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Activated dormant *Cryptococcus gattii* infection in a Dutch tourist who visited Vancouver Island (Canada): a molecular epidemiological approach

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An ongoing outbreak of *Cryptococcus gattii* caused infections, which emerged on Vancouver Island and the Pacific Northwest, has affected more than 200 of the islands' residents, of whom eight died. While *C. gattii* infections are rarely described in travelers, we report a case of cryptococcosis caused by *C. gattii* in a patient treated with high dose corticosteroids for systemic lupus erythematosus induced autoimmune hemolytic anemia. She acquired the disease during a visit to Vancouver Island one year before the onset of the symptoms. This indicates that *C. gattii* may cause a dormant infection that can be activated during treatment with corticosteroids.

Keywords *Cryptococcus gattii*, cryptococcosis, latent infection, travel, molecular epidemiology

Introduction

The primary pathogenic basidiomycetous yeast *Cryptococcus gattii* causes a life-threatening disease of the central nervous system, lungs and skin in humans and animals [1]. The fungus occurs in tropical and sub-tropical regions of South America, Africa, Asia and Australia [2,3]. An ongoing outbreak of *C. gattii* AFLP6/VGII infections emerged in 1999 in the temperate climate of Vancouver Island (British Columbia, Canada). This outbreak affected more than 200 humans and many hundreds of animals [3–6]. Recently, it was shown that *C. gattii* has also colonized the mainland of British Columbia (Canada) and the Pacific Northwest of the United States [3,7–10]. The infection rate of *C. gattii* among citizens and visitors of Vancouver Island is approximately 39 times higher than in Northern Australia, where *C. gattii* is endemic [3,11]. It has been shown that more than 80% of the tested Vancouver Island residents have antibodies against *C. gattii*, as opposed to only 25% positivity among Vancouver Island-based miners tested before the onset of the outbreak [12]. Based on annual *C. gattii* infection rates, Chambers *et al*. [13] suggested that exposure to *C. gattii* may be higher in certain popular tourist destinations on Vancouver Island, such as the surroundings of Nanaimo and Parksville. A number of apparently healthy tourists who visited Vancouver Island for a short period of time became infected with *C. gattii* (14–16; F. Hagen, K. Tintelnot, T. Boekhout, unpublished results). In a recent study MacDougall *et al*. [17], reported that the median incubation time of infections due to *C. gattii* among residents and visitors to Vancouver Island was between 6 and 7 months. However, Lindberg *et al*. [16] published a case report concerning a previously healthy Danish tourist who developed symptoms of cryptococcosis six weeks after he returned from a one week stay on Vancouver Island. This suggested a much shorter incubation time than was previously thought for the outbreak-specific genotype of *C. gattii*.

Here we challenge the generally believed supposition that *C. gattii* is a primary pathogen [18–20] by describing a patient who visited the affected area of Vancouver Island for two weeks and developed symptoms of cryptococcosis one year later after treatment with corticosteroids.

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Detailed molecular typing demonstrated that she was infected by a *C. gattii* isolate of genotype AFLP6/VGII identical to those causing the outbreak.

Case

In August 2007, a 42-year-old woman presented with progressive headache of two weeks duration. Eight months earlier, she was diagnosed with autoimmune haemolytic anaemia as her the first manifestation of systemic lupus erythematosus (SLE), and at that time was treated with prednisolone 60 mg QD PO. However, at the time of admission the dose had been tapered to 17.5 mg QD. On examination, no nuchal rigidity or focal neurological deficit was seen and magnetic resonance imaging (MRI) of the brain was normal. Lumbar puncture showed an elevated opening pressure of 50 cm H_2O and examination of cerebrospinal fluid (CSF) showed predominantly lymphocytic pleocytosis (lymphocytes 77/µl) with normal levels of protein and glucose. Because of the immunocompromised state of the patient, an Indian ink stain was performed which demonstrated the presence of encapsulated yeast cells similar to *Cryptococcus* spp. CSF cryptococcal antigen was positive with a titre of 1:128, and a *Cryptococcus* spp. was isolated in culture from the CSF. Further blood tests on admission revealed severe lymphopenia with a CD4-count of 20/mm^3. A combined HIV-antibody and p24-antigen test was negative. Treatment with intravenous amphotericin B 0.7 mg/kg QD and flucytosine 25 mg/kg QID was started and continued for two weeks, followed by fluconazole 400 mg QD PO for ten weeks. Thereafter, fluconazole was continued, as prophylaxis, at a dose of 200 mg QD PO until 13 January 2009. Because of signs of elevated intracranial pressure, repeated lumbar punctures were performed at two and nine days after admission. On day nine, the cryptococcal antigen titer in the CSF had decreased from 1:128 at admission to 1:4 and cultures were negative. Her headache resolved after the last puncture. To reduce the risk of recurrent cryptococcal infection, prednisolone was replaced by azathioprine 125 mg QD. The patient was in good clinical condition and was discharged from the hospital without neurological sequelae. There was no reoccurrence of the infection during more than two years of follow-up studies.

