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Indirect Enzyme-Linked Immunosorbent Assay for Immunoglobulin G and Four Immunoassays for Immunoglobulin M to *Toxoplasma gondii* in a Series of Heart Transplant Recipients

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***Toxoplasma gondii* infections in heart transplant recipients were monitored by indirect enzyme-linked immunosorbent assay for immunoglobulin G (ELISA-IgG), indirect ELISA-IgM in serum IgM fractions, antibody capture ELISA-IgM, IgM-immunosorbent agglutination assay (ISAGA), and IgM immunoblotting. Basic immunosuppression consisted of cyclosporine and low-dose steroids. Before transplantation, 26 of 43 recipients showed serological evidence of infection. In serum samples from 15 (35%) recipients, specific antibodies were not detected. Approximately 50% of the heart donors were toxoplasma seropositive. Eight of the fifteen seronegative recipients received hearts from toxoplasma-seropositive donors. In four of the eight recipients, seroconversion could be demonstrated with all tests used. In three of these four patients, clinical disease developed. One patient with strong serological evidence of toxoplasmosis died, but toxoplasma parasites and antigens were not detected at autopsy. In two patients, toxoplasma cysts were found in cardiac biopsies. Seroconversion was not prevented by the use of spiramycin prophylaxis in two recipients. Reactivations of latent infections or reinfections were detected by indirect ELISA in six (23%) seropositive recipients, but symptoms and signs of active *T. gondii* infection were not seen. Seroconversion and reactivation of infection were readily found by a combined use of indirect ELISA-IgG and ELISA-IgM and antibody capture ELISA-IgM. Discrepancies in results could be examined by immunoblotting. IgM-ISAGA retained stable positive values longer than IgM-ELISAs did. Cyclosporine treatment did not hamper detection of seroconversion but could cause antibody levels to remain relatively low in primary infections. Seronegative recipients should receive antitoxoplasma treatment on seroconversion.**

In immunocompromised patients, *Toxoplasma gondii* infections may constitute a cause of life-threatening disease. The persistence of viable parasites in tissues after a primary infection is considered the major cause of morbidity of toxoplasmosis in these patients, but transmission via leukocyte transfusions and organ transplants has also been reported (10, 11, 28). In heart transplant recipients, disseminated toxoplasmosis has been described to occur at a relatively high frequency compared with the occurrence of the disease in recipients of liver or kidney transplants (6, 12, 20). Active infections found in transplanted hearts indicate the donor heart as the most likely source of infection (12, 19, 29).

Cyclosporine in combination with low-dose steroids is presently routinely used as immunosuppressive therapy in transplantation. Depression of toxoplasma-specific antibody responses after cyclosporine administration and an antiparasitic activity of the drug have been described (20, 22). Therefore, it is important to determine the value of serology for the diagnosis of active toxoplasma infection in cyclosporine-treated heart-transplant recipients. We report our experience with indirect enzyme-linked immunosorbent assays (ELISAs) for specific immunoglobulin G (IgG) and IgM (ELISA-IgG and ELISA-IgM), two IgM antibody capture assays, and an IgM immunoblotting test in a series of 43 heart transplant recipients.

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MATERIALS AND METHODS

Patients. Between June 1984 and July 1987, 42 patients underwent orthotopic heart transplantation at the University Hospital Dijkzigt in Rotterdam. One patient needed a retransplantation after 9 months because of chronic rejection. Two patients did not survive 1 week after surgery and were excluded from this study. Another three patients that received transplants at other institutions were included. The follow-up period ranged from 9 to 45 months.

Immunosuppressive therapy. Basic immunosuppressive therapy consisted of cyclosporine and low-dose prednisone. Preoperatively, cyclosporine was administered with a starting dose of 3 mg/kg per day. One week postoperatively, this dose was adjusted according to renal function and targeted to blood levels of 150 to 200 ng/ml for the remainder of the first year and to 100 to 150 ng/ml after that. Perioperatively, patients received prednisolone intravenously followed by oral prednisone as soon as possible in doses of 1 mg/kg per day tapered in 6 weeks to 10 mg/day as a maintenance dose.

