	9, blood and BAL	8/9 (1 ND)	1/8	2/9[b]	4/9
	31, BAL	13/31	0/13	ND	0/31
	16, blood	9/16	0/9	1/15 (1 ND)	1/16
Non-AIDS patients (*n*, 40) under immunosuppressive therapy Age: 22–73 yr Male/female: 30/10	6, blood and BAL	5/6	2/5	1/6[b]	2/6
	31, BAL	18/31	0/18	ND	0/31
	3, blood	3/3	2/3	0/3	2/3
Total 148 patients	86, blood 40, CSF 77, BAL	89/147 (1 ND)	6/89	5/76 (72 ND)	24/148

[a] Serology results refer only to serum specimens tested; samples negative for IgG were not tested for IgM. The number of positive samples over the total number of samples available is indicated. ND, not determined.

[b] The numbers shown represent the results of animal inoculation of both blood and BAL samples.

ples of cerebrospinal fluid (CSF), accompanied by whole blood and serum samples, were obtained from these patients. From the remaining 12 patients in this group, only blood and serum samples were available, as lumbar puncture was inadvisable because of increased intracranial tension. Group II consisted of 96 immunocompromised patients, including 56 individuals with AIDS CDC IV, 29 transplant recipients (8 renal, 3 hepatic, 4 lung, 2 bone marrow, and 12 heart transplants), 9 patients with malignancy receiving cytotoxic drugs (5 with non-Hodgkin's lymphoma and 4 with chronic myeloid leukemia), and 2 patients receiving high-dose corticosteroid therapy. Among the 56 AIDS patients, 13 presented fever and progressive atypical pneumonitis that did not respond to broad-spectrum antibiotics or antiviral drugs, 9 had septic fever of unknown origin together with positive IgG antibodies to *T. gondii*, and 34 patients showed evidence of abnormalities in chest radiography only and were therefore subjected to bronchoscopy. From the remaining 40 immunosuppressed non-AIDS patients, 6 (5 heart and 1 lung transplant recipients) were affected by fever and pneumonitis, 4 heart transplant recipients developed postoperative *T. gondii* seroconversion, and 31 (20 organ transplant recipients, 9 patients with neoplasms, and 2 corticosteroid therapy patients) showed abnormalities only in chest radiography and were also submitted to bronchoscopy. Therefore, in total 65 patients were first screened for the presence of *T. gondii* DNA in their bronchoalveolar lavage (BAL) fluid. Blood was further analyzed by PCR or mouse inoculation only when the parasite was detected in the BAL fluid by PCR. The remaining 31 subjects from group II not screened for specific DNA in BAL fluid were, on the basis of clinical symptoms, directly suspected of having extracerebral or pulmonary toxoplasmosis. Thus, blood samples were obtained from all of them. BAL fluid was obtained in some of these cases when the lung was involved. Moreover, additional blood samples were obtained up to 4 weeks after specific therapy had begun in five cases from group II (see Table 3). A separate group of patients without acute toxoplasmosis served as controls. Samples obtained and tested by both PCR and mouse inoculation included 20 blood samples from 20 asymptomatic AIDS CDC IV patients with *T. gondii* IgG antibodies, 10 blood and CSF samples from 10 AIDS CDC IV patients with cerebral abnormalities other than toxoplasmosis, and 8 BAL fluid samples from 8 immunocompetent patients with chest abnormalities (2 with bacterial pneumonia and 6 with cancer).

Serology. Patient sera and CSF samples were screened by a direct agglutination test (DA; bioMerieux, Marcy l'Etoile, France). Only positive sera were further tested in the Sabin-Feldman dye test (4) and enzyme-linked immunosorbent assays (EIA) for IgG (EIAG) and IgM (EIAM) (VIDAS-IgG and VIDAS-IgM; bioMerieux).

Mouse inoculation. Mouse inoculation was performed concurrently with PCR if the samples contained sufficiently fresh material (Table 1). Five milliliters of EDTA-treated blood was first treated with lysis buffer (10 mM $KHCO_3$, 155 mM NH_4Cl, 0.1 M EDTA; pH 7.4). The leukocytes obtained by centrifugation at 3,000 × *g* for 10 min were washed twice with phosphate-buffered saline, pH 7.4, and the final pellets were

