

Specific serum immunopatterns in clinical phases of allergic bronchopulmonary aspergillosis

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Immunoblotting, radioallergosorbent test (RAST), and enzyme-linked immunosorbent assay (ELISA) were performed to determine specific IgE and IgG responses to Aspergillus fumigatus (Af) allergens (IgE-Af; IgG-Af). Serology results were compared in patients with allergic bronchopulmonary aspergillosis (ABPA) (n = 43), patients with Aspergillus fumigatus-associated asthma (Af-asthma) (n = 26), and healthy individuals (n = 3). In patients with different clinical phases of ABPA, three specific immunopatterns were found by immunoblotting. It is proposed to classify ABPA into the active, intermediate, and remission phase with respect to the specific immunoresponse to Af-allergens and asthma symptoms. First, the active phase of ABPA is characterized by a fully developed specific immunoresponse to Af-allergens and severe asthma. Second, the intermediate phase includes patients with elevated specific immunologic findings without asthma symptoms. Third, the remission phase is characterized by a weak specific immunoresponse to Af-allergens after a long-term asymptomatic period. No correlation occurred between specific immunopatterns and irreversible bronchopulmonary lesions. The IgE-Af RAST and IgG-Af ELISA titers of patients with ABPA in the active and intermediate phase were significantly higher compared with patients with ABPA in remission phase and with patients with Af-asthma. In particular, the demonstration of positive IgG-Af ELISA titer generally allows the serologic discrimination of patients with asthma and ABPA from patients with Af-asthma in clinical practice. The present study revealed that immunoblots of most patients with Af-asthma were negative. Immunoblotting demonstrated an IgG reactivity exclusively to low molecular weight (MW) Af-allergens in 8 out of 26 patients with Af-asthma and in the three healthy individuals, and this IgG response may reflect naturally occurring antibodies. (J ALLERGY CLIN IMMUNOL 1992;90:589-99.)

Key words: Allergic bronchopulmonary aspergillosis, allergic asthma, Aspergillus, immunoblotting, major allergens, specific IgE, specific IgG, specific immunopattern

Aspergillus fumigatus (Af) causes a range of pulmonary disorders, including allergic asthma and allergic bronchopulmonary aspergillosis (ABPA), invasion infection in immunocompromised hosts, and

saprophytic colonization in chronic bronchopulmonary disease.¹⁻³ Clinically ABPA manifests as bronchial asthma with transient pulmonary infiltrates that may proceed to proximal bronchiectasis and lung fibrosis.⁴⁻⁷ Laboratory findings include sputum and blood eosinophilia, immediate and late skin reactivity to Af-allergens, elevated total serum IgE level, positive IgE-Af, and IgG-Af and precipitins with Af-allergens.⁷⁻¹³

The diagnosis of ABPA may be difficult because most features are not specific, and as a consequence of the intermittent course, not all criteria are fulfilled at the same time.^{5, 9-13} Furthermore, the pathognomonic central bronchiectasis is only detected in the late stage of ABPA and not seen routinely on chest radiography.^{6, 14} The demonstration of positive precipitins with Af-allergens is of limited value be-

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cause the result may be nonspecific in chronic pulmonary disease or in various forms of allergic asthma.^{2, 3, 15, 16} The measurement of elevated total serum IgE has been an important immunologic finding to confirm the diagnosis of ABPA,⁹⁻¹¹ but many patients with asthma and complicating atopic dermatitis also show high total IgE levels.¹⁷ The determination of IgG-Af allowed the diagnosis of an early stage of ABPA.^{12, 14, 18-21} The individual course of ABPA and clinical manifestations differ largely. Recurrent exacerbations of severe asthma are observed, which alternate with prolonged asymptomatic periods.^{13, 22} Furthermore, chronic progression of asthma occurs.^{7, 13} The clinical course of ABPA does not correspond in each case with the disease activity indicated by serologic and cellular findings,^{5, 23, 24} although total serum IgE antibodies and the blood eosinophil count best reflect the disease activity.^{9-11, 25, 26} Patterson et al.¹³ have identified five stages of ABPA based on clinicolaboratory, therapeutic, and radiologic criteria, beginning with acute stage I.¹³ The remission stage II follows 6 months after discontinuation of successful systemic corticosteroid treatment of acute stage I and exacerbation stage III. The remission stage is free from asthma or characterized by mild asthma that can be controlled by treatment with bronchodilators. The exacerbation stage III has recurrent flares of acute ABPA with all characteristics. The corticosteroid-dependent asthma stage IV includes patients with ABPA who cannot have systemic corticosteroid treatment stopped without the occurrence of severe asthma. The fibrotic stage V is characterized by permanent pulmonary lesions seen on chest radiography, severe asthma, and remaining elevated immunologic values.¹³ In addition, early ABPA stages that have not yet progressed to detectable bronchial or parenchymal lung damage have been defined as seropositive ABPA.¹⁴

The discrimination of patients with ABPA from those with Af-asthma can be difficult because both show a broad spectrum of immediate allergic reactions to various airborne allergens, an immediate skin reactivity to Af-allergens, and positive IgE-Af RAST titer. Immunoblots of patients with ABPA have been described by several authors.²⁷⁻³³ The present study evaluates the specific immunoresponse to Af-allergens by means of RAST, ELISA, and immunoblotting in patients with different stages of ABPA compared with patients with Af-asthma.

