Skin Testing with *Mycobacterium avium* Sensitin to Identify Infection with *M. avium* Complex in Patients with Cystic Fibrosis


We sought to determine if patients with cystic fibrosis and sputum cultures positive for *Mycobacterium avium* complex (MAC) have delayed-type hypersensitivity to an *M. avium* sensitin. Seventeen (33%) of 51 selected patients had MAC isolated from at least one sputum culture. Skin tests with purified protein derivative and *M. avium* sensitin demonstrated that five (10%) of 51 patients were anergic, and anergy was correlated with use of systemic steroids. Sixteen (35%) of 46 nonanergic patients had *M. avium*-dominant skin test reactions. Twelve (75%) of these 16 patients with cultures positive for MAC had *M. avium*-dominant skin tests; the specificity of skin testing was 87%. These data suggest that most patients with cystic fibrosis and sputum cultures positive for MAC have infection rather than colonization with MAC. Skin testing with *M. avium* sensitin is a sensitive and specific method for screening these infections.

Recent reports from the United States and Europe confirm that infection with organisms of the *Mycobacterium avium* complex (MAC) occurs in patients with cystic fibrosis [1-4]. Surveys from cystic fibrosis programs in North America show that as many as 14% of these patients have sputum cultures positive for MAC [5, 6]. Although case reports confirm that some patients with cystic fibrosis and sputum cultures positive for MAC have invasive infection and are therefore candidates for antibiotic therapy [7], it is not known whether most of these patients have invasive infection with MAC.

We used optimal sputum culture techniques and intradermal skin testing with an *M. avium* sensitin and purified protein derivative (PPD) to determine whether patients with cystic fibrosis and sputum cultures positive for MAC have delayed-type hypersensitivity (DTH) to MAC as would be expected with invasive infection. Further, we determined the sensitivity and specificity of *M. avium* skin testing for identification of patients with current or prior isolation of MAC from the sputum.

**Methods**

Patients were recruited from cystic fibrosis programs at the Dartmouth-Hitchcock Medical Center (Lebanon, NH) and the University of North Carolina (Chapel Hill, NC). Patient enrollment at Dartmouth was nonselective. Patient enrollment at North Carolina was selective, with preference given to patients with known prior sputum cultures positive for MAC [5]. Respiratory secretions were obtained by expectoration or by bronchoscopy. All specimens were decontaminated by the previously described two-step technique with use of N-acetyl-L-cysteine and sodium hydroxide followed by oxalic acid [8]. Specimens at Dartmouth were inoculated onto Löwenstein-Jensen, Middlebrook 7H10, and selective 7H11 media (BBL, Cockeysville, MD). Specimens at North Carolina were inoculated into BACTEC 7H12B broth (Becton Dickinson Microbiology Systems, Cockeysville, MD) and Löwenstein-Jensen slants (BBL). Specimens were processed by standard methods; acid-fast bacilli were identified by means of DNA probes for MAC (Gen-Probe, San Diego).

Patients were tested with intradermal injections of 0.1 mL of *M. avium* sensitin (*M. avium* sensitin [serovar 2] PPD RS 10/2, 0.1 µg/0.1 mL; filling lots 61 and 62, Statens Seruminstitut, Copenhagen), 0.1 mL of PPD (5 TU, 0.1 µg/0.1 mL; Tubersol, Connaught Laboratories, Ontario, Canada), 0.1 mL of *Candida albicans* skin test antigen (1:100 Dermatophyton O, Miles, Elkhart, IN), and 0.1 mL of mumps skin test antigen (U.S.P., Connaught Laboratories, Swiftwater, PA). Skin tests were read as millimeters of induration at 48-72 hours by one of the authors who was blinded to the identity of the antigen at a particular site. Anergy was defined as ≤3 mm of induration in response to all four skin tests. A skin test was defined as *M. avium*-dominant if there was a minimum reaction of ≥10 mm to *M. avium* sensitin and this reaction was at least 3 mm greater than the reaction to PPD.

**Results**

A total of 51 patients (24 from Dartmouth and 27 from North Carolina) were entered in the study. The age range of the
patients was 9–52 years (mean, 26 years); 30 patients (59%) were female, and none had a history of tuberculosis or BCG immunization. Fifteen patients (two at Dartmouth and 13 at North Carolina) had at least one known culture positive for MAC before study entry. Four of these patients had been treated for MAC infection, including one who died during the course of the study.

