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Severe *Toxoplasma gondii* I/III Recombinant-Genotype Encephalitis in a Human Immunodeficiency Virus Patient▽

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The reactivation of an uncommon type I/III recombinant-genotype *Toxoplasma gondii* strain resulted in unusually severe encephalitis and chorioretinitis associated with a cerebral salt wasting syndrome in an African human immunodeficiency virus patient. This observation suggests an influence of the parasite genotype on disease expression in immunocompromised patients.

CASE REPORT

A 50-year-old man had a 15-day history of lethargy, weight loss, and fever. Originating from Ghana, he had been living in France for 5 years. He visited his native country in 2002 for 1 month. At hospital admission, in November 2005, clinical examination revealed fever, splenomegaly, hepatomegaly, oral and genital ulcerations, and bilateral acute chorioretinitis compatible with toxoplasmosis. Results from neurological and pulmonary examinations were unremarkable. Abnormal laboratory findings were as follows: hemoglobin level, 7.9 g/dl (normal range [NR], 11.5 to 15.5 g/dl); leukocyte count, 980/mm^3 (NR, 4,000 to 11,000/mm^3); lymphocyte count, 337/mm^3 (NR, 1,000 to 4,000/mm^3); platelet count, 54,000/mm^3 (NR, 150,000 to 500,000/mm^3); alanine aminotransferase level, 448 IU/liter (NR, 8 to 40 IU/liter); aspartate aminotransferase level, 507 IU/liter (NR, 6 to 53 IU/liter); serum lactate dehydrogenase level, 750 IU/liter (NR, 125 to 250 IU/liter); serum alkaline phosphatase level, 433 IU/liter (NR, 53 to 128 IU/liter); and plasma C-reactive protein level, 14 mg/liter (NR, <5 mg/liter). Human immunodeficiency virus (HIV) serology results were positive. There were 10 CD4$^+$ cells/mm^3 and 273,454 HIV genome copies/ml of plasma. Blood cultures for the detection of bacteria, fungi, mycobacteria, *Leishmania* species, and cytomegalovirus antigenemia and PCR assays for the detection of *Leishmania* and cytomegalovirus in the blood were negative. The Platelia *Aspergillus* enzyme-linked immunosorbent assay galactomannan index was 3.95 (NR, <0.5). One week after hospital admission, the patient developed right-side hemiplegia and confusion. He was transferred into an intensive care unit for mechanical ventilation. Abdominal computed tomography demonstrated splenomegaly and hepatomegaly but no enlarged lymph nodes. Magnetic resonance imaging (MRI) of the brain showed widespread lesions, enhanced with gadolinium, throughout the left occipital, temporal, and frontal lobes with marked surrounding edema and mass effect. Cerebral spinal fluid (CSF) analysis yielded the following results: leukocyte count, 13/mm^3 (80% mononuclear cells); glucose level, 74 mg/dl; and protein level, 241 mg/dl.