Acute rejection episodes detected by endomyocardial biopsy were treated with methylprednisolone, 1 g on three consecutive days. For early or ongoing rejections, a 21-day course of rabbit antithymocyte globulin (4 mg/kg per 6 h; doses were adjusted according to the number of T cells).

Prophylaxis and treatment. Cytomegalovirus-negative recipients received hyperimmune globulins for the first 3 postoperative months.

After our first experience with acute disease with *T. gondii* in a seronegative recipient of a heart from a seropositive

donor, the next of these recipients received spiramycin, 3 g/day, for 3 months as prophylaxis for the development of myocarditis and dissemination of infection. Antitoxoplasma therapy consisted of a combination of sulfadiazine, 50 mg/kg per day, plus pyrimethamine, 50 mg/day, for the first 3 days and 25 mg/day thereafter, usually for at least 3 weeks.

Serum collection. Serum specimens were obtained on acceptance of the patient for transplantation and before surgery. Serum from heart donors was acquired when available. Postoperatively, patients were monitored serologically for toxoplasma infection in weeks 1 and 2 after transplantation and then every other week till 12 weeks, subsequently after every month till 6 months after surgery, and thereafter every 6 months. Sera were tested in duplicate when they became available. Portions of all sera were stored at −70°C and assayed again in parallel at a later date. Within-run reproducibility of serologic tests was 100%, and run-to-run reproducibility was approximately 100%.

Records of transfusions and administration of hyperimmune sera were reviewed with regard to times of changes in serologic test titers.

Serologic methods. ELISA-IgG was performed as described by Ruitenberg and van Knapen (27) with freeze-thawed sonificated mouse peritoneal tachyzoites of strain RH of *T. gondii* as antigen (15) and peroxidase-conjugated goat immunoglobulins to human IgG (γ-chains) (Institut Pasteur, Paris, France), with 5-amino-2-hydroxybenzoic acid in combination with H_2O_2 as substrate. End-point titers were obtained via twofold serial dilutions from 1:20 onwards. A serially diluted highly positive serum specimen and five negative reference serum specimens run in duplicate served as controls. Negative controls were pooled sera negative in Sabin Feldman reaction obtained via the blood bank from healthy blood donors. Seroconversion or a fourfold increase in antibody titer was considered significant.

Indirect ELISA-IgM was carried out on serum IgM fractions, obtained by Bio-Gel A-5m (200 to 400 mesh; Bio-Rad Laboratories, Munich, Federal Republic of Germany) filtration as described by Pyndiah et al. (25), in essentially the same way as the indirect assay for IgG, with peroxidase-conjugated $F(ab')_2$ fragment of rabbit immunoglobulins to human IgM (μ-chains) from Dakopatts a/s, Glostrup, Denmark.

Antibody capture ELISA-IgM (acELISA-IgM) with peroxidase-labeled toxoplasma antigen as described by van Loon et al. (36) was performed with the same (labeled) antigen preparation as used in indirect ELISA. Microdilution plates were coated with rabbit immunoglobulins to human IgM (μ-chains) (Dakopatts a/s). 2,2′-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS; Boehringer GmbH, Mannheim, Federal Republic of Germany) was used as substrate. All sera were tested in duplicate. Sera were single-diluted (1:40). A highly positive serum sample and a negative reference serum sample served as controls. A low-positive reference serum, obtained from an individual convalescent from acute toxoplasmosis and positive in IgM immunoblot test, was used to establish a guideline absorbance value. Results were given as the ratio between mean absorbances of test serum and low-positive control. A modified cutoff value of 0.80 (mean plus 3 standard deviations) was obtained by establishing ratios of absorbancy with a panel of 100 untreated serum specimens negative when their IgM fractions were assayed in indirect ELISA-IgM, and the low-positive reference serum.

Positive reactions were checked for the occurrence of false-positivity due to patient antibodies against rabbit immunoglobulins by replacing the rabbit anti-human IgM with a similar antiserum prepared in goats (Nordic Laboratories, Tilburg, The Netherlands), but identical results were obtained.