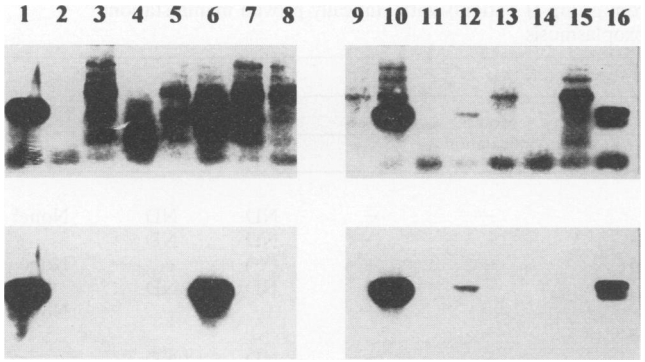

FIG. 1. Southern blot hybridization showing PCR detection of *T. gondii* from blood samples. Cell lysates were subjected to PCR as described in Materials and Methods with primers that amplify a 223-bp DNA-fragment from the *B1* gene. The blots were hybridized with a 190-bp probe directly labeled with horseradish peroxidase, detecting the 223-bp fragment by enhanced chemiluminescence. Blots in the upper panels show insufficiently stringent wash conditions (washed with 32% [wt/vol] urea at 42°C); thus, these blots were reprobed, increasing the urea concentration and the stringency wash temperature to a maximum of 50°C, the maximum tolerated by the peroxidase (optimal conditions used for the lower panels were 42% [wt/vol] urea and a temperature of 48°C). Lanes: 1, 12, and 16, positive-control DNA from 25, 1, or 5 *T. gondii* tachyzoites, respectively; 2 and 14, negative controls (no DNA added); 6 and 10, positive blood samples from patients 17 and 19 (see Table 3); 3 to 5, 7 to 9, 11, and 13 to 15, negative blood samples.

TABLE 2. Results of PCR, serology, and mouse inoculation from group I patients with clinically proven cerebral toxoplasmosis

Patient no.	Sample	Test result[a] for:						Therapy[b]
		Antibody test (serum and CSF)				Mouse inoculation	PCR	
		DA	SFT (IU/ml)	EIAG	EIAM			
1	Blood	+	4	+	−	−	−	None
	CSF	−				−	−	
2	Blood	+	250	+	−	−	−	None
	CSF	+	1	+	−	−	−	
3	Blood	−				ND	−	2 days
	CSF	−				ND	+	
4[c]	Blood	ND				ND	+	None
	CSF	ND				ND	+	
5	Blood	+	4	+	−	ND	−	1 wk
	CSF	+	1	+	−	ND	(+)	
6	Blood	−				−	−	5 days
	CSF	−				−	−	
7	Blood	+	4	−	−	−	−	4 wk
	CSF	−				−	−	
8	Blood	+	16	−	−	−	−	None
	CSF	+	1	−	−	−	−	
9	Blood	+	4	+	−	−	−	4 wk
10	Blood	+	4	+	−	−	−	2 wk
11	Blood	+	16	+	−	−	−	4 wk
12	Blood	+	250	+	+	+	+	None
13	Blood	−				−	−	None
14	Blood	+	4	+	−	−	−	None
15	Blood	+	4	+	−	−	−	None

[a] SFT, Sabin-Feldman dye test; +, positive; −, negative; (+), PCR positive signal detected only after hybridization. ND, not determined. Samples with negative results in DA were not tested by the other serological assays.
[b] Refers to duration of antitoxoplasma therapy before the samples were taken.
[c] The patient died 3 days later from septic shock and lethal encephalitis.

then resuspended in 2.5 ml of PBS. Each sample was divided into two portions which were injected intraperitoneally into two 10-week-old NMRI mice. In the case of CSF or BAL fluid, 0.5- and 1-ml samples, respectively, were injected directly intraperitoneally into each of two mice. Mouse inoculation was considered positive if dissection of the mice revealed intraperitoneal tachyzoites or brain cysts or if the mice developed antitoxoplasma antibodies detected by the dye test (titer, ≥1,000 IU/ml).