Empirical treatment with sulfadiazine (6 g/day), pyrimethamine (100 mg/day), and voriconazole (400 mg/day) in association with a glucocorticoid (methylprednisolone at 1 mg/kg of body weight/day) was started the day after the brain MRI. A brain biopsy was performed 2 days later because of the severe neurological symptoms and the atypical MRI images of the cerebral lesions. Histological examination of the brain biopsy sample showed areas of coagulative necrosis surrounded by chronic inflammation. There were free and May-Grünwald-Giemsa- and periodic acid-Schiff-stained intracellular tachyzoites and cysts filled with ovoid bradyzoites suggestive of *Toxoplasma gondii* (Fig. 1). Real-time PCR targeting a repetitive 529-bp DNA fragment of *T. gondii* (GenBank accession no. AF487550) in both the brain biopsy and CSF samples was positive. The number of *T. gondii* tachyzoites was estimated to be 1,000/ml of CSF. Direct genetic characterization of five microsatellite loci (*TUB2*, *TgM-A*, *W35*, *B17*, and *B18*) of the organisms in the brain biopsy sample was done in a multiplex PCR assay and revealed a mixture of type I and type III alleles (1). The serological tests for *T. gondii* showed high immunoglobulin G levels (858 UI/ml) with no detectable immunoglobulin M. Bacterial, fungal, and viral cultures of CSF were negative. A real-time panfungal PCR assay and mycological cultures of the CSF and brain biopsy samples were negative. Five days after admission into the intensive care unit, the patient had acute hyponatremia (sodium concentration, 130 mmol/liter of plasma) associated with decreased central venous pressure and increased daily urine output (4.52 liters). There was massive natriuresis (114 mmol of sodium/liter) with elevated urine osmolality (414 mmol/liter). Serum osmolality and the blood urea nitrogen level were normal. Cerebral salt wasting syndrome was diagnosed based on intracranial lesions, hyponatremia with polyuria, and natriuresis. After 6 days of water and electrolyte replacement, plasma and urinary electrolyte levels returned to the normal ranges. The cerebral lesions markedly improved as demonstrated by the computed tomography scan. Persistent pancytopenia and cholestasis prompted a bone marrow analysis that showed a hemophagocytic syndrome and macrophages contain-

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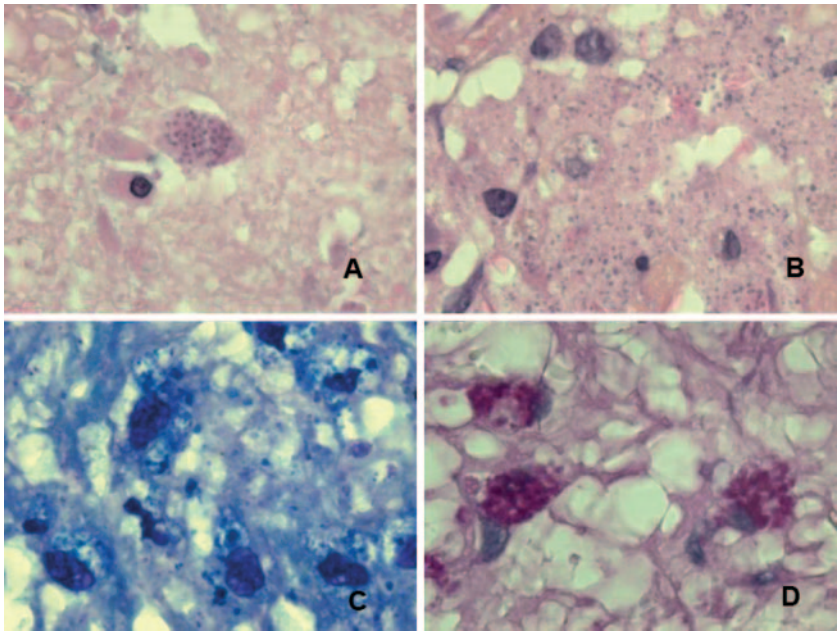

FIG. 1. Histology of the patient's brain biopsy sample. (A) *T. gondii* cyst (stained with hematoxylin and eosin). (B) Extracellular *T. gondii* tachyzoites (stained with hematoxylin and eosin). (C and D) May-Grünwald-Giemsa (C)- and periodic acid-Schiff (D)-stained *T. gondii* tachyzoites within macrophages. Original magnification, ×1,000.

