

The primary interaction of antibody to components of aspergilli

II. Antibodies in sera from normal persons and from patients with aspergillosis

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The capacity of antibodies in 102 human sera to bind components of aspergilli was studied. Sera were from patients with pulmonary aspergillosis or a variety of other clinical disorders and from healthy individuals. The antigens employed were anionic mucopolysaccharide components derived from A. fumigatus and A. nidulans and were not precipitated by antibodies in either human or rabbit sera. The antigens were soluble in 10 per cent trichloroacetic acid and 50 per cent saturated ammonium sulfate, and antigen binding was measured by radioimmunodiffusion and the ammonium sulfate test. Sera from patients with aspergillosis, particularly the mycetomal form, bound significantly greater amounts of the antigens than did the other sera. Binding was associated with IgM and IgG, was to the F(ab)'2 portion of immunoglobulin, and was immunologically specific. Inhibition studies demonstrated the presence of shared antigens between aspergilli and a variety of other fungi, mycobacteria, non-acid-fast bacteria, and commercial house dust concentrates. The minimal but significant binding observed with sera from normal individuals is probably the result of antigenic stimulation by aspergilli as well as other fungi and organisms in the environment.

Fungi of the genus *Aspergillus* may induce a variety of clinical syndromes in human beings, depending upon the clinical status of the patient.¹⁻¹⁰ They can colonize pre-existing pulmonary lesions and often occur as a mycetoma in pulmonary tuberculosis, cystic fibrosis, and bronchiectasis.¹¹⁻¹⁶ An invasive form of aspergillosis is not infrequent in patients with lymphoproliferative disorders or those with altered immune responses.¹⁷⁻²³ In addition, these fungi may be etiologic agents of bronchial asthma, with or without pulmonary eosinophilia.²⁴⁻³⁰ Inhalation of large numbers of spores, e.g., *Aspergillus clavatus*, by malt workers may result in extrinsic allergic alveolitis.³¹ Aspergilli have been implicated as the cause of microangiopathic hemolytic anemia³² and endocarditis on an aortic homograft³³ and as contaminants on mechanical heart valves.³⁴

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Despite an increased awareness of the diverse clinical presentation of these infections, establishing a rapid and secure diagnosis continues to be a problem.^{6, 19, 31-35} For example, only 9 per cent of 98 patients with documented aspergillosis complicating neoplasms had more than a single positive antemortem culture.¹⁹ Furthermore, even when aspergilli are isolated from sputum cultures, a direct relationship to clinical manifestations is frequently not possible, since aspergillus spores may also be inhaled and expectorated by the population at large.^{2, 36, 37} Radiograms are typical in some forms of pulmonary aspergillosis^{3, 6, 12} but cannot be relied upon exclusively for diagnostic purposes.

The principal serologic methods currently employed for the detection of antibodies to aspergilli are: immunodiffusion, immunoelectrophoresis, and complement fixation tests.^{1, 3, 8, 24, 26, 38-42} These tests may fail to detect the primary interaction between antigens and antibodies, and negative tests do not necessarily indicate the absence of antibodies.⁴³⁻⁴⁶ Accordingly, studies were undertaken to measure the binding of components of aspergilli to antibodies in sera from human beings with aspergillosis as well as with other clinical conditions. The antigen employed was isolated from a soluble, sonic fraction of whole aspergillus mycelia as previously described.⁴⁷

MATERIALS AND METHODS

Antigens

Antigens were prepared from a variety of fungi and other microorganisms as described in detail in MATERIALS AND METHODS of the previous paper.⁴⁷ The relatively crude and purified antigens that were derived from *A. fumigatus* and *A. nidulans* are referred to as SS-Fum, TCA-S-Fum, SS-Nid, and TCA-S-Nid, respectively. These antigens were anionic mucopolysaccharides and were soluble in 10 per cent trichloroacetic acid (TCA) and in half-saturated ammonium sulfate (SAS/2). In many of the experiments these antigens were labeled with ¹³¹I or ¹²⁵I and are referred to as ¹³¹I-SS-Fum, ¹³¹I-TCA-S-Fum, etc.^{47, 48}