Immunosorbent agglutination assay (ISAGA) for the detection of toxoplasma IgM antibodies (IgM-ISAGA) (8) was performed as described by the manufacturer (bioMérieux, Charbonnières les Bains, France). IgM antibodies in serum were captured by anti-human IgM monoclonal antibodies coated onto wells of microdilution plates. ISAGA index was read in values from 0 to 12 according to the instructions of the manufacturer: 0 to 5, negative reaction; 6 to 8, borderline reaction; and 9 to 12, positive reaction.

For the IgM immunoblotting assay, polyacrylamide gel electrophoresis of toxoplasma RH strain antigen preparation and subsequent electrophoretic transfer of separated polypeptides to nitrocellulose membrane (0.45-μm pore diameter; Schleicher & Schuell, Dassel, Federal Republic of Germany) as described by Towbin et al. (32) was carried out as described by Herbrink et al. (14). Reactive antibodies to 6-kilodalton (kDa) antigens were detected with peroxidase-conjugated rabbit immunoglobulins to human IgM (μ-chains) (Dakopatts a/s) and diaminobenzidine–9-chloronaphthol–H_2O_2.

When sera were available, detection of toxoplasma circulating antigens and circulating immunecomplexes was undertaken by ELISA at the Laboratory for Parasitology and Mycology, National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands, as described by van Knapen et al. (33, 35).

Histology. A two-step indirect peroxidase technique with Mayer hematoxylin as counterstain (at the Department of Pathology) was used on biopsy and autopsy material. Also a direct immunofluorescence assay, peroxidase-antiperoxidase method (4), on autopsy material and an ELISA on homogenized tissue supernatants (34) with appropriate controls were carried out at the National Institute of Public Health and Environmental Protection.

RESULTS

Before transplantation, 26 (60%) patients showed serological evidence of infection with antitoxoplasma IgG or IgM or both. In sera of two of these recipients, serologic evidence was found only with indirect ELISA-IgM. For these patients, similar results were obtained after transplantation; specific IgG did not appear (29 to 33 months after transplantation).

Antitoxoplasma antibodies were not detected in sera of 15 (35%) patients. Three patients underwent heart transplantation at other institutions; no preoperative serology from two of the three recipients or from any of the three donors was available. At admission to our center, sera of the two recipients with unknown preoperative serology contained antitoxoplasma antibodies.

The use of rabbit antithymocyte globulin was found not to affect serology in this study.

Seronegative recipients. (i) Serologic results. IgG was found immediately after transplantation in sera of 9 of the 15 seronegative recipients with indirect ELISA titers of up to 1:160. This IgG could not be detected any more after 4 to 6 weeks and was apparently passively acquired by blood transfusion, since none of these patients received cytomegalovirus hyperimmune serum.

Eight (53%) of the fifteen seronegative recipients (Table 1) received hearts from seropositive donors.

TABLE 1. Distribution of assay values for antitoxoplasma antibodies among sera from heart transplant recipients after transplantation

Serology[a] of:		No. with serology values after transplantation[b]										
Recipient (*n*)	Donor (*n*)	indELISA-IgG titer		indELISA-IgM titer			acELISA-IgM ratio			IgM-ISAGA value		
		<20	≥20	<20	20–40	≥80	≤0.80	0.80–1.00	≥1.00	0–5	6–8	9–12
Neg. (15)	Pos. (8)	4	4	4	1	3	4	1	3	4	0	4
	Neg. (6)	6	0	6	0	0	6	0	0	6	0	0
	NA (1)	1	0	1	0	0	1	0	0	1	0	0
Pos. (26)	Pos. (10)	0	10	3	6	1	9	0	1	6	2	2
	Neg. (8)	2	6	4	3	1	8	0	0	8	0	0
	NA (8)	0	8	2	3	3	8	0	0	5	2	1
NA (2)	NA (2)	0	2	1	1	0	2	0	0	1	0	1

[a] Pos., Seropositive to *T. gondii*; Neg., seronegative to *T. gondii*; NA, serologic status not available. Determinations were based on indirect ELISA-IgG and -IgM titers of <1:20 (negative) and ≥1:20 (positive). Recipient status before transplantation is given.
[b] Indirect ELISA (indELISA) titers are given as reciprocals.

