Chronic Infection and Reactivation in a Pulmonary Challenge Model of Histoplasmosis

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Reactivation may be a mechanism for the development of histoplasmosis in AIDS. In this study, histoplasmosis was reactivated by the depletion of CD4 and CD8 lymphocytes in mice. CD4 and/or CD8 depletion beginning 1 month after intratracheal infection and continuing for 2 months caused reactivation with a 2.1 log/g increase in the lungs and a 1.5 log increase in the spleen of B6C3F1 mice. Because control animals showed persistent infection, a subsequent experiment sought to determine the long-term outcome in competent mice. Twelve of 32 immunocompetent mice died at weeks 26–52 of infection, and 4 survivors appeared to be clinically ill; all ill mice had high fungus burdens, whereas cultures were sterile in the healthy mice. Eight of the surviving healthy-appearing mice underwent autopsy 2 years after infection, and cultures were sterile. Thus, 16 of 32 immunocompetent mice exhibited progressive infection. CD4 and/or CD8 depletion exacerbated infection, but a chronic progressive and ultimately fatal infection occurred in half the immunocompetent mice.

Disseminated histoplasmosis in the immunocompromised host most commonly results from exogenous exposure, but reactivation of latent infection and transmission by an infected allograft may occur [1]. Evidence for reactivation is indirect. Davies et al. [2] attributed histoplasmosis in organ transplant recipients to reactivation because histoplasmosis was not endemic in Minneapolis, but 10% of residents are skin test-positive for histoplasmosis, and outbreaks have been reported near Minneapolis. Demonstration of the South American genotype in Latino immigrants to New York City [3] provides stronger evidence for reactivation, but exposure after visits to the endemic region cannot be excluded.

Experience in Indianapolis suggests that reactivation is rare. If reactivation occurs, the case rate in persons with AIDS in endemic areas should be high, since more than half the residents are infected with Histoplasma capsulatum. But, in fact, only 1%–3% of persons with AIDS residing in endemic areas exhibit histoplasmosis. Cultures of pulmonary or hepatic granulomas rarely grow organisms [4], which suggests that normal defense mechanisms eradicate the infection over time, reducing the risk for reactivation. The purpose of this study was to establish a reactivation model of histoplasmosis.

Methods

Experimental model and fungus burden measurement. The model, preparation of inoculum, T cell depletion, and measurement of fungus burden are described elsewhere [5]. Six-week-old female B6C3F1 mice were infected intratracheally. Clone 2.43, rat anti-mouse Lyt 2.2 (ATCC TIB 210), was used to deplete mice of CD8 lymphocytes, and clone GK1.5, rat anti-mouse L3T4 (ATCC TIB-207), was used to deplete mice of CD4 lymphocytes. Anti-CD4 and anti-CD8 monoclonal antibodies were administered intraperitoneally as 0.1 mL of clarified ascites fluid every 7 days at a concentration that was determined to maintain immunosuppression for ≥2 weeks. Organ homogenates were serially diluted in increments of 1:10, were plated on brain-heart infusion agar containing 10% sheep blood, and were incubated at 30°C for 10 days. Histoplasma antigen was measured by EIA. Data were expressed as units, with >1 U being positive.

Statistics. We performed a 1-way analysis of variance (ANOVA) on the ranks of the antigen levels and quantitative cultures [6]. When the data were significant by the ANOVA, we performed a Student-Newman-Keuls adjustment for multiple comparisons among members of the data group being analyzed. An overall significance level of α = .05 was used to test all hypotheses.

Results

Demonstration that CD4 and/or CD8 depletion increases fungus burden. To establish that CD4 and/or CD8 depletion could be used as a method to induce reactivation, mice infected with 10^4...
**H. capsulatum** yeast were allowed to recover from the primary infection for 1 month and then were randomized to serve as immunocompetent controls \((n = 8)\) or to receive anti-CD4 and/or CD8 monoclonal antibodies once weekly for 2 months \((n = 7)\).

Urine samples were collected from individual mice and were pooled for the entire group for days 0, 7, 14, and 21 before randomization to the depleted or control groups. After randomization, urine samples from depleted and control mice were pooled separately. Elevated concentrations of *Histoplasma* antigen were first detected at day 7, peaked at day 14, and then declined (figure 1). After randomization, antigen concentrations increased in the depleted mice but remained low in the control mice \((P = .031)\).