Sera

Human test sera. One hundred and two serum samples were obtained from selected persons and divided into 7 major categories, as indicated in Table I. None of the sera exhibited hypogammaglobulinemia as judged by quantitation of IgG, IgA, IgM, and, in most cases, IgD by radial immunodiffusion. Some of the sera were from employees and patients at this institution. Other sera, together with corresponding clinical information, were kindly provided by Dr. J. D. Gerber, Center for Disease Control (Ecological Investigations Program), Kansas City, Kan.; Dr. C. E. Reed, University of Wisconsin, Madison, Wis.; Dr. J. Ciszek, National Tuberculosis Research Institute, Warsaw, Poland; and Dr. Leo Kaufman, Center for Disease Control, Atlanta, Ga.

Antisera to human immunoglobulins. These were prepared by injection of goats with purified preparations of IgG, IgA, and IgM. Rabbit antiseretory IgA was kindly provided by Dr. H. Grey of this institution. Antiserum to human IgD was obtained from Melpar, Inc.

Skin testing

An aqueous commercial test mixture derived from aspergilli (Hollister-Stier Labs.) was diluted 1:100, 1:1,000, and 1:10,000 in buffered saline. Each patient in Group VI and those subjects in Group VII who were at this institution were given intradermal injections of 0.02 ml. of the highest antigen dilution. A wheal 5 mm. in diameter was considered a positive reaction. If there was no reaction at 72 hours, the test was repeated with the next

TABLE I. Classification of patient population

Group	Classification	No.	Total No.
I	Normal control subjects		18
	Tuberculin positive	8	
	Tuberculin negative	8	
	BCG vaccinated	2	
II	Autoimmune disease		12
	Systemic lupus erythematosus with nephropathy	4	
	Systemic lupus erythematosus with rheumatoid factor > 1:64	2	
	Rheumatoid arthritis	3	
	Periarteritis nodosa	1	
	Dermatomyositis	1	
	Scleroderma	1	
III	Active pulmonary tuberculosis		12
IV	Active pulmonary infection due to atypical mycobacteria		12
	With <i>M. intracellulare</i>	6	
	With <i>M. kansasii</i>	6	
V	Active mycotic infection		12
	Blastomycosis	2	
	Coccidioidomycosis	2	
	Cryptococcosis	2	
	Histoplasmosis	2	
	Disseminated candidiasis	1	
	Chronic mucocutaneous candidiasis	1	
	Histoplasmosis and active pulmonary tuberculosis	1	
	Coccidioidomycosis and active pulmonary tuberculosis	1	
VI	Bronchial asthma		12
	Type I skin reactivity to aspergillus*	4	
	Type IV skin reactivity to aspergillus*	1	
	Type III skin reactivity to aspergillus*	1	
	No skin reactivity to aspergillus	6	
VII	Pulmonary aspergillosis†		24
	Aspergilloma‡	16	
	Allergic bronchopulmonary aspergillosis	6	
	Aspergilloma‡ with allergic bronchopulmonary aspergillosis	2	

*Aspergillus mixture, Hollister-Stier Labs., Dallas, Texas.

†All patients infected with *A. fumigatus*, the diagnosis being established by radiographic features, skin reactivity to aspergillus antigen, isolation of the organism from the sputum and a positive precipitin and/or complement fixation test.

