

showed 100% similarity with *B. spielmanii*. *B. garinii* was detected in the heart of 2 animals (from Berlin and Karlsruhe); *B. afzelii* in 3 animals (in the kidney of 2 from Hamburg and Karlsruhe and in the bladder of 1 from Rhineland-Palatinate). A single animal (from Karlsruhe) had *B. afzelii* in the kidney and bladder and *B. garinii* in the heart. Preliminary results have also shown that ticks collected from hedgehogs from the Karlsruhe site were infected with *B. afzelii* (an *I. hexagonus* nymph and an *I. ricinus* female) and with *B. spielmanii* (an *I. ricinus* female, a nymph, and a larva) (Skuballa et al., unpub. data).

These results show, that hedgehogs harbor at least 3 of the 5 recognized *Borrelia* genospecies found in Germany, all of which are known (*B. afzelii*, *B. garinii*) or are strongly suspected (*B. spielmanii*) of being pathogens for humans (9,10). To our knowledge, ours is the first report of *B. spielmanii* from hedgehogs, a *Borrelia* sp. that is usually associated with rodents, especially with garden and hazel dormice (10). That *Borrelia* spp. infections commonly occur in European hedgehogs is likely. However, questions remain about the role of these pathogens in regulating the populations of European hedgehogs and about the status of these common synanthropic mammals as a reservoir host of *B. burgdorferi* s. l. in periurban and rural environments.

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## Invasive Cryptococcosis and Adalimumab Treatment

**To the Editor:** Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) antagonists are immunosuppressants that have shown efficacy in treating inflammatory disorders. However, a recent meta-analysis of controlled trials has shown evidence of increased risk for serious infections in patients with rheumatoid arthritis treated with TNF- $\alpha$  antagonists (1).

Adalimumab is a human monoclonal antibody to TNF- $\alpha$  approved by the US Food and Drug Administration (FDA) for treatment of rheumatoid arthritis. The Spanish registry of adverse events of biologic therapies in rheumatic diseases reported that 1,080 patients were treated with adalimumab from 2003 through 2006 and no cases of cryptococcosis were recorded (2). No cases of cryptococcosis have been detected in 10,050 treated patients in the US postmarketing database for adalimumab (3). We report invasive cryptococcosis in a patient receiving adalimumab. This case underscores the relationship between TNF antagonists and emergence of severe and difficult-to-treat opportunistic infections.

A 69-year-old woman with rheumatoid arthritis diagnosed in 2002 was referred to our hospital for severe acute inflammation of the second finger of the left hand. She had been treated with oral corticosteroids (prednisone, 7.5 mg/day) and several disease-modifying antirheumatic drugs, including chloroquine, methotrexate,

and sulfasalazin, without improvement. One year before the current episode, therapy with adalimumab, 40 mg subcutaneously every 2 weeks for 52 weeks, was started and she showed an acceptable clinical response. She had no recent trauma.

Examination showed severe tenosynovitis of the digital flexor tendon with intense edema and compartmental signs (online Appendix Figure, available from [www.cdc.gov/EID/content/13/6/953-appG.htm](http://www.cdc.gov/EID/content/13/6/953-appG.htm)). She had an axillary temperature of 36.7°C and an admission leukocyte count of 5,900 cells/ $\mu$ L. Results of a neurologic examination and a chest radiograph were normal. Early surgical decompression was performed. Intraoperative findings indicated extensive subcutaneous cellulitis with infiltration of vasculonervous bundles and flexor tendon synovitis. Culture of extracted material from 4 samples, including a biopsy specimen of subcutaneous tissue, identified *Cryptococcus neoformans* susceptible to amphotericin B, azoles, and flucytosine. Results of cerebrospinal fluid analysis were normal. A cranial computed tomographic scan showed no focal lesions. Results of a serum cryptococcal latex test and

HIV serologic analysis were negative. Magnetic resonance imaging of the finger showed inflammation of soft tissues, including the flexor tendon, but no signs of arthritis or osteomyelitis. Treatment with adalimumab was discontinued.

Intravenous liposomal amphotericin B, 300 mg once a day, and intravenous flucytosine, 2.5 g 3 $\times$  a day, were administered for 7 days. Treatment with intravenous fluconazole, 400 mg twice a day for 21 days, was then started. Inflammatory signs decreased. Because residual soft tissue necrosis was extensive, reconstructive surgery was not performed, and her second finger was amputated during the third week after admission. A pathologic examination showed chronic necrotizing granulomatous inflammation with typical encapsulated fungal forms of *Cryptococcus* spp. inside multinucleated giant cells. These forms were observed by staining specimens with hematoxylin and eosin and Mayer mucicarmine (Figure). After an uneventful postoperative period, the patient was discharged and received oral fluconazole, 200 mg once a day for 6 months. Two years later, the patient remains asymptomatic and receives

therapy with methotrexate, salazopyrin, and prednisone.