PATIENTS IN THE STUDY AND METHODS

Patients with ABPA

Patients with ABPA at the Hochgebirgsklinik Davos-Wolfgang were classified according to clinical, serologic, and radiologic criteria as previously described.⁴⁻¹³ A positive

Abbreviations used

Af:	<i>Aspergillus fumigatus</i>
ABPA:	Allergic bronchopulmonary aspergillosis
Af-asthma:	<i>Aspergillus fumigatus</i> -associated asthma
IgE-Af:	Specific IgE directed against <i>Aspergillus fumigatus</i> allergens
IgG-Af:	Specific IgG directed against <i>Aspergillus fumigatus</i> allergens
PRIST:	Paper radioimmunosorbent test
RAST:	Radioallergosorbent test
ELISA:	Enzyme-linked immunosorbent assay
MW:	Molecular weight
kd:	Kilo dalton

immediate and late skin reactivity to Af-allergens was demonstrated in all patients with ABPA. The specificity of the late skin reaction to intracutaneously applied Af-allergens was controlled by simultaneously testing other molds (see herein) to which there was no late skin reactivity. An immediate allergic hypersensitivity to all tested airborne allergens was demonstrated by prick testing in all patients with ABPA. The sera were collected during suspected disease exacerbations with severe asthma or during routine serologic monitoring without asthma symptoms. The average age of the 43 patients with ABPA whose serum was examined was 45 years (24 to 67 years). ABPA was diagnosed at an average age of 35 years, (21 to 67 years), whereas the average age of first asthma symptoms of all patients with ABPA was 10 years (2 to 32 years). The patients with ABPA were grouped with respect to eosinophil count in blood and total serum IgE level. The cellular and serologic parameters best reflect the disease activity.^{9-11, 25, 26} Furthermore, symptomatic asthma as a cardinal clinical sign was considered for classification of patients with ABPA into active, intermediate, and remission phases (Table I).

Active phase

Sixteen patients were grouped in the active phase of ABPA because of severe asthma, blood eosinophilia over 400/mm³ up to 2550/mm³ ($n = 15$), and elevated total serum IgE levels over 1000 IU/ml up to 35000 IU/ml ($n = 13$). Transient lung shadows on chest radiography were detected in 9 out of 16 symptomatic patients with ABPA (Table I). According to the classification of Patterson et al.¹³ one patient in the acute stage I, two patients in the exacerbation stage III, one patient in the corticosteroid dependent stage IV, and 12 patients in the fibrotic stage V belonged to the active phase of ABPA. The patient in the corticosteroid-dependent stage IV (no. 4, Table I) had no blood eosinophilia. Patients with minimal to extensive fibrotic changes were classified as stage V.

Intermediate phase

Nine patients without asthma symptoms were grouped in the intermediate phase of ABPA. The asymptomatic patients

TABLE I. Clinical and laboratory findings of patients with ABPA

ABPA phase	Subject no.	ABPA stage (13)	Eos/mm ₃	Total IgE PRIST IU/ml	IgE-A/ RAST PRU/ml	IgG-AF ELISA abs
Active phase of ABPA	1	I	2550	>1000	63.50	1.540
	2*	III	1068	1920	n.d.	0.461
	3	III	1070	>1000	67.59	0.267
	4	IV	310	5260	58.95	0.758
	5*	V	2360	5960	20.31	0.732
	6*	V	1770	590	44.08	1.234
	7	V	1314	6725	76.18	1.260
	8*	V	900	9450	n.d.	0.655
	9*	V	2320	4145	62.40	0.216
	10*	V	950	11025	80.66	1.498
	11*	V	1098	35000	23.40	1.570
	12*	V	2020	6880	27.89	1.475
	13	V	710	2167	18.78	1.024
	14	V	990	586	6.02	1.012
	15	V	790	600	5.95	1.170
	16*	V	1320	3085	56.98	0.314
Intermediate phase of ABPA	17	V	360	2562	54.82	0.959
	18	V	340	4193	61.69	1.139
	19	V	960	1335	21.43	0.207
	20	V	276	940	9.08	0.432
	21	V	580	639	6.94	0.634
	22	V	600	1330	8.83	0.890
	23	V	440	1490	7.40	0.906
	24	V	290	1580	9.30	1.246
	25	V	560	617	8.75	1.061
Remission phase of ABPA	26	II	160	354	6.03	0.112
	27	II	310	594	3.18	0.112
	28	II	410	625	5.85	0.236
	29	V	10	307	4.59	0.068
	30	V	290	158	3.24	0.564
	31	V	250	192	2.79	0.563
	32	V	90	31	1.59	0.268
	33	V	48	115	n.d.	0.258
	34	V	300	131	3.11	0.365
	35	V	280	35	0.72	0.630
	36	V	n.d.	333	8.65	0.161
	37	V	300	179	0.75	0.332
Remission phase of ABPA with symptoms of other allergic disease	38†	II	820	180	3.70	0.305
	39‡	II	384	1775	n.d.	0.227
	40‡	V	240	1560	2.13	0.180
	41‡	V	730	755	9.44	0.278
	42‡	V	220	12150	10.99	0.184
	43‡	V	440	5170	9.81	0.244

ABPA stage, Defined by Patterson¹³; EOS/mm³, eosinophil count in blood; IU/ml, international units per ml; PRU/ml, Phadebas RAST units per ml; abs, absorbance values at 405 nm; nd, not done.

*Transient lung shadows.

†Acute seasonal rhinitis.

‡Active atopic dermatitis.

showed evidence of disease activity, indicated by blood eosinophilia over 400 mm³ (*n* = 5) and elevated total serum IgE levels over 1000 IU/ml (*n* = 6). None of the patients had any other allergic disease that could explain the elevated eosinophil count in blood or high total serum IgE levels. Two nonasthmatic patients (nos. 17 and 25; Table I) were still treated with high-dose systemic corticosteroids after an exacerbation of ABPA with severe asthma less than 3 months

ago. Chest radiography demonstrated persistent pulmonary lesions in all nine patients (stage V, 13).