‡All patients with the mycetomal form of disease either had a history of previous active tuberculosis or were currently under treatment for active tuberculosis.

more concentrated dilution. Test sites were read at 30 minutes and 6, 24, 48, and 72 hours, and the reactions were described as Types I, III and IV according to Coombs and Gell.⁴⁹

Measurement of antibodies

Qualitative tests. Immunodiffusion, immunoelectrophoresis (IEP), indirect radioimmunodiffusion (RID), and indirect radioimmunoelectrophoresis (RIEP) were carried out as previously described.^{47, 50, 51}

Quantitative tests. These were performed with the use of the ammonium sulfate test.^{47, 52, 53} Tests to inhibit ¹²⁵I-TCA-S-Fum antibody reactions by excess amounts of

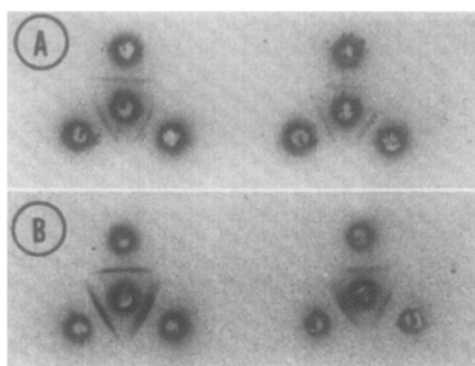


FIG. 1. Results of indirect RID with ^{131}I -SS-Fum and sera from 6 healthy individuals (**A**) and from 6 patients with pulmonary aspergilloma (**B**). Intensity of radioactive bands is greater in **B**.

unlabeled SS-Fum and -Nid antigens were also performed. Potential inhibitory substances were SS preparations of fungi, mycobacteria, non-acid-fast bacteria, staphylococcal protein A, C-reactive protein (CRP), and bovine serum albumin (BSA).

Pepsin digestion of IgG. IgG was isolated from sera of 2 healthy individuals, from a patient with tuberculosis, and from a patient with pulmonary aspergillosis by diethylaminoethyl cellulose (DEAE) column chromatography⁵⁴ by using 0.01M phosphate buffer, pH 7.0. IgG was then subjected to pepsin digestion.⁵⁵ Completeness of digestion was confirmed by immunodiffusion with antisera specific for human Fab and Fc. F(ab)'_2 preparations were tested for their capacity to bind ^{131}I -SS-Fum and ^{131}I -SS-Nid by RIEP. Binding of labeled TCA-S-Fum by 1:5 dilutions of whole serum, by IgG, and by F(ab)'_2 at 2 mg. of protein per milliliter, all derived from a patient with aspergillosis, was also studied.⁵⁶ The capacity of unlabeled SS-Fum and SS-Nid antigens and an unrelated protein, BSA, to inhibit binding in these qualitative and quantitative tests was likewise evaluated.

RESULTS

Measurement of antibodies

Qualitative tests.

IMMUNODIFFUSION TESTS. These were performed on 6 sera selected from each of the 7 categories in Table I. With the use of SS-Fum and SS-Nid as antigens, one or more precipitin bands were developed by every serum tested from Group VII, as well as by some of the sera from Groups I to VI. Distinct precipitin bands were produced by sera from patients with aspergilloma in contrast to the frequently diffuse and halo-type reactions produced by sera from other groups. None of these precipitin bands involved the ^{131}I -labeled components in either SS-Fum or TCA-S-Fum when direct RID was used. This was in accord with the results of experiments using rabbit sera⁴⁷ in which precipitin bands were not associated with antibodies which bound the radioactive test antigens.

INDIRECT RADIOIMMUNODIFFUSION (RID). Six sera were selected from persons in each of the 7 categories in Table I and were studied by the indirect RID test. As summarized in Table II, 41 of the 42 sera tested had antibodies that bound ^{131}I -SS-Fum when the precipitin bands were produced by anti-IgG. Examples of these reactions are illustrated in Fig. 1. Most of the sera from Group I resulted in weakly positive reactions (Fig. 1, A), whereas the majority of reac-

TABLE II. Results of indirect radioimmunodiffusion tests with ^{131}I -SS-Fum antigen