Processing samples by PCR. DNA was prepared and amplified as previously described by Roth et al. (36). Briefly, DNA was released from the cell pellets obtained after lysis and centrifugation of 500 μl of EDTA-treated blood as mentioned above or centrifugation of 1 ml of CSF or BAL fluid by means of proteinase K digestion. The supernatant of the resulting crude cell lysate was analyzed for the presence of the *T. gondii B1* gene, previously described by Burg et al. (5). Three oligonucleotide primers were used: P3, 5′-CTTCAAGCAG CGTATTGTCG-3′, corresponding to nucleotides 661 to 680 of the sense strand of the gene; P7, 5′-TAAAGCGTTCGTG GTCAACT-3′, corresponding to nucleotides 864 to 833 of the antisense strand; and P8, 5′-GGAACTGCATCCGTTCA TGA-3′, corresponding to nucleotides 694 to 713 also of the sense strand of the gene. P3 and P7 initiate the amplification of a 223-bp fragment, whereas P8 and P7 generate a 190-bp fragment partially internal to the first fragment. The first fragment was used as the target for the amplification reaction, while the second purified fragment was used as the detecting probe. The amplification reaction was done in a 50-μl reaction mixture containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5 mM $MgCl_2$, 0.01% gelatin, 250 μM (each) deoxynucleotide triphosphates (dATP, dGTP, dCTP, and dUTP), 12.5 pmol of each primer, 1 U of *Thermus aquaticus* DNA polymerase (all reagents were from Boehringer Mannheim GmbH, Mannheim, Germany), and 10 μl of crude DNA. The reaction was performed by incubating the reaction mixture for 36 cycles in a thermal cycler (bio med, Theres bei Haßfurt, Germany). Each cycle consisted of denaturation at 95°C for 1 min, annealing at 55°C for 50 s, and extension at 72°C for 30 s, with an initial step of 1 min at 95°C and a final step of 10 min at 72°C. Twenty-microliter aliquots of the amplified products were analyzed by electrophoresis on a 4% agarose gel. The bands were visualized by staining with ethidium bromide. The specificity of the amplified products was analyzed by Southern blotting onto nylon Hybond-N^+ membranes, followed by hybridization at 42°C with the horseradish peroxidase-labeled 190-bp probe, using the enhanced chemiluminescence detection system as recommended by the supplier (Amersham, Braunschweig, Germany). A specially optimized hybridization buffer supplied in the system ensures appropriate stringency by inclusion of 6 M urea. This formulation is equivalent to 50% formamide in reducing the thermal melting point of hybrids. To increase stringency, this buffer was further supplemented with 2 M NaCl. Moreover, high-stringency washing of the blots was performed with the recommended washing buffer in the presence of a higher concentration of urea (42% [wt/vol]) and by increasing the temperature to 48°C. In every run, DNAs extracted from 25 or 5 tachyzoites were used as positive controls. Negative controls consisted of negative human DNA and non-DNA samples. The results were considered valid only if the control results were as expected. Each sample was tested twice, on two separate occasions. Each time, the sample was tested at two different dilutions in order to minimize the possibility of an inhibition of the reaction by the sample. In addition, each sample was coamplified with DNA extracted

TABLE 3. Results of PCR, serology, and mouse inoculation from group II patients with clinically proven manifestations of extracerebral toxoplasmosis

Subgroup and patient no.	Sample	Test result[a] for:								Therapy[b]
		Serology				Mouse inoculation	PCR			
		DA	SFT (IU/ml)	EIAG	EIAM		0	1	4	
AIDS										
16	Blood	+	ND	+		−	−	ND	ND	None[c]
	BAL					−	+	ND	ND	
17	Blood	+	4	−	−	−	+	(+)	−	None
	BAL					−	+	ND	ND	
18	Blood	+	250	+	−	+	+	+	−	None
		+	250	+	+[d]					
	BAL					+	+	ND	ND	
19	Blood	+	1	+	−	+	+	ND	−	None
	BAL					+	+	ND	ND	
20	Blood	+	16	+	−	+	+	ND	−	None
Non-AIDS										
21	Blood	+	1	+	−	+	ND	+	−	None[c]
		+	1,000	+	+[d]					
	BAL					+	+	(+)	−	
22	Blood	+	1,000	+	+	−	−	ND	ND	None
	BAL					−	(+)	ND	ND	
23	Blood	+	1,000	+	+	−	ND	ND	−	4 wk
		+	4,000	+	+[d]					
24	Blood	+	250	+	+	−	ND	ND	−	4 wk
		+	1,000	+	+[d]					

[a] PCR results are divided by the duration of antitoxoplasma therapy: none, 1, or 4 weeks (0, 1, and 4, respectively). SFT, Sabin-Feldman dye test; ND, not determined. Symbols are as defined in Table 2, footnote *a*.

[b] Duration of antitoxoplasma therapy before the first sample was taken.

[c] At the time of examination of the first samples, patients 16 and 21 were under sulfamethoxazole therapy because of originally suspected *Pneumocystis carinii* pneumonia.