Study sputum specimens were collected from all 51 patients; 45 had two specimens collected, and six had one specimen collected. A total of 17 patients (33%) had sputum cultures positive for MAC, including 12 study cultures and five prestudy cultures. Twelve patients with sputum cultures positive for MAC had two study cultures performed; both cultures were positive for three patients, and only one of two cultures was positive for nine patients. The sensitivity of a single sputum culture for detecting MAC in these 12 patients was 63%. A sputum culture positive for MAC was associated with increasing age (P = .01; two-sided unpaired t test).

Skin tests were performed on all 51 patients, and five (10%) were anergic, including the patient who died while receiving treatment for MAC infection. Anergy was present in three (50%) of six patients receiving systemic steroid therapy compared with two (4%) of 45 patients not receiving steroid therapy (x² distribution, 7.81; P = .005). Twenty-three (50%) of 46 nonanergic patients had M. avium skin test reactions of ≥10 mm, including seven patients (15%) with reactions of 5–9 mm and 16 patients (35%) with reactions of ≥10 mm. All seven patients with M. avium skin test reactions of 5–9 mm and all 16 patients with M. avium skin test reactions of ≥10 mm had reactions that were at least 3 mm greater than the corresponding reactions to PPD (i.e., M. avium–dominant skin test reactions).

The results of sputum and skin tests are summarized in Table 1. M. avium–dominant skin test reactions were present in 12 (75%) of 16 patients with at least one culture positive for MAC, including eight (89%) of nine patients with two cultures positive for MAC and four (57%) of seven patients with only one culture positive for MAC. The specificity of an M. avium–dominant skin test for at least one culture positive for MAC was 87%.

Discussion

We have demonstrated that nonanergic patients with cystic fibrosis and current or prior sputum cultures positive for MAC exhibit DTH to an M. avium sensitin. In this respect patients with cystic fibrosis are similar to patients with other forms of underlying chronic lung disease who have invasive MAC infection requiring multiple drug treatment [9]. The development of DTH to M. avium suggests that most patients with cystic fibrosis and cultures positive for MAC have been infected with these organisms rather than merely colonized. This possibility is consistent with limited data implying that morbidity or mortality is increased in patients with cystic fibrosis who are infected with nontuberculous mycobacteria [1, 10].

The present study also demonstrates that dual skin testing with PPD and an M. avium sensitin provides a sensitive method for assessing nonanergic patients with current or past sputum cultures positive for MAC. Although positivity of sputum cultures for MAC may be intermittent, 89% of all patients with M. avium-dominant skin tests had two sputum cultures positive for MAC and had an M. avium–dominant skin test. Because the background rate of M. avium-dominant skin test reactions of ≥10 mm is 8% among healthy populations in the United States [11], most patients with cystic fibrosis will have negative M. avium skin tests before infection with MAC. Subsequent change of the skin test from negative to positive would suggest infection with MAC. In the present study four (13%) of 31 patients with cystic fibrosis and no current or prior sputum culture positive for MAC had M. avium–dominant skin tests. Although this rate is not significantly different from background rates among healthy populations [11], some patients with cystic fibrosis may have had undocumented pulmonary or other focal infections with MAC. Anergy was documented in 10% of the patients in the present study and was associated with use of systemic steroids.

The relative prevalence of pulmonary infection due to MAC in the Dartmouth and North Carolina populations cannot be inferred from the present study because at the North Carolina site mycobacterial cultures were performed more frequently and preference was given to patients with known sputum cultures positive for MAC. Skin test studies conducted >30 years ago in the United States suggested that exposure to or subclinical infection with MAC was more common in the southeastern United States than in other regions of the country [12]. However, to our knowledge, regional differences in rates of symptomatic pulmonary disease due to MAC in the United States have never been proven; they have been inferred only from passive nonuniform state surveillance programs [13].

The present study suggests that M. avium skin testing may have a role in identifying patients with cystic fibrosis who are at risk for having MAC isolated from a sputum culture and in assessing the likelihood that these patients have invasive pulmonary disease due to MAC. Prospective clinical studies employing mycobacterial sputum cultures, M. avium skin test-
ing, pulmonary function studies, and serial CTs are needed to formulate clinical criteria that will be useful in determining the need for specific antimycobacterial therapy for patients with cystic fibrosis and sputum cultures positive for MAC and other nontuberculous mycobacteria.

References