Remission

Eighteen patients without asthma symptoms were grouped in the remission phase of ABPA. Twelve asymptomatic patients showed normal eosinophil counts and total serum IgE levels below 500 IU/ml (Table I). Six out of 18 nonasthmatic patients with ABPA had symptoms of other allergic diseases: five patients had currently active atopic dermatitis that was associated with elevated total IgE levels over 1000 IU/ml ($n = 4$); one patient had acute seasonal rhinitis that was associated with blood eosinophilia over 400/mm³ (no. 38; Table I). Chest radiography demonstrated minimal-to-extensive fibrotic changes in 13 out of 18 nonasthmatic patients.

Patients with Af-asthma

Twenty-six patients were characterized as having Af-asthma, on the basis of positive immediate skin reactivity to Af-allergens and positive IgE-Af RAST titer. The sera of all patients were collected during current asthma symptoms. All patients were examined with prick and intracutaneous testing (see herein), and positive immediate skin reactivity to airborne allergens was demonstrated in all patients. No late skin reactivity to Af-allergens or other molds was observed. Thirteen out of 26 patients with Af-asthma had symptoms of active atopic dermatitis that was associated with blood eosinophilia over 400/mm³ and elevated total serum IgE levels over 1000 IU/ml to 9880 IU/ml.

Healthy individuals

Three sera of healthy individuals without evidence of sensitization to various allergens were examined. The total serum IgE level of these patients was below 150 IU/ml.

Skin tests

Prick testing was performed with extracts of tree and grass pollen, house dust, and molds (Bencard; Smithkline Beecham, Neuss, Germany) with standard technique.³⁴ Intracutaneous testing was carried out with purified mold extracts (Bencard; Beecham): *Alternaria* (M1), *Aspergillus* group (M2), *Cladosporium* (M3), *Neurospora* (M4), *Mucor* (M5), *Penicillium* (M6), M10, M11, A13. The different mold allergens were tested simultaneously in all patients with ABPA and Af-asthma.

Eosinophils

Total eosinophil counts were accurately measured with an automated blood analyzer (Hematology-system H*1; Technicon Instruments Corp., Tarrytown, N.Y.).

Total IgE

Radioimmunoassay to determine total serum IgE levels was done with use of Phadebas PRIST (Pharmacia Diagnostics AB, Uppsala, Sweden); The cellulose disks coated with anti-IgE were incubated with 1:10, 1:100, and 1:1000 diluted sera in Phadebas buffer. The mean counts per minute for all three diluted sera were calculated. The international

units (IU/ml) were determined by regression analysis with standard sera (Pharmacia).

IgE-Af

RAST was used to determine the quantitative IgE-Af titer of Phadebas RAST (Pharmacia); the cellulose disks coated with Af-antigen were incubated with 50 mm³ of undiluted serum in Phadebas buffer. Sera were also tested in a 1:5 dilution because individual sera can reach the maximum levels of the assay if tested diluted. All sera were simultaneously tested. The counts per minute were measured on a gamma counter (LKB, Pharmacia). The counts per minute were log transferred before statistical analysis. The Phadebas RAST units were determined by regression analysis with reference sera (Phadebas).

IgG-Af

Quantitative IgG-Af titer was measured by one-layered antibody ELISA according to Kauffman et al.²⁰ and adapted by van der Heide as described below. The batch of Af culture filtrate (no. 0074; a 32-day culture filtrate provided by the laboratory of Allergology of Groningen)³⁵ was diluted to 1 mg/ml in coating buffer (0.1 mol/L NaHCO₃, pH 9.6). Wells of microtiter plates (Maxisorp F96; Nunc Laboratories, Wiesbaden, Germany) were coated with 120 mm³ Af-antigen and incubated overnight at 4°C. After washing three times with each 250 mm³ phosphate-buffered saline containing 0.05% Tween 20 and 0.2% gelatin in the Dynatech (Dynatech Laboratories, Inc., Chantilly, Va.) washing equipment, the wells of immunoplates were incubated with 250 mm³ 1:1000 diluted sera for 1 hour at 37°C (sera dilution in phosphate-buffered saline containing 2% bovine serum albumin, 0.05% Tween 20 and 0.2% gelatin). After washing three times with phosphate-buffered saline, the wells were incubated with 1:1000 diluted alkaline phosphatase antihuman IgG (Dako Corp., Carpinteria, Calif.) in incubation buffer for 1 hour at 37°C. After washing four times with phosphate-buffered saline, incubation was performed with substrate (250 mm³ p-nitrophenyl phosphate 1 mg/ml in 10% diethanolamine buffer and 0.5 mmol/L MgCl₂, pH 9.8). The enzyme reaction was stopped by addition of 50 mm³ 3N NaOH, and the absorbance at 405 nm was measured on a Dynatech spectrophotometer. All sera were simultaneously tested in duplicate; the mean absorbance values were corrected for blank values (without serum).

The data of RAST and ELISA were statistically evaluated by Student's *t* test on a personal computer (IBM Corp., Armonk, N.Y.) by means of the statistical package (CSS, Statsoft, Tulsa, Okla.).

Immunoblotting

Af-antigen. A reference antigen preparation (BN 524) was generously provided by Dr. J. L. Longbottom, Department of Allergy and Clinical Immunology, Brompton Hospital, London. The Af-antigen was a freeze-dried, dialyzed, 4 to 5 week culture-filtrate extract grown in a synthetic medium.^{36, 37} Af-antigen extract (5 mg/ml) was dissolved in sample buffer (pH 7.4) containing 2% sodium

dodecyl sulfate (SDS), 3% dithiothreitol, 10% glycerol, and 0.005% Bromphenol blue as tracking dye (Bio-Rad Laboratories, Ltd., Watford [Hurt] England). Eight molecular weight (MW) standards (Bio-Rad) with MW of 200 kd, 116 kd, 97 kd, 66 kd, 43 kd, 31 kd, 22 kd, and 15 kd were used in sample buffer. All samples were heated at 100° C for 5 minutes.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