[d] The results shown represent the serology of a second serum sample obtained 2 weeks after the first one.

from five tachyzoites. Strict measures to avoid contamination were employed (25). To avoid the possibility of carryover contamination, dUTP was used instead of dTTP in the reaction mixture (26). Positive samples were retested after being treated with uracil-DNA-glycosylase (GIBCO, Eggenstein, Germany). Samples which yielded positive results at least two times and after treatment with glycosylase were considered positive.

RESULTS

Specificity and sensitivity of PCR. The method of directly labeling long nucleic acid probes with peroxidase leads to the introduction of an average of one peroxidase complex every 25 bases. To ensure a high sensitivity, we tried to produce as long a probe as possible. As a result, the probe was not completely internal to the fragment amplified in clinical samples. However, experiments to optimize the stringency of the Southern hybridization method demonstrated that specificity was not impaired (Fig. 1). To assess the sensitivity of the PCR, DNA was extracted and amplified from serial dilutions of *T. gondii* tachyzoites ranging from 10^3 to 0.5 parasites with or without 1 μg of contaminating negative human DNA (corresponding to 3×10^5 leukocytes). In most experiments, DNA corresponding to 1 parasite in the absence and to 10 parasites in the presence of contaminating DNA was detected. In some experiments, DNA corresponding to as few as one parasite in the presence of human DNA was also detectable. Another approach was attempted, in which serial dilutions of the amplified and purified target double-stranded DNA (223 bp), ranging from 1 pg to 0.005 fg (with or without 1 μg of contaminating negative human DNA), were amplified. As little as 0.01 to 0.02 fg of the target DNA in the presence of 1 μg of contaminating negative human DNA could be detected by PCR. Assuming that the haploid genome of *T. gondii* (7.7×10^7 bp) is equivalent to 0.1 pg of DNA (9) and that the *B1* gene is a 35-copy gene (5), detection of 0.01 fg of the 223-bp fragment is equivalent to the detection of approximately one parasite. Moreover, *T. gondii* DNA was detected by this PCR procedure in all blood samples from 10 mice 3 days after intraperitoneal inoculation with 1.6×10^6 BK strain tachyzoites (36).

In all the samples tested, crossover contamination occurred on only two occasions. These samples were found to be negative after treatment with uracil-DNA-glycosylase.

Serology, mouse inoculation, and PCR for the study groups. The patients studied were divided in two groups on the basis of original clinical suspicion of cerebral or extracerebral toxoplasmosis before a definite diagnosis could be established. Altogether, acute toxoplasmosis was diagnosed in 24 cases, 15 from group I and 9 from group II. Serology revealed the presence of specific IgM antibodies in six of these patients only. Mouse inoculation was positive in five cases, with samples available from 21 subjects. Definite clinical diagnosis was based (i) on the presence of characteristic lesions in computed tomography or magnetic resonance imaging scans or on radiological and clinical improvement after specific antitoxoplasma therapy in 13 patients from group I, (ii) on confirmation of cerebral infection, at autopsy in one case or by biopsy in another, and on the clinical fulminating manifestation of a disseminated toxoplasmic infection with septic shock and lethal encephalitis in a further case (Table 2, patient 4) from group I and from patients in group II (Table 3), (iii) on rapid improvement after

specific therapy in 8 cases (patients 16 to 19 and 21 to 24), (iv) on detection of the parasite in blood by mouse inoculation in 4 cases (patients 18 to 21), or (v) on seroconversion with the appearance of IgG followed by IgM after heart transplantation in 4 cases (patients 21 to 24). The parasite was also demonstrated in Giemsa-stained BAL smears from two patients (patients 18 and 21 [data not shown]). In summary, 11 cases of extracerebral manifestations were considered to be extracerebral toxoplasmosis (2 from group I and 9 from group II). On the basis of clinical data, as described above, pulmonary involvement in toxoplasmosis was believed to have occurred in seven of the nine cases represented in Table 3 (patients 16 to 22).

Examination of samples from patients with cerebral toxoplasmosis in group I by PCR revealed *T. gondii* DNA in the blood of two (patients 4 and 11) and in the CSF of three (patients 3 to 5).