Group	Disease category	No.	Binding of ^{131}I -SS-Fum to	
			IgG	IgM
I	Normal	6	6	2
II	Autoimmune disease	6	5	2
III	Active tuberculosis	6	6	5
IV	Active atypical tuberculosis	6	6	4
V	Active mycotic infection	6	6	4
VI	Asthma	6	6	5
VII	Aspergillosis	6	6	6

TABLE III. Per cent ^{125}I -TCA-S-Fum and ^{125}I -TCA-S-Nid bound by 102 human test sera

Group	Category	No.	% Antigen at 0.01 μg N/ml. bound by 1:5 dilution of test sera			
			^{125}I -TCA-S-Fum		^{125}I -TCA-S-Nid	
			Range	Mean $\pm$ S.D.*	Range	Mean $\pm$ S.D.*
I	Normal	18	15.3-29.8	21.6 $\pm$ 3.9	12.2-24.4	17.9 $\pm$ 3.4
II	Autoimmune disease	12	0.7-26.4	13.7 $\pm$ 7.0	0.7-19.2	13.8 $\pm$ 5.7
III	Active tuberculosis	12	11.8-31.8	23.4 $\pm$ 5.9	17.2-29.7	22.6 $\pm$ 3.4
IV	Active atypical tuberculosis	12	15.0-35.9	23.8 $\pm$ 5.7	7.5-29.6	20.7 $\pm$ 5.8
V	Active mycotic infection	12	1.3-36.6	24.3 $\pm$ 10.1	1.3-31.4	20.3 $\pm$ 8.5
VI	Asthma	12	17.4-34.5	25.0 $\pm$ 8.5	11.8-30.9	20.7 $\pm$ 6.7
VII	Pulmonary aspergillosis	24	20.2-56.8	40.6 $\pm$ 12.0	16.7-57.3	35.6 $\pm$ 12.8

*S.D. = standard deviation.

tions produced by sera from Group VII consisted of prominent radioactive bands (Fig. 1, B). When the goat anti-IgG serum that was used in these experiments was similarly tested for its capacity to bind the ^{131}I -SS antigens, no radioactive bands were noted. Binding to precipitin bands produced by anti-IgM was observed by 28 of the 42 sera tested (56 per cent) and by all 6 sera from Group VII (Table II). Binding to IgA, secretory IgA, and IgD precipitin bands was not detected.

Specificity of these reactions was tested by inhibition studies. For this purpose, an excess of unlabeled SS-Fum and/or BSA was added to the central wells 24 hours prior to the addition of ^{131}I -SS-Fum. All the reactions were inhibited by unlabeled SS-Fum, although some only partially. There was no inhibition by BSA.

Quantitative tests. The per cent of ^{125}I -TCA-S-Fum and ^{125}I -TCA-S-Nid at 0.01 μg N per milliliter that was bound by 0.5 ml. of a 1:5 dilution of each test serum was determined by precipitation of the ^{125}I -antigen-antibody complexes with SAS/2. A summary of the results with 2 labeled antigens is shown in Table III. When ^{125}I -TCA-S-Fum was the test antigen, 18 normal individuals (Group I) bound a mean of 21.6 per cent (± 3.9 per cent). Among 24 individuals with documented aspergillosis (Group VII), 18 had levels of binding greater than 2 standard deviations from the mean of Group I ($p < 0.001$). These 18 sera were from patients with the mycetomal form of aspergillosis and had a mean binding value of 46 per cent (± 8 per cent). Similarly, there were statistically significant differences ($p < 0.001$) between the average values of all

