Invasive pulmonary aspergillosis is an emerging devastating infection in the immunocompromised host that is treated with corticosteroids for neoplastic disease or for organ transplantation. By use of a model of invasive pulmonary aspergillosis in corticosteroid-treated CF-1 mice, prior infection and 2 \textit{Aspergillus fumigatus} vaccine preparations (sonicate and filtrate) administered intranasally and subcutaneously were tested for efficacy in protecting against subsequent lethal \textit{A. fumigatus} infection. The mortality rates were as follows: control subjects, 100%; prior infection, 12.5%; sonicate administered intranasally, 29%; sonicate given subcutaneously, 0%; filtrate given intranasally, 75%; and filtrate given subcutaneously, 50%. Prior infection and \textit{A. fumigatus} sonicate vaccine administered by 2 routes protected corticosteroid-treated animals against subsequent lethal invasive pulmonary aspergillosis. The sonicate vaccine was more protective, but the subcutaneous route was more effective.

Materials and Methods

**Mice.** Female CF-1 mice were purchased from Charles River Breeding Laboratories and were used at ages 7–8 weeks. \textit{A. fumigatus.} A strain of \textit{A. fumigatus} isolated from a patient with invasive pulmonary aspergillosis at the City of Hope National Medical Center (Duarte, CA) was used for vaccine preparations and infection. Conidia were collected in sterile 0.9% saline containing 0.1% Tween 80 from 5–7-day-cultures on potato dextrose agar plates grown at 37°C. Clumps of conidia were dispersed with 3-mm glass beads, and the suspension was washed twice and suspended to the desired concentration with 0.9% saline containing 0.01% Tween 80. This procedure gave mycelia-free suspensions of conidia with >95% single conidia. Conidia were enumerated with a hemocytometer, and viability was assessed by agar plating.

**Vaccines and vaccination.** Mice were vaccinated twice, 2 weeks apart, 3 weeks before infectious challenge, either intranasally with 30 μL or subcutaneously at 2 sites with 100 μL of the following vaccine preparations: 10^9 viable conidia administered intranasally only (prior infection), 7-day liquid-culture-grown hyphal mass disrupted by 3 freeze-thaw and sonication cycles (sonicate), and filter-sterilized and 200-times concentrated 14-day liquid culture supernatant (filtrate). Intranasal administration was done under light ketamine/xylazine anesthesia.

**Treatments.** Cortisone acetate was administered subcutaneously in 2.5-mg doses for 6 consecutive days prior to challenge [3].
Results

Animals that were previously infected or were vaccinated with sonicate vaccine were significantly protected against fatal invasive pulmonary aspergillosis (table 1). Although there was a trend toward protection in animals vaccinated with filtrate vaccine, significant protection was not demonstrated. The sonicate vaccine was more effective than the filtrate vaccine ($P = .01$), and the subcutaneous route of vaccination appeared to be more effective than the intranasal route, but the difference did not reach significance ($P = .16$).

Discussion

Prior infection and 2 $A. fumigatus$ vaccine preparations conferred protection against lethal challenge and overcame the immunosuppressive effects of corticosteroids. The immunologic mechanism by which this occurs is unknown. One possibility is that the sensitized animal, after rechallenge with $A. fumigatus$ antigen(s), is capable of specifically responding with larger quantities of cytokines (e.g., IFN-$\gamma$ and GM-CSF) that might reverse the antimicrobial defect in macrophages and neutrophils induced by corticosteroids [5, 6].

Recently, Cenci et al. [8] demonstrated that vaccination with an $A. fumigatus$ culture filtrate was capable of inducing protection in a neutrophilic murine model of invasive pulmonary aspergillosis. They showed that protection could be conferred by the adoptive transfer of antigen-specific CD4 T cells producing IFN-$\gamma$ and interleukin-2. More significantly, they observed local recruitment of lymphocytes and macrophages, despite a profound leukopenia. Thus, in both models, the mechanism may be similar. During subsequent challenge, antigen-specific CD4 T cells express increased amounts of cytokines, recruiting more cells to the area of infection and enhancing microbicidal activity of granulocytes and overcoming a systemic neutropenia in one model and overcoming the immunosuppressive effect of corticosteroids in the other.

It is of interest that the subcutaneous route of immunization appears to be superior to the intranasal route, the natural portal of entry for this pathogen. But there is precedence for this phenomenon in the field of vaccination against Chlamydia trachomatis genital tract infection [9].

In conclusion, vaccination with an $A. fumigatus$ antigen preparation and prior infection can confer protection against subsequent lethal invasive aspergillosis in the setting of corticosteroid immunosuppression. In this study, the sonicate vaccine was more protective than the filtrate vaccine, and the subcutaneous route appeared to be more effective than the intranasal route.

References