Of the nine subjects with acute toxoplasmosis in group II, PCR was positive for six of six BAL fluid samples and four of six blood samples taken before therapy (week 0). Parasite DNA was also detected in the blood of two patients (patients 17 and 18) and blood and BAL fluid of one patient (patient 21) 1 week after specific antitoxoplasma therapy was started. None of the blood samples were positive when examined by PCR 4 weeks after specific therapy had begun. Patient 21 presented fever and pulmonary symptoms 3 weeks after cardiac transplantation. Since the donor was seropositive (donor data are not shown in Table 3), pulmonary toxoplasmosis was suspected. PCR examination of BAL fluid obtained 34 days and BAL fluid and blood obtained 42 days after transplantation revealed the presence of specific DNA. Serological proof of infection was available only on the 42nd postoperative day (dye test, 250 IU/ml; positive EIAM). The parasite was also detected in BAL fluid (34th postoperative day), by both Giemsa-stained smear and mouse inoculation, and in blood (42nd postoperative day) by mouse inoculation. Patient 22 had low-grade intermittent fever 3 weeks after transplantation. Screening of serum by DA showed no antitoxoplasma antibodies. One month later, the patient complained of cough and dyspnea. Chest radiography showed no abnormalities. Screening of the serum for antitoxoplasma antibodies showed a positive dye test (1,000 IU/ml), positive EIAG, and positive EIAM. Examination of blood and BAL fluid by PCR at this time showed the presence of specific DNA in BAL fluid but not in the blood. Mouse inoculation results were found negative for both blood and BAL fluid. As both the donor and the recipient were serologically negative, the occurrence of pulmonary toxoplasmosis was believed to be due to primary fresh infection. Patients 23 and 24 were asymptomatic, but they were found to have seroconverted 2 and 5 months postoperatively, respectively.

Mouse inoculation results and PCR were negative for all blood, CSF, and BAL fluid samples obtained from the control group patients.

DISCUSSION

Toxoplasmosis constitutes a major cause of morbidity and mortality in patients with AIDS. Methods to obtain definite diagnosis are time-consuming and may not be sensitive enough. Often, presumptive therapy is given following the occurrence of clinical improvement (29, 30, 33). Although this approach has a high positive predictive value of up to 80% (8), it may lead to overdiagnosis of toxoplasmic encephalitis or delay the diagnosis of other causes of brain disease.

In this study, samples from 15 patients with cerebral abnormalities due to cerebral toxoplasmic infection were examined. Three patients were found seronegative by DA (21.4%). This high percentage may be due to the low sensitivity of DA, yet we believe negative serology results rather reflect a failure to produce IgG antibodies or a delay in their development due to profound immunosuppression. Indeed, the disease was confirmed in histopathological sections from brain biopsy in patient 13 and from brain autopsy in patient 6. The occurrence of toxoplasmosis in immunosuppressed patients with negative serology results has been recorded by many investigators (18, 27). Porter and Sande were not able to detect IgG antibodies in 22% of patients with pathologically proven cerebral toxoplasmosis (35). The absence of detectable DNA in the five patients' CSF (negative predictive value of 0.86; 40 patients studied) might be due to the fact that toxoplasmic encephalitis is mainly a parenchymal infection. The parasites may not find their way to the CSF unless the infection is by chance near the meninges (29). In a study by Parmley et al., *T. gondii* DNA was detected in the CSF of four of the nine AIDS patients with toxoplasmic encephalitis examined (32). In another study, DNA was detected by PCR in the CSF of 13 of the 20 patients with AIDS who were suspected of having cerebral toxoplasmosis (37). This higher diagnostic yield might be due to the routine nature of lumbar puncture for all patients with suspected cerebral toxoplasmosis. In the present study, CSF samples were not available for seven patients with cerebral toxoplasmosis listed in Table 2 because lumbar puncture was inadvisable because of increased intracranial tension. In the case of one patient (patient 7), the CSF sample was sent after 4 weeks of therapy, which might have caused the negative result. The disappearance of *T. gondii* DNA after treatment has recently been recorded by Dupouy-Camet et al. (17). Of the blood samples from 15 patients with cerebral manifestation of toxoplasmosis examined that are listed in Table 2, the parasite was detected by PCR in only two (13.3%; negative predictive value, 0.74). The clinical presentations of both patients, one with chorioretinitis and septic fever, the other with septic shock and fulminating lethal encephalitis similar to that previously reported for 16 patients with disseminated toxoplasmosis (1), suggest that concomitant disseminated toxoplasmosis occurred in these cases. In France, Tirard et al. isolated the parasite by tissue culture from the blood of 12 of 31 patients (38.8%) with clinically proven toxoplasmosis (38). In a recent study, 9 of 13 patients (69.2%) with clinically confirmed cerebral toxoplasmosis had *T. gondii* DNA in their blood when samples were examined by PCR (17). In both studies, the samples were tested before the patients received any antitoxoplasma treatment. In our study, 3 of the abovementioned 15 patients were under treatment for 4 weeks before samples were collected. Considering only patients who had not received any therapy or who had been treated for less than 1 week when their blood samples were collected, we find that PCR detected parasitemia in 2 of 10 patients with cerebral toxoplasmosis (20%).