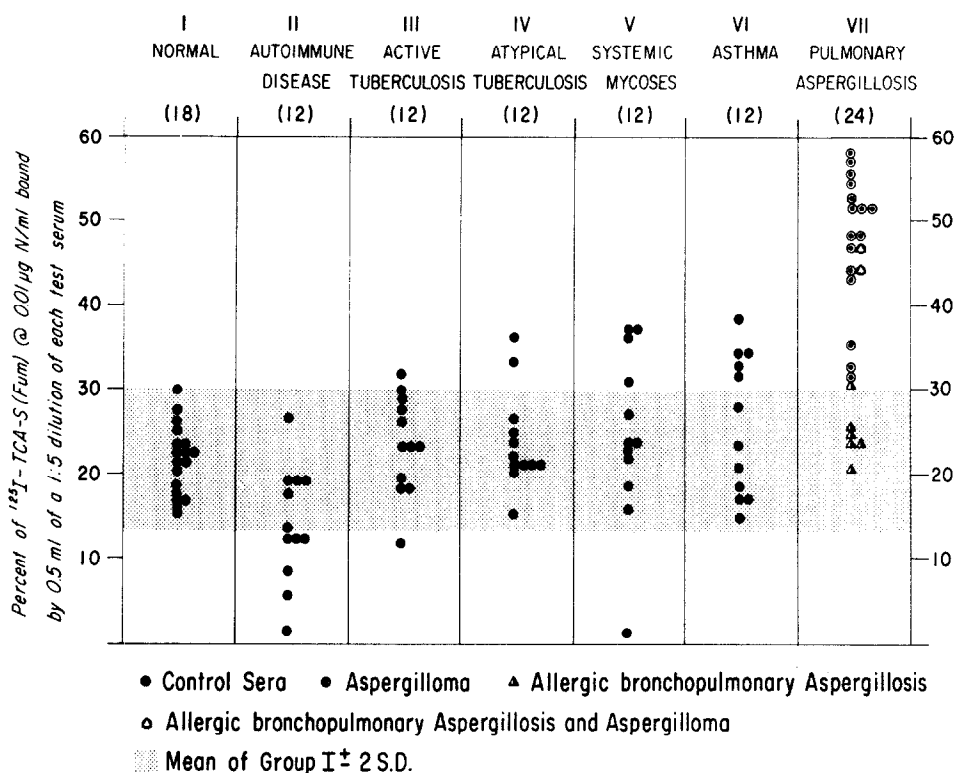


Fig. 2. Graphic presentation of all values showing binding to ^{125}I -TCA-S-Fum at 0.01 μg N per milliliter by 1:5 dilutions of 102 human sera. Shaded area includes the mean values of sera from Group I with 2 standard deviations.

78 sera from Groups I to VI, (21.9 ± 6.8 per cent) and the average value of all 24 sera from Group VII (40.6 ± 12.0 per cent). Individual serum determinations are shown in Fig. 2.

Binding by 12 individual sera from Groups III, IV, V, and VI was more than 2 standard deviations higher than the mean value of Group I. Five of the 12 (in Group VI) were from patients with intermittent reversible obstructive airway disease (asthma). Donors of three of these sera had type I cutaneous reactions to a commercial aspergillus antigen; the 2 remaining patients had received house dust immunotherapy; and one also had severe onychomycosis. When ^{125}I -TCA-S-Nid was the test antigen, relative binding values were similar but slightly lower.

Inhibition studies

Experiments were carried out to study the inhibitory effects of a variety of substances on the binding described above. Twenty-one sera were chosen on the basis of their elevated binding capacities and included 5 sera from Group I and 5 from Group VII. Excess amounts of unlabeled homologous SS antigen, a nonspecific antigen (BSA), and borate buffer only were added to samples of each test serum prior to the addition of ^{125}I -TCA-S-Fum. Some of the data

TABLE IV. Inhibition by unlabeled SS-Fum of binding by sera from normal subjects and patients with aspergilloma

Sera	Per cent ¹²⁵ I-antigen* in precipitates after pretreatment with unlabeled		
	Borate buffer	SS-Fum, 60 µg N/ml.	BSA, 200 µg N/ml.
Normal No. 2	26.0	10.5	25.4
Normal No. 5	23.1	9.5	22.4
Normal No. 15	25.2	9.8	24.9
Normal No. 16	26.9	10.3	27.5
Normal No. 17	32.8	11.0	31.4
Aspergilloma No. 79	47.9	15.5	47.8
Aspergilloma No. 80	49.2	19.4	47.0
Aspergilloma No. 83	43.2	16.7	42.1
Aspergilloma No. 85	38.6	12.8	37.9
Aspergilloma No. 91	46.5	16.3	45.8

*¹²⁵I-TCA-S-Fum at 0.003 µg N per milliliter.

from these determinations that were obtained from 5 sera from Group I and from Group VII are presented in Table IV. All sera were inhibited by the homologous antigen but not by BSA or buffer.