Mice were killed at day 90 of infection, to determine whether depletion induced reactivation of infection, compared with that in nondepleted control mice. Colony counts were higher in the depleted mice than in the control mice (figure 2). Median colony-forming units per gram of lung in control mice was \(6.3 \times 10^3\) cfu/g, compared with \(7.4 \times 10^4\) cfu/g in depleted animals \((P < .001)\). Median colony-forming units per gram of spleen in control mice was \(2.0 \times 10^3\) cfu/g, compared with \(2.5 \times 10^4\) in depleted mice \((P < .001)\).

**Long-term outcome in immunocompetent mice after infection with 10^2 yeasts.** Demonstration of high colony-forming counts in the lungs and spleen of the nonimmunosuppressed control mice at day 90 of infection was unexpected and prompted a subsequent experiment, to determine the outcome of untreated, lower-inoculum infection. For 1 year, we observed 32 immunocompetent mice that were infected with 10^2 yeasts and did not receive treatment or immunosuppression. By month 12 of infection, 12 (37.5%) of the 32 mice had died. Of the 20 survivors, 16 appeared well, and 4 exhibited clinical findings of chronic infection, namely, weight loss, ruffled fur, and reduced movement.

Antigen concentrations in the urine progressively increased in the 12 mice that died and in the 4 that were ill, but remained undetectable in the 16 that were well (figure 3). At weeks 40 and 52, the concentrations in the ill mice were significantly higher than in the healthy mice \((P < .05\) for both). The 4 mice that appeared ill and an equal number of healthy-appearing mice were killed at month 12 of infection, and fungus burden was measured in the lungs and spleen. The mean fungus burden was high in the 4 ill mice (lung, \(6.0 \times 10^6 \pm 4.3 \times 10^5\) cfu; spleen, \(1.3 \times 10^6 \pm 1.1 \times 10^6 \) cfu) but undetectable in the healthy-appearing mice \((P < .001\) for both organs).

The 12 remaining mice were observed for another year, of which 6 died during that time. Of these, tissue samples were cultured from 2 and were found to be sterile in each. The remaining 6 mice were healthy until death at 24 months of infection, and none had positive cultures. For this experiment, the entire liver, spleen, and lungs were cultured for each animal, to permit detection of a single organism per organ. Thus, of the 32 mice that were followed-up for long-term outcome without treatment or immunosuppression, 12 died, 4 exhibited chronic infection at autopsy, and the 16 exhibited spontaneous resolution of the infection.
Figure 3. Urine antigen concentrations in mice with progressive infection vs. healthy-appearing mice. Specimens from individual mice were tested. Nos. in parentheses are no. of animals tested at that time point. There were fewer animals at week 52 in the progressive infection group because of 12 deaths between weeks 36 and 52. Antigen levels in the progressive infection group were higher than in the healthy-appearing animals at weeks 40 and 52 (*P < .05).

Discussion

Depletion of CD4 and/or CD8 cells exacerbated the infection in this experimental pulmonary-challenge model of histoplasmosis, which supports this approach to investigation of reactivation. In mice infected with 10^3 yeasts, CD4 and/or CD8 depletion once weekly for 2 months increased the fungus burden in the lung by 2.1 logs and in the spleen by 1.5 logs. Of importance, the immunocompetent animals still had high fungus burdens at day 90 after infection, which indicates that they had not cleared the infection. This unexpected observation prompted subsequent studies to determine the long-term course in immunocompetent mice infected with a lower inoculum.

In that experiment, half the nondepleted animals infected with only 10^2 yeasts exhibited a progressive fatal infection when followed beyond 6 months. Urinary antigen concentrations predicted the poor outcome in the chronically infected animals, increasing after the third month of infection. The long-term course of histoplasmosis in mice has not been previously reported. We previously reported reduction in fungus burden by day 35 of infection [7] and assumed that the infection would progressively clear. The findings of the current investigation indicate that innate and acquired immune responses transiently control the infection but that defenses subsequently fail, which permits progressive fatal disease in half of the animals.

Factors that explain the disparate outcome after low-inoculum pulmonary challenge in immunocompetent mice remain to be investigated. In experimental paracoccidioidomycosis, strains of mice exhibiting a greater interferon-γ response experienced a favorable outcome [8]. Studies are planned to compare the immune response in animals that successfully cleared the infection with that in animals experiencing progressive disease and in different strains of mice, including the 2 parental strains for the B6C3F1 hybrids used in this study.

References