

Rabbit Antithymocyte Globulin Causes *Blastomyces* and *Histoplasma* Antigenemia

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Rabbit antithymocyte globulin (rATG) is known to yield false-positive *Histoplasma* antigenemia. The fourth generation MiraVista *Histoplasma* antigen assay was modified to block this effect (MiraVista Diagnostics, Indianapolis, Indiana). We report a case of rATG-induced false-positive *Blastomyces* and *Histoplasma* antigenemia in a lung transplant recipient despite modifications of these antigen assays.

Key words: antithymocyte globulin; *Blastomyces*; *Histoplasma*; human anti-rabbit antibody; transplant.

Solid organ transplant patients are susceptible to endemic fungal infections [1–3]. The diagnosis may be difficult, because the clinical findings are not specific and can be confused with other infectious and noninfectious conditions. Antigen detection immunoassays are often used for rapid diagnosis of fungal infections in transplant patients [3]. Such immunoassays are sensitive, are specific, and provide results within 1 to 2 days. However, false-positive results attributed to immunologic responses to rabbit antithymocyte globulin (rATG) complicate their use in transplant patients [4–6].

rATG is a polyclonal antibody produced by immunizing rabbits with human T lymphocytes. It is used in the prevention and treatment of allograft rejection in solid organ transplant recipients. Repeated administration of rATG can induce production of human anti-rabbit antibodies (HARA). These antibodies may cause false-positive results for a variety of antigens [7], including *Histoplasma* [4]. HARA binds to the Fc portions of the rabbit anti-*Histoplasma* IgG capture antibody

and biotinylated rabbit IgG detector antibody. The magnitude of the false-positive result correlates with the HARA level in the serum.

The *Histoplasma* antigen assay was modified to remove the Fc fragment from the detector antibody [5]. This modification reduced false-positive results in patients treated with rATG. These specimens were taken from patients who participated in a research study to determine the course of the rATG antibody response [4].

A subsequent study assessed other modifications to reduce false-positive results [6]. False-positive results were suspected in 12 patients who had negative results in urine, but who had positive results in the serum accompanied by high levels of HARA. The serum was treated with ethylenediaminetetraacetic acid (EDTA) at 104°C for 6 minutes to dissociate antigen-antibody complexes and denature HARA. EDTA heat treatment eliminated antigenemia in 8 of the 12 and markedly reduced it in 4. This modification was incorporated into the MiraVista antigen assays in 2007 (MiraVista Diagnostics, Indianapolis, Indiana).

More recently, normal rabbit serum (NRS) was added to the biotinylated detector antibody to further reduce the HARA effect. NRS adsorbs HARA, preventing it from binding to the capture and detector antibodies (MiraVista Diagnostics, unpublished data).

Here we describe *Blastomyces* and *Histoplasma* antigenemia in a lung allograft recipient despite these modifications, and we review an approach to help establish a correct diagnosis.

CASE REPORT

A 55-year-old male underwent a bilateral lung transplant in April 2014 for chronic obstructive pulmonary disease. Following transplantation, he received tacrolimus, mycophenolate, and prednisone along with trimethoprim-sulfamethoxazole and acyclovir for antimicrobial prophylaxis and azithromycin for immunomodulation. He had an uneventful posttransplant course until October 2017, when, during routine pulmonary function testing, his forced expiratory volume in 1 second (FEV1) was decreased to 75% when compared to his best posttransplant FEV1 along with reduction in forced expiratory volume in 1 second/forced vital capacity (FEV1/FVC) ratio to 60%. He was, however, asymptomatic. His chest radiograph was unremarkable, and the bronchoscopy did not show anastomotic complications. Bronchoalveolar lavage (BAL) bacterial, fungal, and mycobacterial cultures were negative, and a transbronchial biopsy showed no evidence of acute cellular rejection. Mycophenolate was switched to azathioprine for immunomodulation, but the allograft dysfunction worsened.

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His worsening lung function was attributed to bronchiolitis obliterans syndrome (BOS). In early January 2018, he received 6 doses of rATG (50 mg on Day 1 and Day 2; and 100 mg on Day 3, Day 4, Day 5, and Day 6) following premedication with methylprednisolone (1mg/kg on Days 1–4 and 0.5 mg/kg on Days 5 and 6) for management of BOS.

Six days after the last dose of rATG, he noted a diffuse body rash, myalgia, arthralgia, and fever of 39.1°C. Hepatomegaly and splenomegaly were absent on examination. His complete blood count showed leukocytosis of 12 700/mm³, hemoglobin of 12.5 g/dL and platelets count of 178 000/mm³. Basic metabolic and hepatic test results were normal. A chest radiograph was negative for pulmonary infiltrates. Vancomycin, cefepime, and oseltamivir were initiated empirically. However, his clinical symptoms were more consistent with rATG-induced serum sickness for which he received a single dose of methylprednisolone 40 mg. The clinical findings improved within 24 hours with resolution of fever and improvement of the rash, myalgia, and arthralgia. Blood cultures and a nasopharyngeal swab for influenza were negative. The antimicrobial treatment was discontinued and the patient was discharged on the fourth hospital day, receiving prednisone 7.5 mg daily and tacrolimus 2 mg twice daily.

At follow-up 1 week later, the patient was asymptomatic. However, the serum submitted during hospitalization was positive for the *Blastomyces* antigen at 0.9 ng/mL (normal range: none detected), but negative for the *Histoplasma* antigen (Table 1). A computed tomographic scan of the chest was unremarkable. Serum 1,3 β-D-glucan and *Aspergillus* galactomannan were negative. The clinical findings and response to corticosteroids were more consistent with serum sickness than fungal infection and the positive *Blastomyces* antigen was suspected to be falsely positive. Antifungal therapy was withheld and an additional evaluation for fungal infection was pursued. Repeat *Blastomyces* antigen testing in the serum was undetectable, but the *Histoplasma* antigen was weakly positive at 0.4 ng/ml (normal range: none detected) (Table 1). Both were negative in the urine.