Additional inhibition studies were performed on 6 sera selected because they had elevated binding to ¹²⁵I-TCA-S-Fum. One was from a healthy control subject, one serum each was from Groups IV, V, and VI, and 2 were from individuals with aspergilloma in Group VII. Excess amounts of unlabeled SS-Fum, SS-Nid, and SS preparations of *A. flavus*, *C. albicans*, *T. vulgaris*, *T. rubrum*, *M. kansasii*, *M. tuberculosis*, *L. monocytogenes*, *S. pyogenes*, *S. epidermidis*, house dust concentrate, CRP, and staphylococcal protein A, all of which were prepared as previously described,⁴⁷ were used. The concentrations of the potential inhibitors were 50 µg N for the fungi, house dust, and protein A and 100 µg N for the other SS preparations. CRP contained sufficient C protein to produce a 3+ tube precipitin reaction with specific antisera. Inhibitors in 0.5 ml. volumes were added to 0.5 ml. of 1:5 dilutions of the 6 sera. These results are presented in Fig. 3. Binding by all the sera was markedly reduced by SS antigens derived from the *Aspergillus* species and by house dust. There was substantial but less inhibition of binding by SS antigens of *C. albicans*, *T. rubrum*, *M. kansasii*, and *M. tuberculosis* and a slight degree of inhibition by SS from *L. monocytogenes*, *S. pyogenes*, and *S. epidermidis*. As noted in experiments with hyperimmunized rabbit sera,⁴⁷ there was no inhibition by antigens derived from *T. vulgaris*, staphylococcal protein A, or CRP.

Pepsin digestion of IgG

IgG was isolated from 4 sera that had elevated binding of ¹³¹I-TCA-S-Fum. Two sera were from healthy individuals, one from a patient with tuberculosis, and one from a patient with pulmonary aspergillosis. When F(ab)₂ preparations were made and tested by RIEP, binding of both ¹³¹I-SS-Fum and ¹³¹I-SS-Nid by all the preparations was observed. The reactions were inhibited by unlabeled homologous antigens but not by BSA. Binding by whole serum, IgG, and F(ab)₂ that were derived from the same patient with aspergillosis was