Definite seroconversion was observed with IgG and all IgM assays in four of the eight patients that received hearts from seropositive donors and occurred 4 to 6 weeks after transplantation (Table 2). Two of the seroconversions were found after methylprednisolone was given for acute rejection. Occurrence of IgM was noticed before occurrence of IgG in serum of one recipient. IgM and IgG occurred at the same moment in sera of three recipients, with IgM occurring in one case (recipient 18) in the form of circulating IgM complexes. IgM assays did not differ with regard to the detection of the moment of seroconversion. Indirect ELISA-IgG and ELISA-IgM titers and acELISA-IgM ratios reached were low in individual cases (Tables 1 and 2). IgM-ISAGA index values (Table 1) increased gradually in sera of three recipients, but positive values of 9 to 12 were eventually reached in all four cases.

Plateau values for IgG and IgM titers and IgM ratio were maintained until the death of patient 23. In the other three recipients, indirect ELISA-IgM titers dropped significantly from peak titers and acELISA-IgM reached negative ratios in 4 to 8 weeks. IgM-ISAGA retained positive index values from 8 (recipient 5) to more than 72 (recipient 37) weeks. In sera of the latter recipient, anti-6-kDa antibodies could also be detected with IgM blot for at least a year, although in diminishing staining intensity.

In another seronegative patient (recipient 38), the heart donor serum showed a low indirect ELISA-IgM titer; acELISA-IgM and indirect ELISA-IgG were negative. However, a strongly staining 6-kDa band was detected in the IgM blot. When the results became known, spiramycin was replaced by sulfadiazine-pyrimethamine treatment for 8 weeks. Neither seroconversion nor signs or symptoms compatible with toxoplasma infection developed.

(ii) Clinical findings. Findings compatible with *T. gondii* infection are summarized chronologically in Table 3. Three patients developed signs and symptoms that could be attributed directly to active toxoplasma infection at or shortly after seroconversion. Patient 4 (recipient 37) developed fever and drowsiness during week 6 after a second cardiac transplantation with a heart from another seropositive donor. Computed tomographic brain scan showed a hypodense mass lesion right frontotemporal. Toxoplasma could not be established as causative agent with certainty, since before transplantation a brain lesion had been noticed and serology did not indicate changes in antibody level at this time.

TABLE 2. Serologic test titers in primary and reactivated *T. gondii* infections in heart transplant recipients[a]

Recipient group[b]	Patient no.	Titer before transplantation by indELISA		IgM blot (6 kDa)	Seroconversion (wk after transplantation) by indELISA		Peak titer by indELISA		IgM blot (6 kDa)	Peak titer (wk after transplantation) by indELISA		Donor serology by:		
												indELISA		IgM blot (6 kDa)
		IgG	IgM		IgG	IgM	IgG	IgM		IgG	IgM	IgG	IgM	
1	5	<20	<20	NT	5	5	80	40	NA	5	5	40	<20	NT
	18	<20	<20	NT	5	10	1,280	160	NA	24	15	160	<20	NT
	23	<20	<20	NT	12	4	80	320	Pos.	12	11	160	<20	NT
	37	<20	<20	NT	6	6	320	160	Pos.	12	7	160	20[c]	Neg.
2	24	1,280	20[c]	NA			5,120	NR	NT	11		80	<20	NT
	34[d]	320	160[c]	Neg.			5,120	320[c]	Neg.	22	22	40	<20	NT
	28	160	<20	NT			1,280	NR	NT	7		<20	<20	NT
	40	80	<20	NT			NR	320[c]	Weak		11	<20	<20	NT
	2	40	<20	NT			320	160[c]	Neg.	5	9	NA	NA	NA
	27	1,280	<20	NT			NR	80[c]	Neg.		21	NA	NA	NA

[a] Abbreviations: indELISA, indirect ELISA (expressed as reciprocals); NT, not tested; NA, not available; Pos., positive; Neg., negative; NR, no significant rise in titer.
[b] Group 1, Seronegative recipients of hearts from donors who were seropositive for *T. gondii*, group 2, recipients who were seropositive before transplant.
[c] acELISA negative.
[d] In recipient 34, the indirect ELISA-IgM initially dropped to 1:40.