Table 1. Antigen Results at Baseline and Follow-up

Test	Week 0	Week 2	Week 6
<i>Histoplasma</i> antigen-EDTA ^a , NRS ^c	0	0.4	0
<i>Histoplasma</i> antigen-no EDTA ^b , NRS ^c	3.3	1.3	0.4
<i>Histoplasma</i> antigen-no EDTA ^b , NRS ^c	3.3	QNS	QNS
<i>Histoplasma</i> antigen-no EDTA ^b , no NRS ^d	>19.0	QNS	QNS
<i>Blastomyces</i> antigen-EDTA ^a , NRS ^c	0.9	0	QNS
<i>Blastomyces</i> antigen-no EDTA ^b , NRS ^c	2.6	1.8	0.8

Abbreviations: EDTA, ethylenediaminetetraacetic acid; NRS, normal rabbit serum; QNS, quantity not sufficient.

^aEDTA treatment performed, results as ng/ml.

^bEDTA treatment not performed, results as ng/ml.

^c3% NRS added to anti-*Histoplasma* detector antibody.

^dno NRS added to detector antibody.

The *Histoplasma* antigen was negative in the serum at follow-up at 6 weeks, but there was insufficient serum to repeat the *Blastomyces* antigen test. *Histoplasma* antibody testing by immunodiffusion and complement fixation were negative, but *Blastomyces* antibody testing was not performed.

MiraVista Diagnostics was consulted about the possibility of falsely positive antigen results. Additional studies were conducted to determine if the results were caused by the immunological response to rATG.

METHODS

Histoplasma and *Blastomyces* antigen detection was performed as previously described [8, 9]. Procedures to reduce the effect of HARA included adsorption of the biotinylated anti-*Histoplasma* antibody with normal rabbit serum (NRS) [4] and pretreatment of the serum with EDTA at 104°C [10].

RESULTS

Elimination of EDTA pretreatment caused an increase of the *Histoplasma* antigen from 0 ng/mL to 3.3 ng/mL in the week 0 serum (Table 1). Exclusion of NRS from the biotinylated detector antibody caused a further increase from 3.3 ng/ml in the presence of NRS to ≥19 ng/ml in the absence of NRS. Specimens from week 2 and week 6 were insufficient to evaluate the effect of EDTA pretreatment and NRS.

The elimination of EDTA pretreatment increased the *Blastomyces* antigen from 0.9 to 2.6 ng/mL in the week 0 specimen, and antigenemia persisted for 6 weeks. There was not adequate specimen to evaluate elimination of NRS in the *Blastomyces* antigen assay.

DISCUSSION

Blastomycosis and histoplasmosis in transplant recipients are usually treated with a lipid formulation of amphotericin B for a few weeks followed by triazoles for at least a year. The treatment can be complicated by amphotericin-B-associated adverse effects, including nephrotoxicity and electrolyte abnormalities. Other less serious adverse effects include triazole-related transaminitis, rashes, a prolonged QT interval, and drug interactions. The consequences of an incorrect diagnosis of a fungal infection and treatment with potentially toxic antifungal agents may be serious.

Several factors suggested that the antigen results may have been falsely positive in our patient. The donor did not have evidence of blastomycosis or histoplasmosis. Clinical findings of disseminated histoplasmosis such as colitis, hepatomegaly, splenomegaly, focal skin or mucosal lesions, and central nervous system findings were absent [3]. Our patient's findings were rash and fever. Laboratory findings of disseminated histoplasmosis such as pancytopenia and hepatic laboratory abnormalities were absent [11].

Histoplasma antigenuria, found in 95% of transplant patients with histoplasmosis, was absent [3]. Antibodies to *Histoplasma* were also absent, although the antibody response is impaired in transplant patients [3].

Most patients with disseminated histoplasmosis require at least a few days of therapy to show clinical improvement [12]. Our patient improved within 24 hours of receiving methylprednisolone. Most importantly, our patient has not developed histoplasmosis or blastomycosis after a year of observation. Untreated disseminated histoplasmosis is rapidly fatal in immunocompromised patients [13].

Additional testing was helpful in establishing that the results were likely falsely positive. Demonstration that elimination of pretreatment of the serum with EDTA at 104°C and exclusion of NRS from the biotinylated detector antibody markedly increased the antigen concentration provides strong evidence that the positive result was caused by HARA.

Cross-reactions in other mycoses must also be considered in arriving at the correct diagnosis. Complete cross-reactivity occurs in histoplasmosis and blastomycosis, high-level cross-reactivity in paracoccidioidomycosis and *Talaromyces marneffei* infection [14], and low-level cross-reactivity in coccidioidomycosis [15]. Although histoplasmosis causes false-positive results for *Aspergillus* galactomannan in the serum, the converse has not been reported, except in BAL [16, 17].

CONCLUSION

Despite modification to the MiraVista antigen assay, false-positive results still occur in patients who have received rATG. Physicians are reminded to consider the possibility of false-positive results if clinical, radiographic, and laboratory findings do not support the diagnosis. Consultation with the laboratory performing the test is recommended for additional testing for HARA interference.

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critical review, and interpretation of test results; M.M.D. for interpretation of the test results; and M.E. and J.K. for revision and critical review of the manuscript.

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