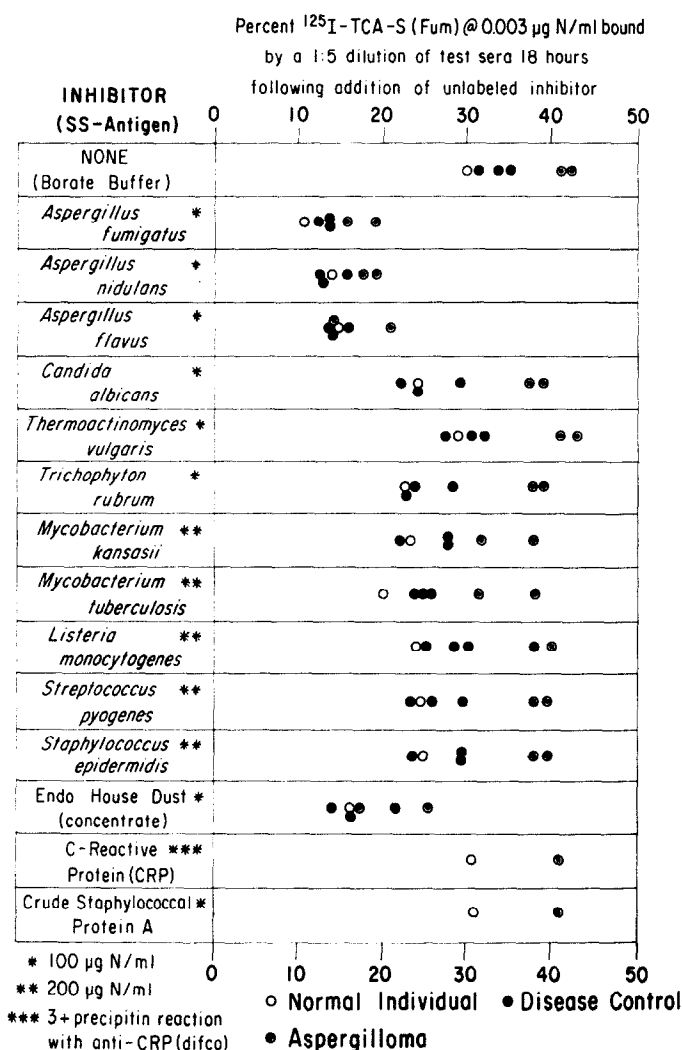


FIG 3. Graphic presentation of the results of inhibition studies. Six selected sera were preincubated with 14 unlabeled SS antigens derived from fungi, mycobacteria, other bacteria, and house dust. Two sera were preincubated with preparations of C-reactive protein and staphylococcal protein A. ^{125}I -TCA-S-Fum at 0.003 μg N per milliliter was then added to all sera.

studied with the ammonium sulfate test. As seen in Table V, there was a comparable amount of binding to the radiolabeled antigen by all three preparations.

DISCUSSION

The results of the experiments described above demonstrate that nearly everyone had some circulating antibody to the labeled antigens that were derived from aspergilli. Sera from 18 patients with the mycetomal form of aspergillosis bound greater than 2 standard deviations above the mean binding observed in sera from a similar number of normal individuals (Fig. 2). Sera from 6 patients

TABLE V. Inhibition by unlabeled SS-Fum of binding by serum and serum components

Serum and serum components	Per cent ¹³¹ I-TCA-S-Fum* in precipitates after pretreatment with unlabeled		
	Borate buffer	SS-Fum	BSA
Whole serum 1:5 dilution	47.9	15.5	47.8
IgG (2 mg. protein/ml.)	45.7	13.8	43.2
F(ab)' ₂ (2 mg. protein/ml.)	33.1	4.3	35.1

*¹³¹I-TCA-S-Fum at 0.002 μ g N per milliliter.

with allergic bronchopulmonary aspergillosis did not show a similar increase in binding. This is consistent with reports of smaller amounts of precipitating antibody in these patients.^{4, 9} The decreased binding by sera from these 6 patients may also be secondary to therapy with corticosteroids.²⁶

Sera from 12 subjects with respiratory diseases other than pulmonary aspergillosis also bound increased amounts of labeled antigen. These sera were from tuberculous and asthmatic patients at this institution as well as from subjects with disseminated mycotic disease from other centers who had negative sputum cultures for aspergilli. It was not possible to rule out subclinical infections with aspergilli not detectable by repeated sputum culture. The frequent association of aspergillosis with tuberculosis and other chronic pulmonary diseases¹³⁻¹⁶ suggests that these elevated values may represent early subclinical infection or inapparent colonization. It is of interest that 3 of 5 sera in Group VI with increased binding also had positive type I cutaneous reactions to an *Aspergillus* antigen. One of the 3 also had severe onychomycosis, and all had been exposed to previous skin testing and/or immunotherapy with house dust. House dust was previously shown to contain components which share antigens with aspergilli.⁴⁷

The relatively low binding values in some sera from patients with autoimmune diseases (Group II) may have been the result of suppressive effects on circulating antibodies by corticosteroids and azathioprine.⁵⁷ This possibility was supported by the low binding observed by serum from a patient in Group V with disseminated candidiasis. This patient was receiving azathioprine and corticosteroids for Goodpasture's syndrome and is represented in Fig. 2 as the single low value in Group V.