Sodium dodecylsulfate gel electrophoresis was carried out with a discontinuous buffer system, as described by Laemmli.³⁸ A vertical slab gel electrophoresis unit was used (Midget; Pharmacia). A 3.75% polyacrylamide stacking gel was placed on top of a 12.5% polyacrylamide separation gel. The stacking gel buffer contained 1.25 mol/L Tris-HCl, pH 6.8; the separating gel buffer contained 1.857 mol/L Tris-HCl, pH 8.8. One hundred cubic millimeters of 5 mg/ml pretreated *Af*-antigen was loaded on a one-well comb with a Hamilton (Hamilton, Bonaduy, Switzerland) microliter syringe. In a second small well 5 mm³ of pretreated protein standards were added. The electrophoresis was run at constant current of 40 mA/2 gels and at a constant temperature of 5° C of the electrode buffer (Multitemp II; LKB) for 4 hours (Macrodrive 1 Power supply; LKB). Before transferring onto nitrocellulose, one part of each gel was protein stained with Coomassie brilliant blue (Bio-Rad) to control the separation of *Af*-antigen components and protein standards.

Blotting

The electrophoretic transfer of separated *Af*-antigen components onto nitrocellulose with pores 0.45 µm in size (Schleicher & Schuell, Kassel, Germany) was performed in a discontinuous semidry system between two graphite electrodes at high constant current 100 mA for 1 hour.³⁹ (Multiphor II Novablot; LKB); cathode buffer contained 20% methanol and 6-amino-n-hexanoic acid, pH 7.6; anode buffer 1 and 2 contained 20% methanol and 1:25 mmol/L, 2:300 mmol/L Tris, pH 10.4 according to the manufacturer's instructions. To control the transfer the gels were stained with Coomassie brilliant blue. The transparency of the gel indicated complete transfer of all *Af*-components. The nitrocellulose membranes were washed for 5 minutes in distilled water, air-dried, and stored at 4° C in filter paper (Schleicher & Schuell). A part of the nitrocellulose membrane was protein-stained with amido black (Bio-Rad) to detect the separated *Af*-antigens in relation to the molecular standards.

Immunologic detection of IgE-*Af* and IgG-*Af*

The nitrocellulose membrane was cut into strips and placed in eight-channel tubes (Inotech, Wohlen, Switzerland) filled with blocking buffer containing 1% bovine serum albumin, 5% fetal calf serum, 0.5% Tween 20, 0.01% merthiolate, 0.1 mmol/L MgCl₂, and 10 mmol/L Tris.⁴⁰ After incubation of the nitrocellulose strips in blocking buffer on a horizontal shaker for 30 minutes, diluted patient sera were added to separate nitrocellulose strips. Serum to

be probed for IgG was diluted to 1:100, whereas serum to be probed for IgE was diluted 1:10 in blocking buffer. The sera were incubated overnight on a horizontal shaker at room temperature. Strips were washed twice in blocking buffer for 5 minutes and a third time in Tris-buffered saline solution containing 0.05 mol/L Tris, 0.15 mol/L NaCl, and 0.01% merthiolate. The substrate was then added.

IgG-*Af* immunoblot

Nitrocellulose strips were incubated with 1:100 diluted peroxidase-labeled goat antihuman IgG (Dako) for 3 hours on a horizontal shaker at room temperature. After washing the strips three times for 5 minutes, the substrate and color reagent was added to each strip (3% H₂O₂ and 4-chloro-1-naphthol in methanol; Bio-Rad). The color development was stopped after 10 minutes by washing the nitrocellulose strips with water. After being extensively washed with water the strips were air-dried, and the developed bands were compared semiquantitatively by several investigators. The incubation and enzyme reaction was done simultaneously with 43 sera from patients with ABPA, 26 sera from patients with *Af*-asthma, and three sera from healthy individuals.

IgE-*Af* immunoblot

Nitrocellulose strips were incubated with 1:10 diluted isotope labeled (125-I) antihuman IgE (Phadebas RAST) overnight at room temperature. After decanting the antihuman antibodies and washing three times with triethanolamine-buffered saline, the strips were air-dried, fixed on a plastic foil and placed on X-ray film (Hyperfilm Betamax, Amersham Corp., Arlington Heights, Ill.). The exposure was performed in an X-ray cassette equipped with an intensifying screen (Agfa Gevaert, Leverkusen, Germany) for 2, 4, and 8 days at -70° C. The autoradiographs were compared semiquantitatively depending on time of exposure. The incubation of the strips and exposure to X-ray film was done simultaneously with all 43 sera from patients with ABPA and 26 sera from patients with *Af*-asthma.

RESULTS

IgE-*Af* RAST titer

Positive IgE-*Af* RAST titers were observed in all patients with ABPA and *Af*-asthma. The RAST titers of diluted sera were expressed in log counts per minute in Fig. 1, A and summarized in Phadebas RAST units in Table I. The patients in the active phase of ABPA showed the highest mean IgE-*Af* RAST titers, whereas the patients with *Af*-asthma showed the lowest IgE-*Af* RAST titer (Fig. 1, B). There was a significant difference of IgE-*Af* RAST titer if the results of the patients in the active phase were compared with patients in the remission phase ($p = 0.001$) or with patients with *Af*-asthma ($p = 0.001$). Furthermore, a significant difference of IgE-*Af* RAST titers was found if the results of the patients in the intermediate phase of ABPA were compared with patients in the remission phase of ABPA ($p = 0.01$) or with patients with *Af*-

