

called in as a consultant, suspected extrinsic allergic alveolitis, and made a visit to the patient's home. He did not find any humidifiers or air conditioners but he did find 3 gerbils. On two separate subsequent occasions the patient was removed from the home, did very well, was placed back into the home, and 6 to 8 hr later developed all the symptoms previously mentioned.

Dr. Korenblat then placed the asymptomatic patient in the cab of a pick-up truck with the 3 gerbils and asked him to drive around the streets of St. Louis one evening with the windows closed. Seven hours later the doctor received a call from the gasping patient, which established the etiology. Because of this

finding, we did not feel it necessary to include another bronchial challenge. It was a true bronchial challenge we thought, albeit not as elegant as one might do in the laboratory, and we were reluctant to challenge this patient again and make him ill. Immunologic studies include marked wheal and erythema response to gerbil serum and gerbil pelt extract and positive precipitins to gerbil serum and rat serum.

In summary, while thermophilic, fungal, and avian antigens seem to be the most common and important causes of HP or extrinsic allergic alveolitis, it is very important to broaden our scope and consider other possible sources, such as rodents.

Culture and antigen variants of *Aspergillus*

E. J. Bardana, Jr., M.D. Portland, Ore.

Antibodies to the genus *Aspergillus* can be measured by a variety of techniques. The majority of currently utilized methods are qualitative in nature and frequently fail to discriminate between the patient with opportunistic colonization with aspergilli and individuals with clinically active disease. Our objectives in pursuing studies with this fungus were to: (1) isolate and characterize reactive antigens from *Aspergillus* which could be adapted to a quantitative primary binding test; (2) evaluate whether such an assay would have diagnostic and prognostic clinical significance; (3) compare this assay with other serologic techniques; and (4) capitalize on the versatility of the radioimmunoassay technique to explore shared antigenicity between the genus *Aspergillus* and other related and unrelated microorganisms.

Relatively crude and purified antigens were isolated from sonically disrupted mycelia of *A. fumigatus* and referred to as the sonicated supernatant (SS-Fum) and trichloroacetic acid supernatant (TCA-S-Fum) antigens, respectively. Similar antigens were isolated from *A. nidulans* and *A. flavus*. The major radio-labeled ligand of the TCA-S antigen (^{125}I -TCA-S-Fum) was soluble in 10% TCA and in half-saturated ammonium sulfate and, therefore, suited to the ammonium sulfate precipitation test.⁴² Studies of this antigen by differential staining subsequent to analytic nonlinear gradient polyacrylamide electrophoresis showed it to be an immunologically nonprecipitating anionic mucopolysaccharide which was insensitive to

DNase or RNase, slightly sensitive to pepsin, and very sensitive to periodate oxidation and borohydride reduction.

Initial primary binding studies were carried out in hyperimmunized rabbits and significant differences in binding to ^{125}I -TCA-S-Fum were demonstrated with the use of pre- and postimmunization serum samples. Whole mycelial sonicates were employed to immunize rabbits.

The capacity of antibodies in human sera to bind crude and purified radiolabeled components of *Aspergillus* were also studied with the ammonium sulfate test. All individuals studied were found to have some specific antibody to *Aspergillus*. Sera from individuals with aspergillosis bound significantly greater amounts of antigen than did sera from normal subjects as well as sera obtained from patients with nonmycotic infection, nonaspergillus mycotic infection, and autoimmune and allergic disease. Binding was associated with IgG and IgM, was to the F(ab)₂ portion of the immunoglobulin, and was immunologically specific.⁴³

The ammonium sulfate, complement fixation, and immunodiffusion tests were compared for their efficiency in detection of specific *Aspergillus* antibody in sera from 79 patients with established aspergillosis: 41 patients with aspergilloma, 28 with allergic bronchopulmonary aspergillosis (ABPA), 3 with both aspergilloma and the allergic variant, and 7 with invasive or disseminated aspergillosis. Both mycelial