TABLE 3. Serologic findings and symptoms attributable to *T. gondii* infections in seronegative recipients of hearts from seropositive donors

Patient no.	Wks (posttransplant) of spiramycin treatment	Serology and symptoms	Wk after transplantation
5	None	Eosinophilic infiltrate in endomyocardial biopsy	2
		Seronegative[a]	3
		Fever	4
		Toxoplasma cysts in endomyocardial biopsy; seropositive	5
		Patient recovered with sulfadiazine-pyrimethamine therapy	
18	11–26	Seropositive	5
		Fever	7
		Abnormal liver function tests	8
		Patient recovered while on spiramycin treatment	
		Toxoplasma cyst in endomyocardial biopsy	67
		Sulfadiazine-pyrimethamine therapy	
23	1–15	Subfebrile temperature; seronegative	2
		Seropositive	4
		Fever	6
		Encephalopathy (hemiparesis, aphasia)	10
		Sulfadiazine-pyrimethamine therapy	15
		Patient died; no *Toxoplasma* parasites found at autopsy	16
37	2–13	Seronegative	3
		Seropositive	6
		Retransplantation with heart of seropositive donor	32
		Fever, drowsiness	38
		Patient recovered while on sulfadiazine-pyrimethamine therapy	

[a] Seronegativity was based on indirect ELISA-IgG and -IgM titers of <1:20; seropositivity was based on indirect ELISA-IgG or -IgM titers or both of ≥1:20.

However, the patient recovered while on sulfadiazine-pyrimethamine therapy.

Numerous toxoplasma tissue cysts appeared at the time of seroconversion in sections of endomyocardial biopsies from patient 5. Tachyzoites were not detected. The finding of a single tissue cyst in an endomyocardial biopsy, routinely taken 67 weeks after transplantation from recipient 18, led to administration of sulfadiazine-pyrimethamine.

In patient 23, a computed tomographic brain scan showed hypodense mass lesions in the capsula interna and the subinsular region suggestive of hemorrhagic infarcts and was treated accordingly. After progressive neurologic dysfunction was found, the prophylactic spiramycin was replaced by sulfadiazine-pyrimethamine therapy. Five days later the patient improved, but fever developed again the next day. The patient died after bradycardia and cardiac arrest 10 days after the start of specific therapy.

(iii) Histopathologic results. At autopsy of patient 23, a diffuse panencephalitis was found with massive perivascular lymphocytic infiltrates and glial nodule formation in cerebral hemispheres, cerebellum, and truncus, in addition to extensive necrosis and hemorraghic lesions in cortical and subcortical areas of the occipital lobe and basal ganglia. An extensive search for toxoplasma parasites and antigens was made. Giemsa-stained smears of brain tissue, direct immunofluorescence assay, and antigen ELISA on nonfixed brain material all remained negative. Examination of Formalin-fixed paraffin-embedded tissue sections of multiple samples of brain, lung, myocardium, liver, and muscle by conventional staining techniques, periodic acid-Schiff, Giemsa stain, indirect peroxidase technique, and peroxidase-antiperoxidase method could not demonstrate toxoplasma. *Aspergillus*, *Candida*, *Acanthamoeba*, *Encephalitozoon*, and bacterial infections could not be established. Cytomegalovirus and herpes simplex virus were found only in lung tissue and were not considered the cause of death.

Seropositive recipients. In 3 of the 26 seropositive recipients (Table 1) and 1 of the 2 recipients with unknown serology, IgM-ISAGA showed positive values of 9 to 12 in sera and serum IgM fractions from before transplantation throughout the period described (15 to 32 months). In another four recipients, borderline values of 6 to 8 were found; in three cases these values were maintained for at least 14 to 23 months. Also, acELISA-IgM remained stable-positive in one case. Treatment of the sera with 2-mercaptoethanol before testing turned assays negative. IgM blot did not detect anti-6-kDa antibodies in these sera.

Six patients showed a significant rise in either IgG or IgM indirect ELISA titer or both (Table 2). IgG titers were found to increase gradually, starting 3 to 8 weeks after transplantation and reaching peak titers over a period varying from 3 to 14 weeks. IgM titers started to rise after 6 weeks post-transplantation, in one case after an episode of intensified immunosuppression during week 17, showing a fourfold rise or more over a period varying, as with IgG, from 3 to 14 weeks. With acELISA-IgM or IgM-ISAGA, increases in antibody level were not observed. IgM protein blot detected only weakly reactive antibodies to 6-kDa antigens in the serum with the peak titer of patient 40. Of the six recipients, three showed an increase in antibody level after an episode of increased immunosuppression.