The *Cryptococcus* isolate (CBS10866) was further genotyped using amplified fragment length polymorphism (AFLP) fingerprint analysis, the mating-type was analyzed by PCR and Multi-Locus Sequence Typing (MLST) of seven unlinked nuclear loci (IGS, *CAP10*, *GPD1*, *LAC1*, *MPD1*, *PLB1*, and *TEF1*α; GenBank accession nos. FJ830387-FJ830393) [2,16,21]. These molecular investigations revealed that this strain belonged to *C. gattii* genotype AFLP6A/VGIIa, and was found to be fully identical to isolates representing the highly virulent genotype that caused the ongoing outbreak on Vancouver Island, Canada.

After the diagnosis of the *C. gattii* infection, the patient was questioned about her travel history. At the age of six, she moved from Australia to the Netherlands and had last visited Australia in 1989. In August 2006, she travelled to British Columbia and Alberta for one month, including two weeks on Vancouver Island where she spent most of the time in and around Victoria. In July 2007, she travelled for two weeks in Greece (Athens and Crete) and shortly after her return to the Netherlands became ill as described above.

Discussion

The case presented here shows that a latent infection caused by a *C. gattii* isolate which was identical to the Vancouver Island outbreak genotype AFLP6A/VGIIa, was activated during the period that the patient underwent a treatment with corticosteroids (prednisolone) in order to suppress the autoimmune hemolytic anemia that she developed five months after her visit to Vancouver Island. A limited number of apparently dormant *C. gattii* infections have been reported in the literature. In 1986, Bottone and colleagues [22] described a previously healthy female patient who travelled to Mexico and California, 5 and 13 years, respectively, before the onset of the *C. gattii* serotype B infection. Dromer *et al*. [23] presented a case of cryptococcosis in a 42-year-old apparently immunocompetent Cambodian male who lived for more than 20 years in France. Approximately one year after he returned from a 3.5-year stay in Gabon, he developed symptoms of tuberculosis which was treated with intensive four-drug chemotherapy. He subsequently developed cryptococcosis caused by *C. gattii* serotype B. However, since molecular techniques for the typing of *C. gattii* isolates had not yet been developed, the conclusion that both patients developed cryptococcosis as a result of the activation of a dormant *C. gattii* infection remains speculative. Recent cases of apparent dormant infections due to *C. gattii* with the Vancouver Island outbreak genotype AFLP6/VGII involved subjects who visited or lived in the region colonized by *C. gattii* for 4 and up to 14 years prior to the appearance of cryptococcosis symptoms [9]. One of these patients had an underlying disease (viz. cancer and kidney failure) prior to the development of cryptococcosis caused by the Vancouver Island outbreak specific genotype of *C. gattii*. However, as both patients lived in areas where *C. gattii* has been previously reported, recently acquired *C. gattii* infections could not be entirely ruled out.

In Europe, autochthonous *C. gattii* infections are known to occur almost exclusively in the Mediterranean area, especially in Greece, Italy and Spain. Several veterinary and clinical cases from this region were described and

involved *C. gattii* infections in goats and humans [24–27]. Using molecular techniques, it was found that these serotype B *C. gattii* strains were genotype AFLP4/VGI (F. Hagen, T. Boekhout, A. Velegraki, M.F. Colom and J.M. Torres-Rodriguez, unpublished data). Two recent clinical cases involving *C. gattii* in Greece were found to be caused by strains with genotype AFLP6/VGII. However, these strains had a different MLST genotype than those causing the ongoing Canadian outbreak ([28] F. Hagen, T. Boekhout, A. Velegraki, unpublished data). The fact that our patient was infected with *C. gattii* AFLP6A/VGII, genetically identical to that of the outbreak, strongly suggests that she was infected during her stay on Vancouver Island, rather than during her holiday in Crete, Greece.

Our observations and the cases discussed above indicate that apparently immunocompetent residents of, and visitors to, British Columbia and the Pacific Northwest may become silently infected with *C. gattii*. Clinicians and health practitioners must be aware of the fact that an immunosuppressive treatment may activate a latent *C. gattii* infection in previously immunocompetent residents and visitors to Vancouver Island and the Pacific Northwest. Since a *C. gattii* infection is easily neglected in non-endemic regions, such as Europe, it is essential to obtain a detailed travel history when diagnostic features of cryptococcosis (e.g., persistent headache with or without fever, lethargy and personality changes) are observed and an immunosuppressive treatment is given or considered.

Given that the vast majority of tested Vancouver Island residents have antibodies against *C. gattii* [12], it is likely that more activated dormant infections due to *C. gattii* AFLP6/VGII may appear in the future. It remains to be seen, however, how many visitors will become infected during their stay on Vancouver Island or the Pacific Northwest. The current number of six infected tourists ([14–16] F. Hagen, K. Tintelnot, T. Boekhout, unpublished results) indicates that such infections due occur and, therefore, clinicians and health practitioners should be alert to the fact that activation as a consequence of a subsequently weakened immune status may occur long after the initial infection.

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Conflict of interest: All authors declare that there is no conflict of interest.

Access to data

All authors had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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