TABLE I. Percent ^{125}I -TCA-S-Fum at 0.02 μg N/ml bound by a 1:5 dilution of test sera employing 7.5% polyethylene glycol, MW, 4000

Group	Category	No.	Range	Mean $\pm$ SD
1	Normal individuals	52	4.4 – 26.4	13.9 $\pm$ 5.4
2	Agammaglobulinemia	17	0.1 – 2.3	0.7 $\pm$ 0.6
3	Autoimmune disease	19	1.3 – 28.8	9.0 $\pm$ 6.4
4	Bronchial asthma	17	6.3 – 23.7	13.2 $\pm$ 5.0
5	Pulmonary tuberculosis	15	5.0 – 33.2	15.4 $\pm$ 8.8
6	Non-mycotic pulmonary infection	10	6.3 – 21.4	11.5 $\pm$ 4.9
7	Cystic fibrosis	15	1.8 – 23.6	14.5 $\pm$ 5.9
8	Non- <i>Aspergillus</i> mycotic pulmonary infection	15	2.4 – 30.4	13.7 $\pm$ 8.9
9	Allergic bronchopulmonary aspergillosis	15	33.9 – 52.2	45.6 $\pm$ 5.0
10	Aspergilloma	14	37.5 – 55.8	46.5 $\pm$ 5.6

TABLE II. Clinical applicability of circulating *Aspergillus* antibody levels in relation to total serum IgE, severity of symptoms, and treatment in a patient with allergic bronchopulmonary aspergillosis

Date	Percent ^{125}I -TCA-S-Fum bound by a 1:5 dilution of serum	Total serum IgE levels (WHO U/ml)	Severity of clinical symptoms	Treatment*
10/25/73	50.0	25,000	Marked	A/A-B
11/26/73	50.7	32,000	Marked	A/A-B
12/7/73	49.5	18,000	Marked	A/A-B
1/8/74	46.5	14,500	Marked	A/A-B
2/20/74	36.8	8,000	Moderate	A/A-B; P 60 QD
4/4/74	32.6	10,000	Moderate	A/A-B; P 30 QD
5/14/74	29.3	11,500	Moderate	P 20 QD
7/1/74	34.6	3,800	Moderate	P 15 QD
8/26/74	32.6	6,200	Mild	P 10 QD
11/16/74	25.5	4,300	Mild	P 30 QOD
2/10/75	19.0	3,700	Mild	P 20 QOD
4/21/75	16.3	2,800	Asymptomatic	P 15 QOD
6/30/75	16.2	2,100	Asymptomatic	P 10 QOD
9/15/75	17.7	1,750	Asymptomatic	P 10 QOD
3/15/76	32.1	5,200	Mild	A/A-B; P 20 QD
5/14/76	41.7	11,000	Moderate	A/A-B; P 15 QOD

*A/A-B, aerosolized amphotericin B, 5 mg in 2 ml sterile water by IPPB qid; P, prednisone and number represents dose in mg at the time; patient was also maintained on anhydrous theophylline, 250 mg qid, and terbutaline sulfate 5 mg tid throughout period of observation.

(SS-Fum, ^{125}I -TCA-S-Fum) and culture filtrate (*Aspergillus*-Fum) antigens were used in testing. Each of the techniques was found to have limitations in the detection of *Aspergillus* antibody. No precise correlation was noted between results achieved with the three test systems. However, the primary binding assay appeared to be the most sensitive test. Their simultaneous employment enhanced demonstration of circulating antibody and facilitated accurate diagnosis, since it appeared that certain patients made antibodies that were better represented in the culture filtrate antigen than in mycelial antigen.⁴⁴

Current work has been directed toward modifica-

tion of the ammonium sulfate test with a goal of increasing both its sensitivity and specificity. It was found that the labeled TCA-S-Fum antigen was adaptable to the polyethylene glycol (PEG) radioassay technique.⁴⁵ Because of the ideal solubility characteristics of this antigen in 7.5% PEG (molecular weight, 4,000) as shown in preliminary antigen-binding capacity studies with hyperimmunized rabbit sera, this method is currently being utilized for all investigations.

