Cryptococcus neoformans is one of the most commonly encountered pathogens in AIDS patients (1). Acquired by inhaling basidiospores or desiccated yeast (2), C. neoformans can disseminate from the lung to the central nervous system (CNS), where it causes a life-threatening meningoencephalitis (1). Even with aggressive chemotherapy, patient relapse and mortality rates are high (3). Thus, there is a strong motive to develop a vaccine or immunotherapy for this infection. However, before such immunotherapy can rationally be developed, the mechanisms that function in resistance to this yeast must be understood.

Several components of the host response to C. neoformans have been identified and include T cell–mediated immunity (reviewed in reference 4). Of particular relevance to AIDS are the recent findings of Mody et al. (5), that systemic disease caused by C. neoformans strain 145 is exacerbated in mice treated with an antibody that depletes them of CD4+ T cells. On the other hand, depletion of CD4+ T cells had no effect on the multiplication rate of this yeast at the site of infection in the lung. However, before such immunotherapy can rationally be developed, the mechanisms that function in resistance to this yeast must be understood.

Materials and Methods

Cryptococcus neoformans. Serotype A strain 184 (6) was kindly provided by Dr. Juneann Murphy of the University of Oklahoma Health Sciences Center, Oklahoma City. Organisms in log-phase growth at 37°C were harvested from Sabouraud’s dextrose broth by centrifugation, and washed in PBS. Yeast cells in the inoculum were counted on a hemocytometer and suspended to 10⁶/ml. A sample was plated on Sabouraud’s agar to confirm the concentration of viable organisms.

BALB/c mice obtained from the Trudeau Institute Animal Breeding Facility were infected intratracheally with 10⁶ yeast cells in 100 μl of PBS, as previously described (8).

In Vivo Depletion of T Cell Subsets. The hybridoma GK1.5 (Dr. Frank Fitch, University of Chicago) secreting rat IgG2b anti-L3T4 mAb, and hybridomas 30-H12 and TIB-210 (American Type Culture Collection, Rockville, MD) producing IgG2b anti-Thy-1.2 and anti-Ly-2.2 mAbs, respectively, were grown as ascites in pristane-primed, irradiated BALB/c mice. The rat IgG2b content of ascites was quantitated by ELISA, and the mAbs were stored at -70°C until needed. To deplete T cells, mice were thymectomized at 4–5 wk of age and infused intravenously 1 wk later with 1 mg of the appropriate mAb. One group of control mice received 1 mg of normal, affinity-purified rat IgG (ICN Immunobiologicals, Lisle, IL). Mice were infected with C. neoformans 1 wk after mAb treatment. Recipients of anti-CD4 mAb received an additional 0.5-mg dose of mAb every 10 d (9).

Cytofluorometry. The efficiency of T cell depletions was determined by flow cytofluorometric analysis of cells obtained from the lung by bronchoalveolar lavage. F(ab’)² fragments of the anti-CD4, anti-CD8, and anti-Thy-1.2 mAbs were prepared and conjugated to fluorescein as described by Gutstein and Wofsy (10). A cocktail containing FITC-conjugated goat anti–mouse Ig G, A, and M was obtained from Cappel Laboratories (West Chester, PA).

Cells were incubated with the FITC-labeled reagents,
propidium iodide (to mark dead cells), for 45 min at 4°C, washed, and analyzed with a FACScan® cytofluorometer (Becton Dickinson & Co., Sunnyvale, CA). Data are expressed as the mean number of viable CD4+, CD8+, Thy-1.2+, and Ig+ cells present.

**Enumeration of Viable C. neoformans.** At various times after inoculation, four mice from each experimental group were anesthetized with halothane gas and exsanguinated. The trachea was exposed and intubated, and the lungs were lavaged with 5–10-ml volumes of Ca²⁺- and Mg²⁺-free HBSS supplemented with 3 mM EDTA (7). The cells in the lavage fluids were counted and differential counts were obtained from cytocentrifuge smears stained with Diff-Quik (American Scientific Products, McGaw Park, IL).