log cpm

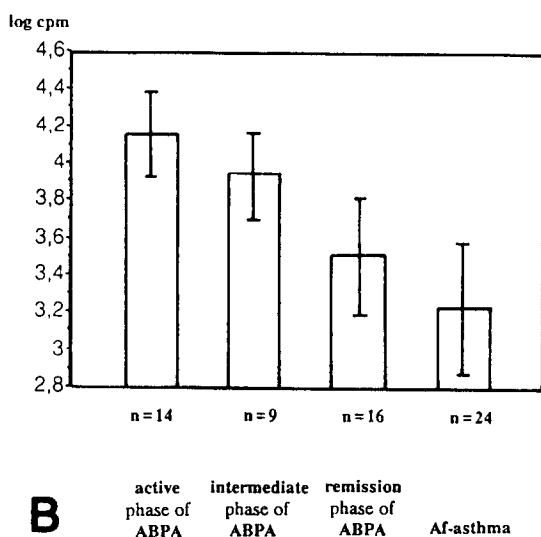
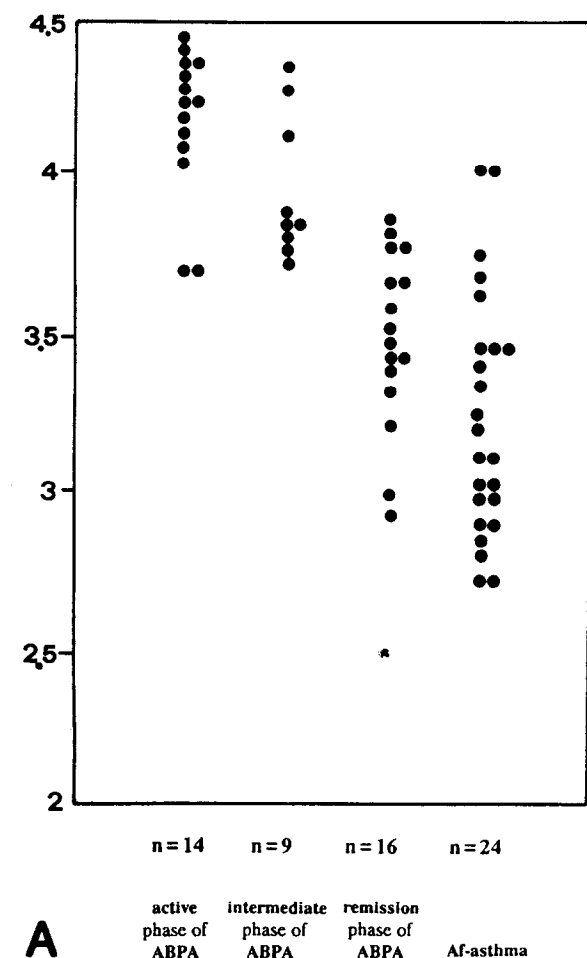


FIG. 1. **A**, IgE-Af RAST titers expressed in log counts per minute (log cpm) of each patient group. **B**, IgE-Af RAST titers expressed in log counts per minute (log cpm) presented as the mean $\pm$ SD of each patient group.

asthma ($p = 0.006$). The IgE-Af RAST titers of the patients in the remission phase of ABPA compared with the patients with Af-asthma did not differ significantly ($p = 0.149$).

IgG-Af ELISA titer

The IgG-Af ELISA titers expressed in absorbance values at 405 nm are demonstrated in Fig. 2, A and summarized in Table 1. The distribution of IgE-Af ELISA titers was continuous. In this study the upper normal limit of IgG-Af ELISA titer expressed in absorbance values at 405 nm: 0.327 was defined as the mean value of 26 patients with Af-asthma with additional two standard deviations. Thirteen out of 16 patients in the active phase of ABPA and eight out of nine patients in the intermediate phase of ABPA showed IgG-Af ELISA titers over the arbitrarily defined upper normal limit, whereas five out of 18 patients in the remission phase of ABPA showed positive IgG-Af ELISA titers in the lower part (Fig. 2, A). The three healthy individuals had negative IgG-Af ELISA titers (Fig. 2, A). The patients in the active phase of ABPA showed the highest mean IgG-Af ELISA titers, whereas the patients with Af-asthma showed the lowest IgG-Af ELISA titers (Fig. 2, B). Patients in the active phase of ABPA ($p = 0.0001$) as well as in the intermediate phase of ABPA ($p = 0.001$) and in the remission phase of ABPA ($p = 0.003$) showed significantly higher IgG-Af ELISA titers if compared with patients with Af-asthma.

IgE-Af and IgG-Af immunoblot

A broad spectrum of IgE and IgG directed against 12 different major Af-allergens in the MW range from about 15 kd to 97 kd were found in the sera of patients with ABPA in the active phase. The overall binding of IgE and that of IgG was directed against similar major Af-allergens, whereas the quantity of the IgE and IgG directed against the corresponding Af-allergens could vary in the sera of each patient. Three types of IgE-Af and IgG-Af patterns were more or less similar and are illustrated schematically in Fig. 3. The IgG-Af patterns were characterized by a first strong response to the high MW Af-allergens (immunopattern 1) followed by an intermediate to strong response to the Af-allergens with MW of 30 kd to 60 kd (immunopattern 2) and subsequently followed by a strong response to the low MW Af-allergens of about 15 kd to 30 kd (immunopattern 3). The IgE-Af patterns did not follow MW order. A major allergen of approximately 35 kd was found in the weak responders (immunopattern 1). This was followed by an intermediate response to the Af-allergens with MW of 30 and 50 kd and finally included a strong response to the high MW and low MW Af-allergens (immunopattern 3).

