Humoral Immune Responses in a Human Case of Glanders

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Within 2 months of acquiring glanders, a patient developed 8-, 16-, and 4-fold increases, respectively, in specific IgA, IgG, and IgM serological titers against *Burkholderia mallei*. Within 14 months of infection, the titers decreased to the baseline. Serum from this patient was also highly reactive against *Burkholderia pseudomallei* whole cells. *Burkholderia mallei* whole cells did not react with sera from patients with other diseases. Therefore, an assay using a *B. mallei* cellular diagnostic antigen may be useful for the serodiagnosis of glanders.

*Burkholderia mallei* is an obligate animal pathogen responsible for the disease known as glanders. Glanders is found primarily in equines, generally causing chronic disease in horses and acute disease in donkeys and mules (8, 17). Although humans are not the natural host, *B. mallei* can cause serious disease in humans and has been classified by the CDC as a category B biotreat agent. This microorganism is particularly infectious by the aerosol route (7), but it can also be acquired orally or through breaks in the skin. There are currently no vaccine countermeasures or sensitive and specific diagnostic tests for this disease. In March 2000, the first case of human glanders in the United States since 1945 occurred in a microbiologist working with the causative microorganism (3, 14). While the actual route of infection was not established, infection most likely occurred via skin contact. The lack of suitable diagnostic assays contributed to a delay in the diagnosis and effective treatment of this infection.

In this single case study, we evaluated whether killed *B. mallei* whole cells can be the basis for a sensitive and specific serological test for glanders in humans. Comparisons with normal human sera and sera from other disease etiologies are qualitative and not based on statistics. The presence of specific antibodies against *B. mallei* was evaluated in an enzyme-linked immunosorbent assay (ELISA). Briefly, *B. mallei* (ATCC 23344) was grown in glycerol (4%) tryptone (Difco) broth culture for 24 h in a shaker incubator at 37°C, killed with 2.1 million rads of gamma irradiation (cobalt 60 source) while frozen on dry ice, washed (three times) with sterile phosphate-buffered saline (PBS), and resuspended in sterile water at a concentration of 1 mg/ml. The *B. mallei* strain used to make the diagnostic antigen was the same as the one that infected the patient. Irradiated whole cells were safety tested for the absence of viability before proceeding. Killed whole cells were added to microtiter plates (1.25 μg/well; Immulon 2 plates), and the assay was conducted similarly to one used to determine the serological response to *Coxiella burnetii* infection (16, 18). We tested for the presence of specific IgA, IgG, and IgM antibodies. Serological titers were the highest dilutions of serum giving a minimum 0.05 optical density unit difference when comparing the test and control (no antigen) wells. ELISA titers were similarly obtained against *Burkholderia pseudomallei* (NCTC 4845) killed whole cells.

Sera described in this study were taken from the patient infected in 2000 (14). After infection with *B. mallei*, patient sera were obtained 64, 88, 122, 169, 245, and 402 days after infection and the antibody responses against *B. mallei* whole cells were determined. Archived sera obtained prior to infection were also available for analysis. Serum collected 64 days after infection had the highest noted IgG and IgA titers, at 32,768 and 512, respectively (Fig. 1). The IgM response peaked 3 months after infection at a titer of 2,048. The maximum titers were 8-, 16-, and 4-fold increases, respectively, over the mean IgA, IgG, and IgM baseline titers. Titers remained at the highest levels at 2 to 4 months after infection and then began to decline. One year after infection, antibody titers were approximately at baseline levels. The patient went on to make a complete recovery from glanders, as evidenced by resolving splenic and liver abscesses and a lack of clinical symptoms of the disease.

In order to confirm that serological titers from the infected patient were indeed elevated compared to titers from healthy, uninfected individuals, we determined anti-*B. mallei* titers using sera from 18 coworkers, most of whom shared the laboratory environment with the patient. All sera were collected within 7 months of patient infection. The mean IgA, IgG, and IgM anti-*B. mallei* titers were 20, 540, and 196, respectively (Fig. 2). Antibody responses in healthy individuals were highly variable, as the ranges of titers were <16 to 64 for IgA, 128 to 1,024 for IgG, and 16 to 2,048 for IgM. The standard deviations were 13, 369, and 465, respectively. The patient titers shown for comparison were from serum collected 88 days after infection. The patient’s IgA and IgG titers were more than 1 log higher than the respective mean titers of sera from healthy individuals.

