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CASE REPORT

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blotting in *Toxoplasma*-seropositive heart

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Abstract Toxoplasmosis is a life-threatening disease in heart- or lung transplant recipients that can result either from the reactivation of a latent infection or from an organ-transmitted infection. The diagnosis of acute toxoplasmosis is easy in cases of seroconversion following a mismatch. However, when the recipient is *Toxoplasma*-seropositive before transplantation, usual serological techniques do not allow the differentiation between endogenous and organ-related reinfection. The aim of this study was to determine whether western blotting could contribute to this differentiation. Sequential sera from two heart- and one liver- and lung transplant patients whose anti-*Toxoplasma* antibody titers strongly increased after transplantation, were analyzed by western blotting. Neosynthesized IgG were observed on blots incubated with the sera from two patients who had received transplants from *Toxoplasma*-seropositive donors, whereas no neosynthesized IgG was detected on blots from the patient who had received a transplant from a *Toxoplasma*-seronegative donor. Our results suggest that the detection of neosynthesized IgG in the recipient may be related to the recognition of a new parasite strain possibly brought by the transplant from a *Toxoplasma*-seropositive donor.

Keywords *Toxoplasma gondii* · Heart transplantation · Reactivated toxoplasmosis · Western blotting · Serology

Introduction

Toxoplasmosis is a life-threatening infectious complication in transplant recipients, particularly in heart-, lung- or bone marrow transplant patients [5, 6, 7, 23]. It can result either from a donor-related, acquired infection or from an endogenous reactivation of a latent infection [3, 14]. Patients at risk for acquired toxoplasmosis are mostly heart- or lung transplant patients [3, 23] since both organs are potential sites for *Toxoplasma* encystement. Besides, toxoplasmic reactivation may occur in *Toxoplasma*-seropositive transplant recipients independently of the type of graft [4, 5, 12, 20, 24]. Acute toxoplasmosis can be diagnosed by the observation of its clinical manifestations, though they are often non-specific (fever, neurological signs, pneumopathy, elevation of biochemical enzymes, imaging...). Less often, the parasite can be detected on bronchoalveolar fluid or tissue samples by PCR [11], mouse inoculation [2], or histopathology [14]. Serological findings may also contribute to the diagnosis, i.e. evidence of toxoplasmic seroconversion or serological reactivation (an at least 3-fold rise in anti-*Toxoplasma* IgG titers, with or without emergence of specific IgM or IgA), but a serum sample

Table 1 Clinical description of the patients. (*M* Male, *F* female, *PMT* pyrimethamine, *CM* clindamycine, *TMP* trimethoprime, *SMX* sulfamethoxazole)

Case no.	Age (y)/Sex	Transplant	Clinical symptoms (delay of onset)	Treatment	Outcome
1W	46/M	heart	–	–	Survived
2M	67/M	heart	cerebral toxoplasmosis myocarditis (4 weeks)	PMT-CM	Died
3C	16/F	liver-lungs	lungs rejection (14 weeks)	TMP-SMX	Survived

Table 2 Serological parameters of the patients. (*TP* transplantation)

Case no.	Serology of the donor	Serology of the recipient: IgG/IgM		detection of neosynthesized IgG by WB(kDa)
		Pre-TP	Post-TP	
1W	+	+ /–	+ /–	+ (105, 66, 64, 53, 43)
2M	+	+ /–	+ / +	+ (37)
3C	–	+ /–	+ /–	–

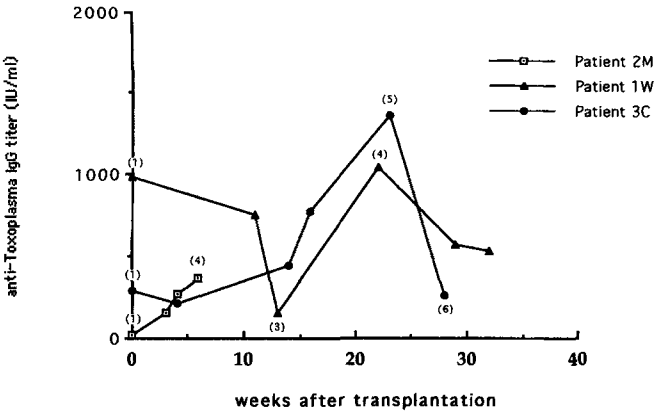

Fig. 1 Kinetics of anti-*Toxoplasma* IgG titers after transplantation in the three transplant recipients. The n° indicated along the curves refer to the sera analysed by western blotting in Fig. 2

collected before transplantation must be available. However, when both donor and recipient are *Toxoplasma*-seropositive, it is not possible to know whether an acute toxoplasmosis is related to an endogenous reactivation of the recipient strain, or to a newly acquired infection by an organ-transmitted strain.