From three recipients (patients 24, 34, and 40), complete series of serum specimens were available for detection of circulating antigens and immune complexes. Circulating immune complexes occurred before the rise in antibody titer in patient 34 and 40 IgG and IgM complexes, respectively. Circulating antigens were not found. In the case of recipient

24, IgG complexes were detected in serum of the heart donor.

No transfusions other than those at surgery were given. Administration of hyperimmune globulins against cytomegalovirus and changes in antibody titer did not correlate.

The medical records of the patients did not show clinical symptoms or signs that could directly be attributed to active infections with *T. gondii*.

DISCUSSION

The common route of *T. gondii* infection in heart transplantation is associated with reception of the heart of a seropositive donor. Approximately 50% of the recipients in the present study received hearts from such donors. This figure corresponds rather well with the average seropositivity of the population in The Netherlands. The about 50% seropositive heart donors and nearly 40% seronegative recipients in our patient series indicate that active toxoplasma infections can be expected to occur relatively frequently in cardiac transplant recipients in The Netherlands.

Another source of infection might be blood transfusions. In processing transfusions buffy coats are removed, but up to 40% of the original number of leukocytes are still left in the preparations. Exacerbations of latent infections or reinfections are thought to occur regularly in healthy blood donors in The Netherlands (35). The occurrence of transitory IgG in 60% of seronegative recipients directly after transplantation indicates the existence of *T. gondii* infections in blood donors used. The transfer of toxoplasma parasites with transfusions is therefore not ruled out (2, 31). However, active toxoplasma infections within the first 2 months posttransplantation in seronegative recipients of the heart of a seronegative heart donor were not detected. The risk of obtaining toxoplasma infection by blood transfusions seems therefore to be much smaller than that of obtaining infection via organ transplants.

Seroconversions in seronegative recipients and onset of increase in antibody levels in seropositive recipients were found to occur during the first 2 months after surgery and in one case after an episode of increased immunosuppression 3.5 months posttransplantation. These observations are in agreement with findings in other heart transplant patients (20, 37). In recipients described by Luft et al. (20), peak titers in dye test were reached as early as 3 to 13 weeks posttransplantation. However, most of their patients received azathioprine in combination with rabbit antithymocyte globulin and corticosteroids. The use of cyclosporine in immunosuppressive treatment in the present study might explain the more gradual rise in antibody levels found (20, 22). The rise in antibody titer 3.5 months after surgery in a seropositive recipient indicates that the possibility of active toxoplasma infection should still be considered outside the direct posttransplant period of the first 2 months during and after episodes of increased immunosuppression. However, clinically apparent disease in reactivated infections appears to be rare (20).

In seropositive recipients, changes in IgM antibody levels were detected only with indirect ELISA. It is not clear why acELISA did not measure these changes, except that indirect ELISA determines the concentration of antitoxoplasma antibodies and acELISA determines the percentage of total IgM reactive with toxoplasma antigen. In establishing endpoint titers in indirect ELISA, a larger range of antibody affinities to toxoplasma antigens may be used than in acELISA, in which measurements of absorbances at low sample dilutions may be more correlated with high affinity antibodies (17). Sensitivity of indirect ELISA-IgM appears to be greater than that of acELISA-IgM in late stages (reactivations) of infection and similar in early (primary) infections. IgM-ISAGA did retain stable positive index values relatively long, and in one seropositive recipient acELISA remained positive. These observations emphasize once more the need for investigation of paired serum specimens in situations in which patients are not strictly monitored for infection.

Small peaks in acELISA can be missed if times of obtaining consecutive serum specimens are widely spaced. Low titers in indirect ELISA are retained longer, but changes in specific IgM level are readily detected. A combined use of both IgM ELISAs therefore appears favorable.