ing intracytoplasmic budding yeasts stained with May-Grünwald-Giemsa stain. These features and the size of the organisms suggested the presence of *Histoplasma capsulatum*. The *Histoplasma* antigen detection test is not available in France and was not done. A real-time panfungal PCR assay of a bone marrow sample was positive. The diagnosis of disseminated histoplasmosis was confirmed 7 days later by bone marrow culture at 25°C on Novy-MacNeal-Nicolle culture medium (intended to grow *Leishmania* sp.) and later on Sabouraud dextrose agar. Tests for the detection of antibody against *Histoplasma* and blood cultures remained negative. Voriconazole (400 mg/day), which was administrated for 2 weeks because of the repetitively elevated Platelia *Aspergillus* indexes, was switched to intravenous liposomal amphotericin B (4 mg/kg/day) for 20 days, followed by oral itraconazole (200 mg/day). After 4 weeks, the patient recovered from *Toxoplasma* encephalitis and disseminated histoplasmosis. Transaminase levels and platelet and leukocyte counts normalized within 4 weeks. The Platelia *Aspergillus* indexes normalized after 2 months. Both antitoxoplasmic and antifungal treatments were sustained. Highly active antiretroviral therapy, including emtricitabine, tenofovir, and efavirenz, was initiated. Despite a persistently low (~14/mm^3) CD4 lymphocyte count, there was no evidence of a relapse of either toxoplasmosis or histoplasmosis. After 5 months of follow-up, the patient died from septic shock complicating *Pseudomonas aeruginosa* pneumonia.

A growing body of evidence suggests that atypical and/or recombinant *T. gondii* genotypes are associated with unusually severe toxoplasmosis at least in immunocompetent patients (5, 6). We report a case of severe encephalitis and chorioretinitis caused by the reactivation of a I/III recombinant-genotype *T. gondii* strain in an immunocompromised African HIV patient. Our patient was born in Ghana and stayed there until 2000. He probably acquired both *T. gondii* and *H. capsulatum* infections in his native country. He presented with atypical, extensive cerebral lesions, a rapidly deteriorating neurological condition necessitating mechanical ventilation, and cerebral salt wasting syndrome that has never before been reported in connection with *T. gondii* encephalitis. His encephalitis was associated with a relatively high density of *T. gondii* tachyzoites in the brain and CSF, as shown by the histological examination of the brain biopsy sample and the quantitative real-time PCR results for the CSF. The hypothesis of a correlation between parasite density, estimated by quantitative real-time PCR analysis of the CSF, and the severity of the encephalitis is plausible and in line with the findings of Romand et al. (9), who reported that an increased risk of severe fetal outcome is associated with a high density of tachyzoites (>100/ml) in the amniotic fluid.

The majority of *T. gondii* strains isolated in Europe and the United States cluster within the three main clonal genotypes: I, II, and III. Several studies have suggested a link between disease severity and *Toxoplasma* strains (8). Type I strains have been associated with high-level virulence in mice (11). They have the ability to cross epithelial barriers rapidly and reach immunoprivileged sites (4). An overrepresentation of type I strains in patients with ocular toxoplasmosis (6) and in immunocompromised patients (8) in some studies with limited sampling has been described previously. Type II strains, avirulent in mice, are the most prevalent in patients with toxoplasmosis and in immunocompromised patients as well as those with congenital toxoplasmosis (2, 7, 8) in Europe and the United States. Type III strains have occasionally been described in association with human toxoplasmosis (2, 7) but are very uncommon, at least in Europe and the United States. The genetic characterization of our patient's isolate yielded an unusual

combination of type I and III alleles, which has been found in other African patients (3). At least in mice, new combinations of alleles can lead to a dramatic increase in virulence (10). Atypical and/or recombinant genotypes are found more frequently among isolates from exotic host species, geographically remote areas, and patients with severe disease (3). These genotypes have been associated with severe toxoplasmosis acquired by immunocompetent patients in French Guiana (5), human ocular toxoplasmosis (6), and severe forms of congenital toxoplasmosis (2).

The unusually severe encephalitis and chorioretinitis associated with the reactivation of a *T. gondii* genotype I/III strain in an immunocompromised patient fuels the hypothesis of strain-related differences in the virulence of *T. gondii* parasites and opens new avenues of research to illuminate this fascinating aspect of host-parasite interactions.

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