With the use of the PEG assay, 187 sera from 10 different patient categories were studied for specific *Aspergillus* antibody. Sera from 52 normal individu-

als had a mean binding of 13.9%, with a range of 4.4 to 26.4%. This was significantly different from the mean binding of 0.7% in 17 sera from patients with agammaglobulinemia, 45.6% in 15 sera from patients with allergic bronchopulmonary aspergillosis, and 46.5% in 14 sera from patients with aspergilloma ($p < 0.001$). The negligible binding in sera from patients with agammaglobulinemia (attributed to regular administration of gammaglobulin) is particularly convincing with respect to specificity of the test system. The mean binding observed in other patient categories was not significantly different from that observed in the normal group. Serum from 5 patients not having documented clinical aspergillosis had binding levels above two standard deviations from that observed in normal individuals.

Experiments were carried out in selected sera from all groups to evaluate the inhibitory effects of a variety of microorganisms on the binding described above. Excess amounts of unlabeled SS-Fum antigen, a similar SS antigen derived from *Candida albicans*, a nonspecific antigen, bovine serum albumin (BSA), and borate buffer alone were added to samples of each test serum prior to the addition of radiolabeled antigen. Binding in all sera tested was totally inhibited by

homologous antigen, partially inhibited by *C. albicans*, and not at all inhibited by BSA or buffer.

Primary binding assays have been criticized as being supersensitive and yielding information which, though immunologically sound, is clinically irrelevant. Serial binding studies were conducted over 3 years in several patients with active pulmonary aspergillosis. Data from a representative 45-yr-old male with allergic bronchopulmonary aspergillosis is shown in Table II. The ability to quantitate specific serum antibody levels assisted in establishing the diagnosis and provided an index to judge the efficacy of medical therapy. In this disease, levels of *Aspergillus* antibody frequently parallel total serum IgE and severity of clinical symptoms. Similar prognostic information was noted following surgery in patients with resectable mycetomal disease.

In summary, all individuals appear to have some circulating antibody to the genus *Aspergillus*. Primary binding tests with the use of a mycelial antigen have been shown to be very sensitive in measuring *Aspergillus* antibodies and demonstrating antigenic similarities and differences between species of *Aspergillus*, other fungi, as well as a variety of microorganisms.

Variability of antigenicity of *Aspergillus fumigatus*

C. Reed, M.D. Madison, Wis.

Clinicians have a number of questions about *Aspergillus* antigenicity that cannot be answered without better knowledge of the biology and chemistry of *Aspergillus fumigatus*. Indeed, we have made little progress in this area since the extensive studies Joan Longbottom carried out more than 14 years ago.

What is the best reagent to use to elicit the dual skin test response that helps in the diagnosis of allergic aspergillosis? Apparently, most of the preparations that are marketed for this purpose are extracts of the mat and spores. They seem to be satisfactory, providing that sufficiently high concentrations of the antigen are employed. Culture filtrates also contain skin test-reactive material, but there are no systematic studies comparing culture filtrate with extracts of the mycelia

or any studies comparing batches of extract in terms of skin test reactivity.

We also know little about the physical or chemical properties of the antigen or antigens that react in the skin, though some clues may be obtained by radioallergosorbent test (RAST) studies performed in the laboratories of Drs. Carl Arbesman, Roy Patterson, and others. Patients with allergic aspergillosis have a very high amount of IgE in the serum, particularly shortly after an acute flareup of the pneumonic infiltrates. With this high IgE level, one might also expect very high RAST values, but such is not the case. In fact, immunoabsorbents prepared with extracts of *Aspergillus* seem to bind only the same magnitude of specific IgE from these sera as do