The lung, liver, and brain were homogenized in ice-cold PBS in thick-walled glass tubes fitted with Teflon pestles. The homogenates of these tissues were appropriately diluted in PBS, and 100-μl aliquots were placed onto Sabouraud’s agar. After 24–48 h of incubation, colonies of C. neoformans were enumerated.

**Statistics.** The number (log_{10}) of viable C. neoformans (CFU) in the tissues of infected mice are expressed as the mean (±SD) of four or five mice. Sample means were compared with the Student’s t test. Each experiment was performed three times.

**Results**

**Resistance to an Intratracheal Challenge with C. neoformans in Mice Depleted of CD4+ or CD8+ T Cells.** BALB/c mice were thymectomized and then treated with anti-CD4 mAb, anti-CD8 mAb, or both mAbs. 1 wk later, these mice and controls treated with normal rat IgG were infected in the lung with C. neoformans. At 6 wk of the infection, the mice were killed and viable yeast cells in their lungs, livers, and brains were enumerated. Results in Table 1 show that normal BALB/c mice were resistant to the opportunistic 184 strain, having eliminated all but 100 of the yeast cells, with no dissemination of the infection to extrapulmonary organs. Depletion of CD8+ T cells and, to a lesser extent, depletion of CD4+ T cells resulted in a significant increase in the number of yeast cells at the site of infection in the lung. However, only CD4+ T cell–depleted mice had disseminated disease, as evidenced by the presence of 10³–10⁴ viable yeast cells in their brains. Mice depleted of both T cell subsets were not able to eliminate the yeast from the primary site of infection in the lung, or to prevent it from establishing metastatic foci of infection in the brain.

**Kinetics of Growth of C. neoformans in the Lungs of Mice Depleted of CD4+ vs. CD8+ T Cells.** The effects of depleting T cell subsets on the pulmonary phase of the disease was examined by infecting mice intratracheally with 10⁶ C. neoformans, and killing groups of infected mice at 1, 3, and 7 d, and at weekly intervals thereafter. It can be seen in Fig. 1 that elimination of the yeast from the lungs of normal mice began at 3 wk of the infection, and the number of viable yeast cells declined progressively thereafter. Fig. 1 also shows that depleting CD4+ T cells had only a slight but significant (p < 0.05) effect on the ability of the host to clear the yeast from the respiratory tract, as evidenced by a 1-wk delay in the onset of elimination of the yeast. In contrast, mice depleted of CD8+ T cells failed to eradicate the organism, and acquired a chronic pulmonary infection. These and the

**Table 1. Number (log_{10}) of Viable C. neoformans in the Lungs and Extrapulmonary Organs of Normal and T Cell-decient Mice at 6 wk after Intratracheal Inoculation with 10⁶ yeast cells**

| Treatment | Lung     | Liver      | Brain     |
|-----------|----------|------------|-----------|
| Rat IgG   | 2.26 ± 0.56 | <1.48     | <1.48     |
| Anti-CD4 mAb | 3.80 ± 0.26 | <0.001*   | 3.78 ± 0.53 | <0.05 |
| Anti-CD8 mAb | 5.06 ± 0.26 | <0.001   | <1.48     | NS†    |
| Anti-CD4 mAb + anti-CD8 mAb | 7.07 ± 0.45 | <0.001   | 5.31 ± 0.61 | <0.001 |

To deplete T cell subpopulations, groups of female BALB/c mice were thymectomized and treated with anti-CD4 mAb, anti-CD8 mAb, or both mAbs. 1 wk after treatment, mice were inoculated intratracheally with C. neoformans. 6 wk later, the mice were killed and viable yeast in the organs were enumerated by plating samples of homogenized tissue on SDA. Data are the mean (±SD) of four or five mice.