The rate of serious infections in the US clinical trial safety database of adalimumab as of April 2005 was 5.1/100 patient-years. This rate is similar to that reported in the general population with rheumatoid arthritis. However, as in our case, some infections associated with adalimumab are severe and difficult to treat (3). Cryptococcosis has not been previously associated with use of adalimumab. Cryptococcal infections have been described in 19 patients receiving TNF- $\alpha$  antagonists other than adalimumab (infliximab or etanercept) in the FDA Adverse Event Reporting System from 1998 to 2002 (4). Three cases of cryptococcosis in patients receiving TNF- $\alpha$  antagonists have been reported (5–7).

The association between cryptococcosis and use of TNF- $\alpha$  antagonists can be explained by the immune response to *C. neoformans*, which relies on effective T-cell host defenses and in which TNF- $\alpha$  has an essential role. TNF- $\alpha$  is involved in maintaining a T-helper cell type 1 immune response because it induces production of interleukin-12 (IL-12) and IL-18, with subsequent production of fungicidal interferon- $\gamma$  (8). In animal models, TNF- $\alpha$  blockers are associated with reduced recruitment of inflammatory cells to the area of infection and an increased risk for cryptococcal dissemination (9). Moreover, *C. neoformans* impairs production of TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 and increases levels of IL-10, which induce a T-helper cell type 2 immune response (10). Cryptococcal virulence factors impart greater dependence upon TNF- $\alpha$  for a sufficient host response (9). Adalimumab may increase immunosuppression, which is required for a cryptococcal infection.

Our patient received a low dose of prednisone. Although corticosteroids are a risk factor for cutaneous cryptococcosis, cases with serious outcomes rarely occur. However, the

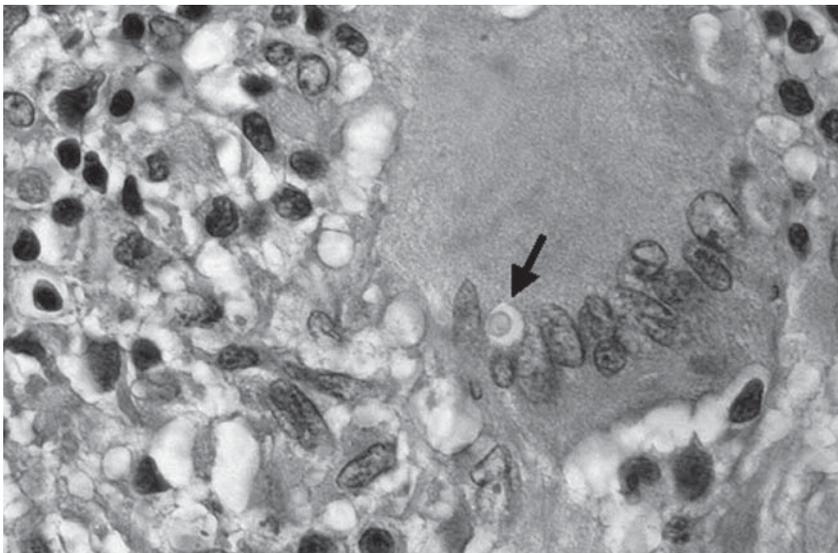


Figure. Histiocytic granuloma with lymphocytes and multinucleated giant cells and an encapsulated intracytoplasmic mucicarmine-positive structure identified as a *Cryptococcus* sp. (arrow) (hematoxylin and eosin- and Mayer mucicarmine-stained, magnification  $\times$ 400).

risk for fungal infection related to low doses of steroids is minimal. Active surveillance, as well as analysis of associated risk factors, is required to detect concurrence of severe opportunistic infections in patients treated with TNF antagonists and to identify patients who could benefit from these therapies with fewer risks.

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## Determining Risk Factors for Infection with Influenza A (H5N1)

**To the Editor:** Novel antigenic subtypes of influenza viruses have been introduced periodically into the human population, resulting in large-scale global outbreaks (1). Highly pathogenic avian influenza (H5N1) viruses reemerged in 2003. Since then, they have reached endemic lev-

els among poultry in several South-east Asian countries, and across Asia, they have caused nearly 300 human infections, with a high rate of mortality (1,2). The results of many studies, including those for one recently conducted by Dinh et al. (3), have been published in an effort to identify the source(s) and modes of transmission of influenza A (H5N1) to humans and to guide the control and prevention of influenza infection.

Although new data regarding influenza A (H5N1) are urgently required, scientific rigor must be maintained during research and analysis to prevent misidentification of exposures as a risk factor for the disease and to prevent creation of iatrogenic panic among the exposed population and the scientific community (4). One point of scientific rigor that must be maintained is the use of adequate statistical analysis. The multivariate model in the study by Dinh et al. (3) was constructed by using a backward, stepwise variable selection strategy, in which variables with  $p < 0.20$  were included in the initial model. However, such a strategy has resulted in a first model and subsequent steps with far more than 10 variables per outcome (e.g., 28 persons with avian flu), resulting in model overfitting (i.e., a statistical model that is too complex for the amount of data), which could result in imprecise estimates or spurious associations (5).

We believe that scientific methods must be meticulously applied when planning, executing, analyzing, and interpreting the results of influenza (H5N1) studies to prevent identification of false risk factors for acquiring infection.

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