In the last few years, many reports concerning the occurrence of extracerebral toxoplasmosis, especially pulmonary toxoplasmosis, in AIDS patients and organ transplant recipients have been published (2, 3, 6, 7, 10, 12–14, 22, 31, 34, 36). In a recent study of 80 AIDS patients at autopsy, extracerebral involvement was revealed in 13 cases (16.2%) (23).

Analysis of the results for patients from group II with extracerebral manifestation of toxoplasmosis shows that *T. gondii* DNA was detected by PCR in 6 of the 77 BAL samples examined (7.7%). Of the seven patients with pulmonary toxoplasmosis, parasite DNA was detected by PCR in the blood of five. This finding indicates that the disease was disseminated

but also demonstrates the value of the examination of blood by PCR in cases of pulmonary toxoplasmosis. This dissemination coincides with the nature of pulmonary toxoplasmosis, which usually results from invasion of the lung by parasites coming from reactivated, distant infection (31) and is usually accompanied by the infection of other organs, as indicated by autopsy studies (23). Of the nine patients with extracerebral toxoplasmosis in group II, parasitemia was detected by PCR in only five (55.5%). This percentage is affected by the absence of parasites in the blood of the last two patients, who had already undergone 4 weeks of antitoxoplasma therapy before the samples were taken. The absence of the parasite in the blood of three of the four non-AIDS patients with extracerebral toxoplasmosis in group II might reflect the better immunological status of these patients, since they all developed IgM antibodies. This immunological response might have contributed to the rapid clearance of the parasites from the blood. Indeed, considering only the AIDS patients with extracerebral infection in both groups (two from group I and five from group II), we find that PCR detected parasitemia in six (all as yet untreated when the samples were obtained), equivalent to 85.7%. In summary, if we consider all nine patients in both groups, either with or without AIDS, with extracerebral manifestation of toxoplasmosis who had not received antitoxoplasma therapy when the samples were collected (two from group I and seven from group II), PCR detected parasitemia in seven (77.8%; negative predictive value 0.98; 141 patients studied).

The results obtained in this study show that if PCR is applied to help establish diagnosis of toxoplasmosis using blood samples, it is important to consider whether cerebral or disseminated manifestation is occurring. We have shown that the sensitivity of the assay increases significantly in cases where a dissemination of the parasites can be expected. Examination of autopsy samples from patients with AIDS in the United States (39) and Germany (23) showed that 14.3 and 29%, respectively, of cases with cerebral toxoplasmosis were associated with extracerebral involvement. This coincides with our finding that analysis of blood in cerebral toxoplasmosis by PCR yielded a low negative predictive value. Therefore, only selected samples should be tested in a clinical situation in order to increase the validity of this diagnostic tool. The PCR method used in the present study detects DNA obtained from cell pellets of the sample; thus, we cannot rule out the possibility of achieving a higher sensitivity if DNA is extracted from the whole sample, including putative free-circulating DNA. On the other hand, since blood samples were obtained only once, low-grade intermittent parasitemia might have caused negative results. Another problem that should be addressed is that patients with chronic *T. gondii* infection may have parasites appearing in the blood at intervals during asymptomatic phases of the disease. This may occur rarely, as shown by the negative results from the asymptomatic control group and by a high specificity of the PCR in this study. Dannemann et al. failed to isolate the parasite by animal inoculation in 111 follow-up blood samples from 33 human immunodeficiency virus-infected patients with IgG antitoxoplasma antibodies (11). Finally, our results confirm that mouse inoculation is less sensitive than PCR for the detection of the parasite in both BAL fluid and blood.

In conclusion, examination of blood by PCR may be useful for the diagnosis of pulmonary and extracerebral toxoplasmosis, since most cases are associated with dissemination of the parasites in peripheral blood. Analysis by PCR of blood from patients with cerebral toxoplasmosis is valuable only (i) if the infection is associated with a disseminated spread of the parasite or severe encephalitis, which is not common since most cases result from local reactivation of latent brain cysts, and (ii) if the patients are not receiving any prophylactic or therapeutic treatment.

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