We report here three cases of *Toxoplasma* serological and/or clinical reactivation in transplant patients (2 heart, 1 lungs-liver) followed at Hôpital Broussais (Paris) from 1995 to 1997. Sequential sera collected before and after transplantation were tested for anti-*Toxoplasma* IgG antibodies by western blotting. The aim of this study was to determine whether heart- or lung transplantation from a *Toxoplasma* seropositive donor, both organs at risk of transplant-transmitted toxoplasmosis, could trigger neosynthesis of specific IgG in the recipient, a fact that might be suggestive of an immune response against new parasitic antigens brought by an infected transplant. Conversely, we expected that heart transplantation from a *Toxoplasma*-seronegative donor would not favour the appearence of new IgG isotypes.

Patients and methods

Cases history

Case no 1 (Patient 1 W) received a heart transplant from a *Toxoplasma*-seropositive donor. *Toxoplasma* serology of the recipient was positive before transplantation, indicating a previous immunization (Tables 1 & 2). Therefore, the patient did not receive any anti-*Toxoplasma* prophylactic treatment. The immunosuppressive therapy consisted of cyclosporine, prednisolone, and azathioprine. Post-transplantation follow-up was uneventful, apart from a moderate renal failure. There was no evidence of acute rejection. Three months after transplantation, a significant rise in anti-*Toxoplasma* IgG titers was observed (Fig. 1), without specific IgM. There were no clinical or biological signs of acute toxoplasmosis. Routinely performed myocardial biopsies and transvenous myocardial blood sampling were tested for *Toxoplasma* by immunohistopathology and cell culture, and all were negative. The patient underwent no specific anti-*Toxoplasma* treatment, and antibody levels decreased spontaneously within 3 months, then re-increased 3 months later without any clinical manifestation.

Case no 2 (Patient 2M), a 67-year-old man with previous immunity from *Toxoplasma*, received a heart transplant from a *Toxoplasma* seropositive-donor (Tables 1 & 2). He underwent the same immunosuppressive regimen as patient 1W with cyclosporine, prednisolone, and azathioprine. Shortly after undergoing transplantation, the patient became febrile and developed bacterial pneumonia and septicaemia. On the 5th postoperative day, he fell into a coma, stage 1. Three weeks later the fever was still running, despite broad spectrum antibiotherapy. A deterioration of renal function requiring dialysis was also observed. On day 29 post transplantation, a myocardial biopsy revealed the presence of *Toxoplasma* cysts and tachyzoites, with evidence of peripheral necrosis and oedema. Treatment with pyrimethamine and clindamycine was started. At the same time, scanning densitometry showed a cerebral abcess evoking cerebral toxoplasmosis. Parasite DNA was detected in circulating blood by PCR using a previously described technique [21]. Concomitantly, there was a twenty-fold increase in anti-*Toxoplasma* IgG titers (Fig. 1), with appearence of specific IgM and IgA. The patient's condition rapidly deteriorated, and he died two weeks later.

Case no 3 (Patient 3C) was a female bipulmonary- plus liver-transplant patient. The donor was *Toxoplasma*-seronegative. The

recipient had a positive antibody titer before undergoing transplantation and was subjected to prophylactic treatment with trimethoprim-sulfamethoxazole, as commonly prescribed in lung transplant recipients, independently of their serological status (Tables 1 & 2). Like the two other patients, 3C underwent an immunosuppressive therapy with cyclosporine, prednisolone, and azathioprine. One month after transplantation, there was a two-fold increase in anti-*Toxoplasma* IgG titers, which peaked two months later in a seven-fold increase (Fig. 1). Specific IgM remained negative. The rise in IgG titers occured concomitantly to a rejection of the lungs, which required a treatment with corticosteroids and antithymocyte globulin. A bronchofibroscopic examination and a bronchoalveolar lavage were performed and were negative for *Toxoplasma* tachyzoites. Specific treatment with trimethoprim-sulfamethoxazole was maintained during one year. Anti-*Toxoplasma* IgG titers returned to a base level 4 months after transplantation. No systemic complication was observed.

Sera

Sera were collected on day 0, just before transplantation, then about every month. As soon as an acute toxoplasmosis was clinically suspected, the serological follow-up was strengthened, and serum was sampled every week.