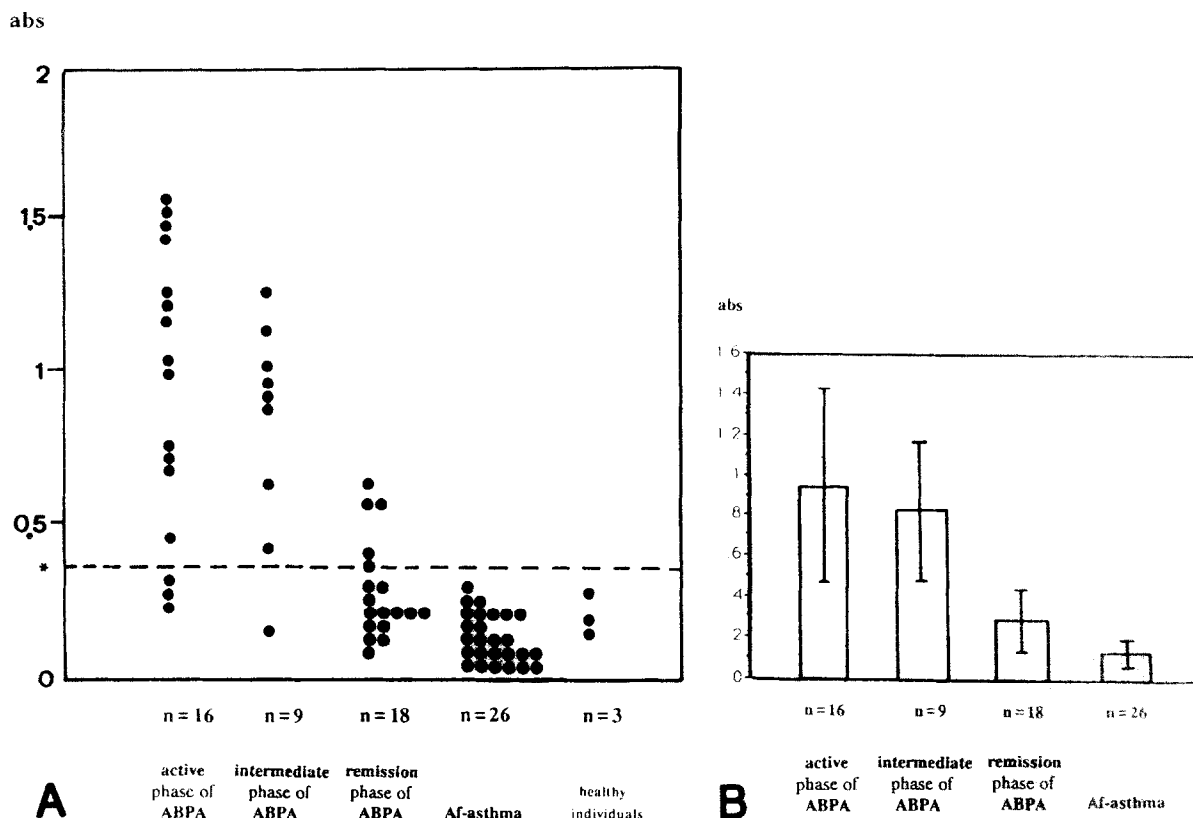


FIG. 2. A, IgG-Af ELISA titers expressed in absorbance values at 405 nm (abs) of each patient group.
* - - - upper limit: mean + 2 SD of 26 patients with Af-asthma. **B,** IgG-Af ELISA titers expressed in absorbance values at 405 nm (abs) presented as the mean $\pm$ SD of each patient group.

The IgE-Af and IgG-Af immunoblots corresponded to the IgE-Af RAST and IgG-Af ELISA titers, respectively.

Immunopattern 3 was found in all sera of patients with ABPA in the active phase (Figs. 3 and 4). An especially strong IgG reactivity to one low MW Af-allergen at approximately 15 kd (band no. 12, Fig. 3) was observed in one patient in the active phase (No. 1; Table I), whereas the corresponding strong IgE reactivity to the low MW Af-allergen was observed in all sera of patients with ABPA in active phase (Fig. 4). The strong IgE and IgG response to several major Af-allergens was reduced if the immunoblots of patients with ABPA in active phase were compared with patients in the intermediate phase of ABPA (immunopattern 2, Fig. 3). The strong IgG reactivity to the high MW Af-allergens between 66 kd and 97 kd (band no. 1 to 3; Fig. 3) in contrast to the weak or negative corresponding IgE reactivity was characteristic for all patients in the remission phase of ABPA. Furthermore, a weak IgE and IgG response to up to six major Af-allergens was found (immunopattern 1, Fig. 3).

Immunoblotting demonstrated no IgE-Af reactivity

in 22 out of 26 patients with Af-asthma and no IgG-Af reactivity in 14 out of 26 patients with Af-asthma. A weak IgG reactivity to up to three low MW Af-allergens without corresponding IgE reactivity was found in eight out of 26 patients with Af-asthma. Negative or very weak IgG-Af immunoblots corresponded with the negative IgG-Af ELISA titers (Fig. 2, A), whereas the negative IgE-Af immunoblots differed from the positive IgE-Af RAST titers (Fig. 1, A). A weak IgE and IgG response to up to four Af-allergens over the whole MW range was found by immunoblotting in four out of 26 patients with Af-asthma.

The immunoblots of the three healthy individuals demonstrated a strong IgG reactivity exclusively to one low MW Af-allergen at about 22 kd (band no. 11, Fig. 5) and a very weak IgG reactivity to the low MW Af-antigen at about 15 kd. No IgE reactivity was detected by immunoblotting.