Detection of circulating immune complexes was undertaken to investigate whether this could increase the sensitivity of the serodiagnostic approach. In a few cases these complexes were indeed found before changes in IgG or IgM titers, but it appears that their detection is only of limited use. Their presence in seropositive recipients illustrates that they are no indication for development of clinical disease and only provide information on parasitic activity (30, 35). Because of insufficient quantities of specific serum specimens, data on the occurrence of circulating antigens in seronegative recipients are incomplete and do not allow conclusions.

IgM immunoblotting of sera from seropositive recipients revealed in one case (patient 40) weakly reactive antibodies to 6-kDa antigens. These antibodies are supposed not to be present in sera from chronic infections and are absent in negative controls (9, 24). Nevertheless, in IgM blots, false-positive results appear not to occur with regard to 6-kDa antigens (26). Discrepancies between results of both IgM ELISAs and IgM blot (e.g., heart donor serum of patient 38) might also be due in part to differences between affinities of measured antibodies in the assays. The relatively long period during which 6-kDa IgM-reactive antibodies were found (patient 37) indicates that detection of recent infections with blotting can be hampered by the long-lasting presence of such antibodies in individuals.

Despite extensive search and use of sensitive techniques to detect parasites or parasitic antigens (4, 21, 34), organisms were not demonstrated at autopsy in patient 23. Histopathological inspection of brain tissue revealed phenomena that were considered more compatible with virus infection than with *T. gondii* infection (1, 3). It is therefore not unlikely that this patient did not have toxoplasmosis at death.

Since fatal infection may develop in the seronegative recipient of a heart of a seropositive donor, it was decided to use prophylaxis in patients belonging to this high-risk group. Prophylaxis is based upon killing or inhibition of proliferation of parasites. However, the effective levels of antiparasitic drugs in serum and body fluids have not been determined. Synergistic action of sulfadiazine and pyrimethamine on parasites outside mature tissue cysts has long been known from experimental infections. Less is known about efficacy of pyrimethamine alone, but it is claimed to prevent toxoplasmosis in heart transplant recipients (12, 38). Also, in vitro studies suggest that sustained high levels of pyrimethamine in blood may be effective against toxoplasmosis (13), but caution must be exerted when extrapolating these data to in vivo efficacy. However, bone marrow toxicity is a disadvantage of using pyrimethamine. Spiramycin was used in the present study as prophylaxis for the development of myocarditis and further dissemination of infection. This

macrolide has been shown to prevent transplacental infection in humans (5), and hepatotoxicity and interference with concomitant administrated drugs have never been described (7, 16, 23). However, despite spiramycin prophylaxis, 3 g/day, seroconversion occurred, and the data suggest that spiramycin should not be used for this purpose. Another serious drawback in the use of spiramycin is that it cannot prevent neurotoxoplasmosis (16, 18). Therefore, pyrimethamine instead of spiramycin should be studied further as the prophylactic drug. It appears justified to treat patients with combination therapy on seroconversion (20) or in situations (as in the case of recipient 38) in which it is desirable to minimize the risks. Clinically apparent disease in seronegative recipients can develop concurrently with seroconversion but may be delayed (20, 29). This was considered in the decision to provide specific therapy when a single tissue cyst was found in an endomyocardial biopsy from recipient 18 and when encephalopathy developed in patient 37, 6 weeks after the second heart transplantation was performed.

Thus, life-threatening primary infections can develop in the seronegative recipient of a heart from a seropositive donor. Reactivated infections apparently rarely lead to clinical disease. Primary infections are detected by all tests used. Cyclosporine treatment does not hamper detection of seroconversion but can cause antibody levels to remain relatively low in primary infections. Therefore, heart transplant recipients should be monitored for *T. gondii* infection from the moment of acceptance of the patient for transplantation, and sensitive assays such as indirect ELISAs for specific IgG and IgM should be used. Primary and reactivated infections are readily distinguished by the combined use of indirect ELISAs for specific IgG and for IgM in serum IgM fractions and acELISA-IgM. IgM-ISAGA retains stable positive values longer than both IgM-ELISAs and is therefore less suitable for the purpose. In case of discrepancies between results of IgM-ELISAs, serum specimens can be examined by immunoblotting. Seronegative recipients should receive antitoxoplasma treatment on seroconversion.

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