Toxoplasma serology

Specific IgG was determined by ELISA (Platelia Toxo IgG, Sanofi-Pasteur Diagnostics, France). Specific IgM was detected by ELISA (Platelia Toxo IgM, Sanofi-Pasteur Diagnostics), and positivity was confirmed by an immunosorbent agglutination technique (Toxo ISAGA, BioMérieux).

Western blotting

Strips were prepared with an antigenic lysate consisting of purified *Toxoplasma* tachyzoites obtained after peritoneal lavage of mice inoculated with the RH strain. Equal amounts of protein lysate were electrophoresed through 12% polyacrylamide gel (Biorad, France), and transferred onto a nitrocellulose membrane (Biorad), as previously described [8]. The membranes were blocked with Tris buffered saline (TBS: 0.05 M Tris, 0.15 M NaCl) containing 2% glycine and 2.5% defatted milk, and were then cut into strips. Strips were incubated with the sera diluted 1:20 in the same buffer for 1 h, and protein recognition by the sera was revealed by incubation for 1 h with a rabbit anti-human immunoglobulin G-alkaline phosphatase conjugate (Biosys, France) diluted 1:800, then with a chromogenic substrate. The reaction was stopped with water. A molecular weight marker (Pharmacia Biotech, France) was simultaneously processed. The patterns obtained with the sequential sera from each patient were compared, and the detection of additional bands was recorded.

Results

The Western blot patterns differed between the patients, regarding the number and the intensity of the bands detected. Despite a dramatic rise in IgG titers, the western-blot patterns obtained by incubation of sera from patient 3C remained stable (Fig. 2). This patient underwent a chemoprophylaxis and received lungs plus liver transplants from a *Toxoplasma*-seronegative donor, and had no clinical sign of reactivated toxoplasmosis. The analysis of Western blot patterns revealed that antibody increase was not accompanied by the synthesis of new IgG isotypes.

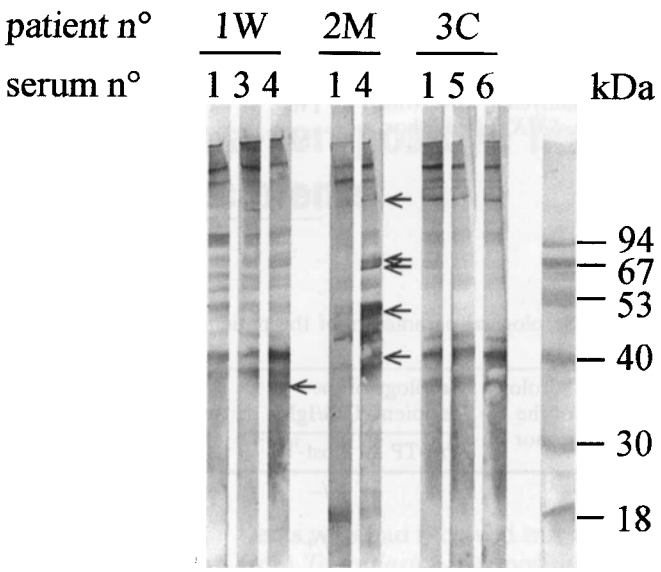

Fig. 2 IgG patterns obtained by western blotting of sequential sera from the three transplant patients. The sera n° tested correspond to the sequential sera, as reported in Fig. 1. The sera named n°1 were sampled shortly before transplantation. Bands corresponding to neosynthesized IgG are indicated with *arrows*

Contrasting with these data, both other patients who received heart transplants from *Toxoplasma*-seropositive donors synthesized new IgG isotypes (Fig. 2). On blots revealed with sera from patient 2 M, several additional bands (105 kDa, 66 kDa, 64 kDa, 53 kDa, and 43 kDa) were detected at the time when antibody titers were increasing, suggesting a neosynthesis of IgG, subsequent to transplantation. Western blot patterns obtained with sera from patient 1 W were very similar apart from one new band of low intensity at 37 kDa that was revealed by the serum sampled 5 months after transplantation.

Discussion

Previous studies have demonstrated the wide clinical spectrum of toxoplasma infection post transplantation as well as the great differences in kinetics of antibody response in the recipients [14]. Clinical disease is frequent in case of toxoplasma mismatches in heart- or lung transplant recipients, particularly in the absence of chemoprophylaxis [7, 24], but is unusual in patients

with anti-*Toxoplasma* antibodies prior to transplantation. However, when a patient is *Toxoplasma*-seropositive before transplantation, it is difficult to determine whether an acute toxoplasmosis is related to an endogenous reactivation or to a reinfection. In this study, we postulated that western blotting might be a tool for differentiating i) an asymptomatic serological reactivation from a serological reactivation associated with the development of an acute toxoplasmosis, and/or ii) the reactivation of a latent infection from a newly acquired infection. A limiting factor could be the numerous hemodialysis and/or blood transfusions to which transplant patients are subjected, and which could interfere with the dosage of antibody. However, the amounts of antibody inferred by blood transfusion are diluted in a large volume and are therefore negligible, compared to the level of antibody already present in the patients' serum.