DISCUSSION

The results of the present study demonstrate that the specific immunoresponse to Af-allergens reflects the clinical situation of ABPA. Three specific im-

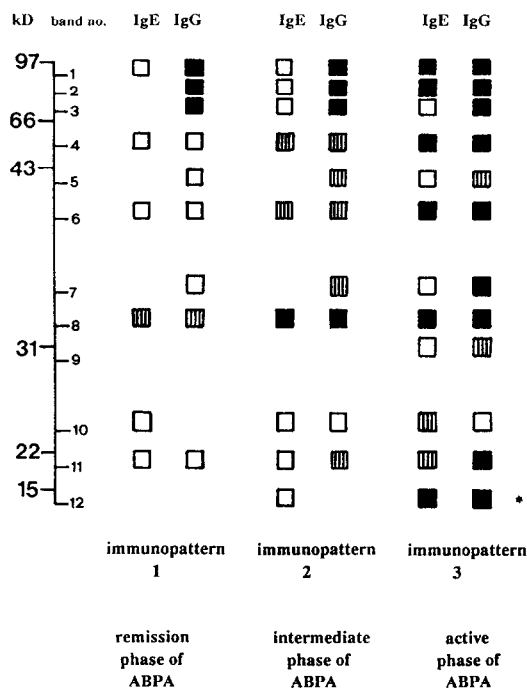


FIG. 3. IgE-Af and IgG-Af immunoblots. IgE-Af and IgG-Af patterns are schematically represented in relation to the clinical phase. MW standards in kD and band number (band no.) of the Af-allergens are indicated on the left side. Open space means no reaction; □ weak reaction; □ intermediate reaction; ■ strong reaction.

*band no. 12: strong reaction exclusively observed in one patient in active phase of ABPA (acute stage I; [13]).

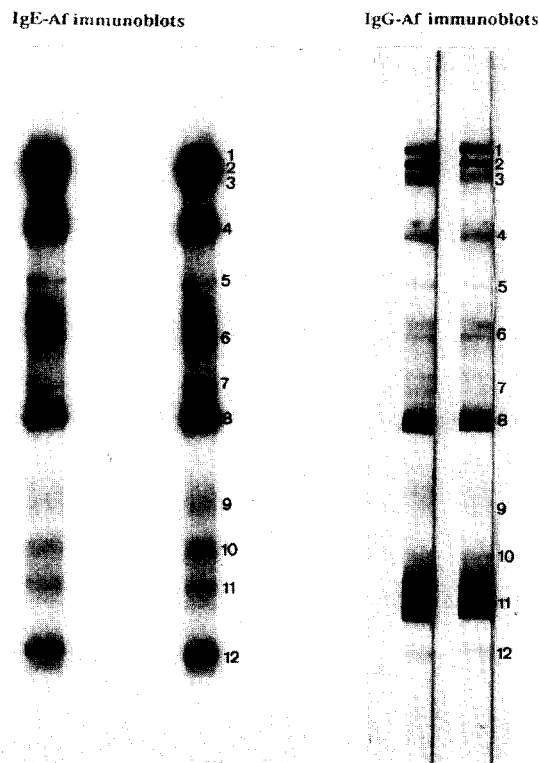


FIG. 4. IgE-Af immunoblots (autoradiography) and IgG-Af immunoblots (enzyme reaction) of one patient in active phase of ABPA, performed in duplicate. The band numbers of corresponding Af-allergens are indicated at the right side.

munopatterns are identified by immunoblotting that correlate with the IgE-Af RAST titer, IgG-Af ELISA titer, total IgE level, and asthma symptoms. We propose to classify ABPA into active, intermediate, and remission phases. The active phase is characterized by a fully developed specific immunoresponse to Af-allergens and severe asthma. The intermediate phase includes nonasthmatic patients with elevated specific immunologic findings and may correspond to a flare of ABPA without asthma symptoms or may precede an exacerbation of ABPA with severe asthma.^{5, 23, 24} Furthermore, treatment of patients in the active phase with systemic corticosteroids causes a transition to the intermediate phase. The remission of ABPA is characterized by a weak specific immunoresponse to Af-allergens after a long-term asymptomatic period.^{13, 22} In the active phase immunoblotting demonstrates a strong IgE and IgG reactivity to up to 12 major Af-allergens (immunopattern 3, Fig. 3). The immunoblots of patients in the intermediate phase are characterized by a reduction of the IgE and IgG reactivity to certain major Af-allergens (immunopattern 2, Fig. 3) in correlation with the decrease of IgE-Af RAST

and IgG-Af ELISA titers. In the remission phase the immunoblotting reveals a weak IgE and IgG reactivity to up to five major Af-allergens and a strong IgG reactivity to three high MW Af-allergens (immunopattern 1, Fig. 3). The correlation of the specific immunopatterns with the asthma symptoms and the significantly higher IgE-Af RAST and IgG-Af ELISA titers of patients in the active and intermediate phase compared with patients in the remissions phase indicates that the specific immunoresponse to Af-allergens reflects the disease activity of ABPA. Nonasthmatic patients with ABPA without radiographic abnormalities, as well as nonasthmatic patients with irreversible bronchopulmonary lesions are often found in the remission phase of ABPA (Table I). The low IgE-Af RAST and IgG-Af ELISA titers in the remission phase indicate that a complete removal of *Aspergillus* from the respiratory tract is possible in all stages of ABPA, since the specific immunoresponse to Af-allergens reflects the contact with the intrabronchial colonizing microorganism. Our results suggest that immunologic grouping of the patients with ABPA defines the clinical situation more accurately than stag-

ing ABPA with respect to fibrotic changes on chest radiography.¹³ This is due to the fact that the specific immunopatterns correlate with the asthma symptoms, whereas there is neither a correlation between the specific immunoresponse to *Af* and the permanent pulmonary lesions nor a correlation between the asthma symptoms and the fibrotic changes on chest radiography. Furthermore, the classification of ABPA into the active, intermediate, and remission phase takes into consideration the possibility of ABPA exacerbations without asthma symptoms.^{23, 24} It is important to know whether minimal or extensive fibrotic changes are present on chest radiography in the advanced stages of ABPA because of prognostic implications. However, we found that the assessment of minimal fibrotic changes on chest radiography may be difficult and largely depends on the experience of the investigator. In addition, central bronchiectasis is a further prognostic factor that can be recognized exclusively by CT scanning.⁴¹ Serologic follow-up of patients with ABPA allows well-timed therapeutic management and therefore may improve the prognosis of ABPA. Possible exacerbation of ABPA without asthma symptoms can only be detected by serial measurement of IgE-*Af* and IgG-*Af*. The increase in specific immunologic parameters in the sera of nonasthmatic patients indicates the transition from the remission into the intermediate phase of ABPA. Such asymptomatic patients may need therapeutic intervention to prevent exacerbations of ABPA with severe asthma and recurrent transient pulmonary infiltrates, and late bronchopulmonary sequelae may be reduced.^{6, 7} Thus it is important to follow specific *Aspergillus*-related immunologic parameters, since the total IgE level and eosinophil count is influenced by the contact with various allergens.