* Data from T cell-depleted mice are compared with rat IgG-treated controls with the Student’s t test, with p < 0.05 considered statistically significant.
† Not significantly different from rat IgG-treated controls.
Changes in the Composition of the Alveolar Exudate during the Expression of Anti-Cryptococcus Immunity in the Respiratory Tract. Microscopic examinations of lavage fluids of normal mice revealed an initial increase in PMN during the first 24 h after inoculation. The numbers of PMN rapidly declined, followed by an increase in macrophages and lymphocytes. As the numbers of yeast cells decreased in the lungs of normal mice, so did the numbers of mononuclear cells. A similar pattern was observed in lavage fluids of mice depleted of either CD4+ or CD8+ T cells.

Fig. 2 shows the changes, against time of infection, in the lymphocyte populations in the lungs of normal and T cell--deficient mice. It can be seen that most of the lymphocytes that accumulated in the alveoli of normal mice were CD4+, with smaller numbers of CD8+ T cells also present. T cells targeted for depletion with mAb were completely absent from the alveolar exudate. Although some Thy-1.2+ cells were found in the lungs of doubly depleted mice, it is almost certain that they were neither CD4+ nor CD8+.

Their surface membrane phenotype was not characterized any further. It is important to note, however, that mice treated with anti-CD4 mAb or anti-CD8 mAb had numbers of Thy-1.2+ cells in their alveolar exudate comparable with that in normal controls. This indicates that there had been an expansion in the undepleted T cell subset in the lung.

Discussion

This paper provides evidence that CD8+ as well as CD4+ T cells are involved in protective immunity against an opportunistic strain of C. neoformans. It shows that CD8+ T cells are important for the protective immune response at the site of infection of the lung, whereas CD4+ T cells appear to be more important for preventing the dissemination of the disease to the brain and other extrapulmonary sites. Thus, the results parallel those published by Mody et al. (5), which showed that in mice infected with a highly virulent strain of C. neoformans, a deficiency in CD4+ T cells increases the risk of cryptococcal meningoencephalitis by allowing the establishment of metastatic foci of infection in the brain.

It is perhaps significant that while CNS infections caused by C. neoformans are a formidable clinical problem, the pneumonia that may precede or accompany meningoencephalitis in AIDS patients is often mild or asymptomatic. One could suggest that their possession of a significant number of CD8+ T cells is the reason why AIDS patients fail to develop progressive pulmonary disease. Our studies in mice clearly show that the generation and the expression of protective CD8+ T cell–mediated anti-Cryptococcus immunity in the lung can occur in the virtual absence of CD4+ T cells.

After recruitment to the lung (13), protective CD8+ T cells, or their precursors, undoubtedly undergo considerable expansion in the respiratory tract, as evidenced by the finding that the number of T cells in the lungs of CD4+ T cell–depleted mice was comparable with the number in infected control mice. It should be noted that expansion of the number of CD8+ (OKT8+) T cells has also been documented in the lungs and peripheral blood in AIDS patients (12, 14, 15). However, because of the generally accepted belief that suppressor T cells can be CD8+, it has been assumed by some that these CD8+ T cells were interfering with a protective immune response (12, 14, 16). Our results in mice illustrate, however, that CD8+ T cells can contribute to, rather than suppress, protective immunity in the CD4+ T cell–depleted host.

How CD8+ T cells function in anti-Cryptococcus immunity and why these cells apparently cannot completely prevent the dissemination of the yeast to extrapulmonary sites remains to be determined. Although it is now well documented that CD8+ T cells can carry out functions previously thought to be performed exclusively by CD4+ T cells (17, 18), it is possible that some yeast cells escape the lung before a therapeutic level of CD8+ T cell–mediated immunity is generated and focused. It is also possible that immune mechanisms mediated by CD8+ T cells are not expressed on a systemic basis and thus may be ineffective against organisms that gain access to the circulation. Nonetheless, our results join an increasing body of evidence that shows that immunity against nonviral infections (19–22), including another opportunistic pathogen, Toxoplasma (23), can depend on CD8+ T cells.
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