Antibody to components of aspergilli was detected in the IgG class of immunoglobulin in 41 of the 42 sera tested. A majority of sera tested also contained antibodies specific for the IgM class of antibody. There was no correlation between IgM or IgG antibodies and active disease (Table II). Although it is known that a variety of disorders involving secretory surfaces manifest increased levels of circulating exocrine IgA,^{58, 59} such antibodies with the capacity to bind *Aspergillus* components were not observed. Binding of labeled antigens by sera from normal persons as well as from patients with aspergillosis was inhibited by homologous antigens and not by unrelated substances such as BSA (Table IV). Labeled antigens also bound to the F(ab)'₂ or antigen combining portion of IgG. These findings strongly suggest that the

binding observed by sera from patients with a variety of disease states, healthy individuals, and patients with aspergillosis represents a true immunologic reaction between antigen and antibody.

The inhibition experiments indicated that some of the binding to *Aspergillus* antigens was a result of exposure to antigenic determinants shared with other microorganisms ubiquitous in the environment. The greatest inhibition of binding by the labeled test antigens was by unlabeled homologous and related *Aspergillus* antigens. Substantial inhibition also occurred with a concentrated commercial preparation of house dust commonly used for skin testing and for immunotherapy of allergic patients. Similar observations were noted using hyperimmunized rabbit sera.⁴⁷ Lesser degrees of inhibition occurred with 2 unrelated fungi (*C. albicans* and *T. rubrum*), mycobacteria (*M. kansasii* and *M. tuberculosis* strain H37Rv), and other bacteria (*L. monocytogenes*, *S. pyogenes*, *S. epidermidis*). Inhibition by the non-acid-fast bacteria was more apparent in sera from subjects in Groups I to VI than in sera from patients with aspergillosis. No inhibition was observed with C-reactive protein, staphylococcal protein A, or *T. vulgaris*. Other laboratories have described shared antigenicity between species of *Aspergillus* and a variety of other fungi.^{2, 4, 14, 26, 41, 42, 60, 61} Our studies suggest in addition that mycobacterial antigens and other bacteria share some antigenic components with aspergilli.

The universal presence of humoral antibodies noted in this study is a reflection of the ubiquity of *Aspergillus* spores in the atmosphere, soil, and other organic material.^{1-3, 36, 37} Its frequent isolation from house dust specimens further substantiates this contention.⁶² It would appear that most, if not all, persons have been functionally immunized to *Aspergillus* species to one degree or another. The antigenic stimulation of antibodies in noninfected individuals may be due to aspergilli as well as other fungal and bacterial components in the environment. It is of interest in this regard to note that antibodies in normal human sera have also been reported to components of mycobacteria,⁶³⁻⁶⁵ *Neisseria meningitidis* and *N. gonorrhoeae*,^{66, 67} *Hemophilus influenzae*,⁶⁸ and enteric gram-negative bacteria.⁶⁹

The experiments in this and the preceding paper indicated that the labeled antigens used in our studies did not precipitate with antibodies to aspergilli.⁴⁷ This is in contrast to results of immunodiffusion studies with the use of antigens prepared by other methods or from culture filtrates.^{1-3, 16, 18, 26, 29, 30, 39-42} It is known that some populations of antibodies may not precipitate with antigen, agglutinate with antigen-coated erythrocytes, or fix complement, and these can best be demonstrated with a primary type of test.⁴³⁻⁴⁶ The primary tests employed to measure the antibodies to components of aspergilli in this study showed significant differences in the amount of binding to our antigens by sera from patients with different disease states. Further studies and experiences with these procedures will be required before the full clinical significance of these antibodies is understood. However, employment of the TCA-S antigen and the ammonium sulfate test has potential diagnostic applications, especially in the mycetomal form of pulmonary aspergillosis.

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