All patients with asthma symptoms and evidence of sensitization of *Af*-allergens, as indicated by positive prick test and RAST, should be screened by ELISA. The additional determination of positive IgG-*Af* ELISA titer generally allows the serologic discrimination of patients with ABPA and asthma symptoms from patients with *Af*-asthma in clinical practice. Hence, the serologic diagnosis of ABPA in early stages is possible without detectable permanent bronchopulmonary lesions by radiologic methods. The immunoblotting confirms the positive IgE-*Af* RAST and IgG-*Af* ELISA titer. Furthermore, patients with ABPA in all phases can be differentiated from patients with *Af*-asthma because the IgE-*Af* and IgG-*Af* immunoblots of most patients with *Af*-asthma were negative. The negative IgE-*Af* immunoblots of patients with *Af*-asthma could not be explained by lower IgE-*Af* RAST titer compared with patients with ABPA in remission

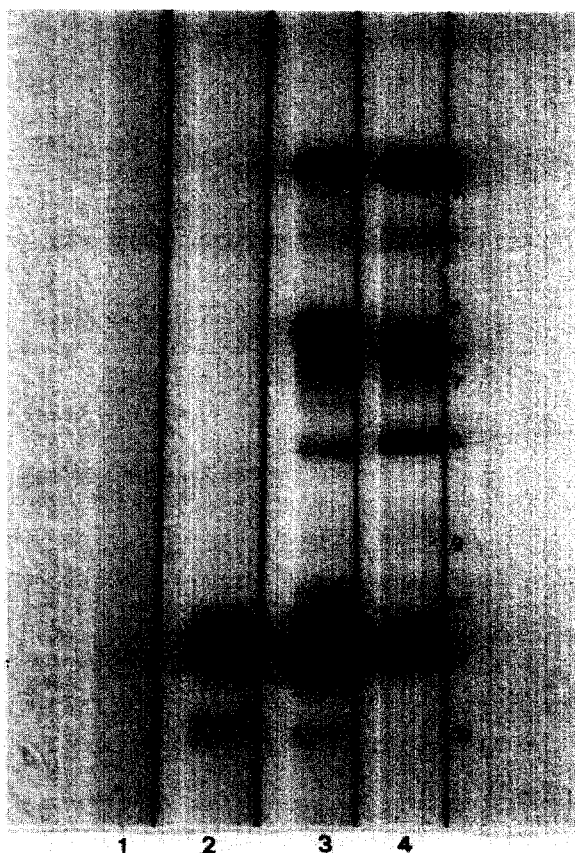


FIG. 5. IgG-*Af* immunoblots (enzyme reaction). 1, one patient with *Af*-asthma: no IgG-*Af* reaction; 2, one healthy individual: strong IgG-*Af* reaction to one low MW *Af*-allergen at 22 kd (band no. 11) and very low reaction to low MW *Af*-allergen at 15 kd; 3 + 4, two patients in active phase of ABPA: strong IgG-*Af* reaction to several *Af*-allergens. Band numbers of the *Af*-allergens are indicated at the right side.

(Fig. 1, A). Thus the nature of the immunoresponse of patients with ABPA must be different from patients with *Af*-asthma. In four out of 26 patients with *Af*-asthma, a weak specific IgE and IgG response to certain major *Af*-allergens is found by immunoblotting. The positive IgE-*Af* and IgG-*Af* immunoblots may be indicative of the development of an early stage of ABPA, which is interpreted as a complication of mold sensitive asthma.^{4, 8}

Immunoblotting demonstrates an IgG response exclusively to low MW *Af*-allergens without corresponding IgE reactivity in eight out of 26 patients with *Af*-asthma and in the three healthy individuals (Fig. 5). This IgG response may reflect a normal immunoresponse after inhalation of *Af*-allergens that are widespread in the environment. The so-called naturally occurring antibodies did not interfere with the applied ELISA technique (Fig. 2, A).^{42, 43}

In summary, we present evidence that ABPA can be usefully classified with regard to specific serology responses in relation to asthma symptoms. Specific IgE and IgG responses to *Aspergillus* can be measured by RAST and ELISA, respectively, whereas immunoblotting provides various specific immunopatterns. The correlation of these specific serum responses with asthma symptoms permits an accurate characterization of ABPA.

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The role of water temperature and laundry procedures in reducing house dust mite populations and allergen content of bedding

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The effects of various laundry procedures on house dust mites and their allergens have been established. All mites were killed by water temperatures 55° C or greater. Killing at lower temperatures was not enhanced by any of the pure detergents or laundry products tested. A cold cycle of laundry washing with or without laundry powder did not remove most live mites from bedding, however, the allergen concentration (Der p I/gm fine dust) was reduced by more than 90%. Dry cleaning did not reduce the allergen concentration of the dust, although most, if not all, mites were killed. (J ALLERGY CLIN IMMUNOL 1992;90:599-608.)

Key words: House dust mites, mortality, water temperature, detergents, laundry procedures

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There is increasing evidence that allergens, and particularly house dust mite (HDM) allergens, are important in the etiology and perhaps the pathogenesis of asthma.¹ Several trials of allergen avoidance have demonstrated significant clinical benefits when methods that are likely to produce substantial reductions of allergen exposure in houses have been applied.^{2,3} However, the concentration of allergen in many houses exceeds the proposed minimum threshold level