The three cases we analyzed raise several lines of reflection. Patient 2M, though previously immunized against the parasite, developed an acute toxoplasmic infection, assessed by the detection of circulating parasite DNA by PCR and tachyzoites in the myocardium biopsy. These biological evidences of acute infection were associated with the observation of a cerebral abcess by scanning densitometry, and signs of toxoplasmic myocarditis. Western blot analysis showed that newly synthesized IgG were detected concomitantly to serological reactivation, suggesting that these antibodies could be synthesized in response to new parasitic antigens of a different strain brought by an infected heart transplant.

Patient 1W had a serological reactivation, with appearence of one newly synthesized antibody isotype on IgG blots. This IgG neosynthesis could correspond either to the recognition of a new antigen from a parasite strain present in the transplant, or to a higher synthesis of one isotype already present in the patient's serum before transplantation, but at an undetectable level. This second hypothesis seems less likely, since the IgG titer before transplantation was high, therefore all isotypes should have been detected on blots at this point in time. The absence of clinical signs in this patient could be related to this high antibody level before transplantation, that could have rapidly killed the parasite and prevented its replication. By contrast, patient 3C, who could not encounter a new parasite strain, since the donor was *Toxoplasma*-seronegative, did not synthesize new IgG isotypes, despite a strong serological reactivation.

In summary, these results may suggest that recipients who receive transplants potentially "at risk" for toxoplasmosis, i.e. heart or lung from seropositive donors, could undergo serological reactivation and synthesize new IgG isotypes, possibly in response to the introduction of a new parasite strain, whereas recipients who receive transplants from seronegative donors do not synthesize new IgG isotypes. In the former case, the increase of antibody titers wouls be due to both higher synthesis of isotypes already present in the serum before transplantation and synthesis of new isotypes. In the latter case, serological reactivation would result only in the higher synthesis of old isotypes. This hypothesis could be reinforced by the observation that in several liver or kidney transplant patients, less commonly at risk for transplant-related toxoplasmosis, we did not observe IgG neosynthesis, despite a strong serological reactivation, suggesting that the recipient did not encounter a new parasite strain (personal data). Another hypothesis would be that patients with reactivating cysts could develop new antibody isotypes against new antigens emerging from cyst rupture. However, such isotypes seems far less abundant than old isotypes, since they are unable to modify the anti-*Toxoplasma* IgG avidity which remains at a high and stable level, suggestive of past immunity [17]. Indeed, McHugh et al. [16] were unable to demonstrate a correlation between an immune response against cyst antigens and a clinical reactivation of latent toxoplasmosis,

The frequency of serological toxoplasmic reactivation is low in our experience (3.7%, 3/81 transplant patients at Hôpital Broussais from 1995 to 1997), regarding the high seroprevalence (around 54%) of toxoplasmosis in France [1]. Besides, serological reactivation was previously reported to be rarely accompanied by clinical signs [13]. This low incidence of acute toxoplasmic reactivation may be due to new immunosuppressive protocols that are known to reduce the infectious complications in transplant recipients [10, 19].

Chemoprophylaxis is systematic in France for lung transplant patients, and, for heart transplant patients, in cases of *Toxoplasma* mismatch only. Our study underlines that such a prophylactic treatment should be prescribed to patients receiving a heart transplant from a *Toxoplasma*-seropositive donor, even if they are previously immunized. The prophylactic treatment most commonly used is trimethoprim-sulfamethoxazole [15, 18], but its efficiency was suspected to be inconsistant [22], as already reported in HIV-infected patients [25]. Prophylaxis with pyrimethamine was suggested by others for heart transplant recipients [9, 24].

In conclusion, our study suggests that i) the detection of neosynthesized IgG in the recipient with serological reactivation may be related to the recognition of a new parasite strain brought by the transplant, ii) by western blotting we are unable to differentiate between acute toxoplasmosis and serological reactivation without clinical signs, and iii) previously immunized patients, too, are at risk of organ-transmitted toxoplasmosis and need a careful follow-up.

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