In order to confirm that killed *B. mallei* ELISA antigens did not nonspecifically react with human disease sera, sera collected from individuals with clinical cases of ehrlichiosis (n = 1), spotted fever (n = 1), murine typhus (n = 1), tularemia (n = 1), chronic Q fever (n = 1), and cutaneous anthrax (n = 1) (15) were tested in an ELISA for reactivity against a *B. mallei* killed cellular antigen. The highest titer (IgG) measured was more than 1 log lower than that found when human glanders serum was assayed (Fig. 3). The patient preinfection titer was similar to titers of patients diagnosed with other diseases.
Although the ELISA did not give comparatively high results when sera from unrelated (i.e., nonglanders) diseases were tested, we assessed whether the assay can be useful in discriminating between glanders and the closely related disease melioidosis.

We observed that human serum collected 64 days after \textit{B. mallei} infection was equally reactive against \textit{B. mallei} antigens (antibody titer of 16,384) and a related whole-cell preparation from \textit{B. pseudomallei} (NCTC 4595) (antibody titer of 16,384). To eliminate the possibility that the anti-\textit{B. pseudomallei} titer was due to previous \textit{B. pseudomallei} exposure rather than serological cross-reactivity, we evaluated pre- and postinfection sera against the \textit{B. mallei} and \textit{B. pseudomallei} diagnostic antigens (Fig. 4). We found that patient preinfection and postinfection titers through day 64 were the same with both antigens, while for later times, the anti-\textit{B. mallei} titers were somewhat higher. Therefore, this assay is not able to differentiate between infections with \textit{B. mallei}, \textit{B. pseudomallei}, and similar microorganisms. In addition, patient titers against the \textit{Burkholderia cepacia} antigen were approximately one-half the titers against the \textit{B. mallei} and \textit{B. pseudomallei} diagnostic antigens (data not shown). Therefore, as with other diseases, potential serological cross-reactivities against closely related microorganisms can present a diagnostic challenge.

Although diagnostic tests have been developed for use in equines (4, 9, 13), no serological tests have been developed to identify glanders in humans. Skin tests using mallein did not become positive in horses until 3 to 4 weeks after infection and therefore may have little value diagnostically (10). The indirect hemagglutination and complement fixation (CF) tests have been used in animals (5, 12), but the CF test, in general, is relatively insensitive and may not detect cases of glanders (16). The indirect hemagglutination assay (IHA) is the most frequently used serological test for human melioidosis (6) and probably would identify glanders cases as well. In melioidosis testing, the failure of the IHA to detect antibody responses despite culture-confirmed disease has been observed (6). Alternate diagnostic methods that detect the presence of specific microorganisms can be useful in the diagnosis of diseases (1, 2), but it is not always possible to obtain clinical specimens containing infectious microorganisms, and obtaining such specimens may involve the use of invasive techniques, such as aspiration and biopsy. Microorganisms are generally very difficult to find, even in acute abscesses (11). Blood cultures are frequently negative until terminal stages of the disease (11).

A large collection of sera obtained from human clinical glanders cases may identify serodiagnostic tests that are statistically valid. Unfortunately, the rarity of this disease in humans makes accumulation of an appropriately sized collection of sera nearly impossible. However, a diagnostic capability for diseases that may be caused by bioterror attacks is critically important, and preliminary assessments of diagnostic assays that may aid in the identification of cases are important. We also were limited in the sera we had available for testing that were collected soon after the presumed infection date. Although serum collected on day 64 was strongly positive, we presume that sera collected earlier in the infection would also have been positive. These preliminary data suggest that an...
ELISA using killed cellular *B. mallei* diagnostic antigens may be useful for the serodiagnosis of human glanders.

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