

Aspergillus fumigatus specific IgE and IgG antibodies for diagnosis of *Aspergillus*-related lung diseases

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IgE and IgG antibodies against *Aspergillus fumigatus* were detected by crossed radio immunoelectrophoresis (CRIE) on the sera of seven patients with aspergilloma, six patients with allergic broncho-pulmonary aspergillosis (ABPA) and 25 patients with extrinsic asthma with *Aspergillus* allergy. IgE-CRIE analysis indicated the presence of *A. fumigatus*-specific IgE in sera of patients with ABPA and *Aspergillus* asthma but not of aspergilloma patients. IgG-CRIE showed that both aspergilloma and ABPA patient sera contained high levels of circulating specific IgG antibodies in contrast to sera of *Aspergillus* asthma patients, which did not show detectable amounts of *Aspergillus*-specific IgG antibodies. Specific IgE binding could be demonstrated for the major allergens Ag-10 and AG-40 in all ABPA patients, in 80 % of *Aspergillus* asthma patients but not in sera from aspergilloma patients. Specific IgG antibodies directed towards the major allergens could be detected in most of the aspergilloma patients, between 30-70 % of the ABPA patients but not in sera from patients with *Aspergillus* asthma.

Key words: *A. fumigatus*; CIE/CRIE; diagnosis; IgE; IgG.

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Aspergillus species and in particular *Aspergillus fumigatus* appears to be the etiological agent in various lung diseases (1, 12, 16). Inhalation of low doses may induce sensitization and asthma in *Aspergillus*-sensitive patients, while inhalation of high doses may cause symptoms of alveolitis and farmer's lung associated with raised levels of IgG antibodies and stimulation of cell-mediated immunity. *A. fumigatus* grows best at body temperature (37°C) and the microorganism may proliferate in the bronchi and induce longer lasting symptoms, such as allergic broncho-pulmonary aspergillosis (ABPA) with chronic or recurrent asthmatic symptoms associated with eosinophilia and X-ray signs of chronic and parenchymal inflammation often leading to fibrotic lung damage. In aspergilloma, fungi grow in preformed cavities in the lung often causing relatively weak symptoms of low grade fever. *A. fumigatus* can also colonize the pleural

cavity causing chronic pleural infection. Finally, in the compromised host *Aspergillus* sepsis may develop.

Skin tests with *A. fumigatus* antigens have been used for diagnosis of ABPA and *Aspergillus* allergy in patients with asthma (8, 14). *In vitro* assays such as RAST, ELISA and immunoprecipitation have been used for diagnosis of ABPA, aspergilloma and pleural infections as specific circulating antibodies of IgG- and/or IgE-type are formed in patients suffering from such disorders (10). One major problem with diagnosis has been the production of relevant *A. fumigatus* antigens. *A. fumigatus* preparations are complex mixtures of more than 50 precipitating antigens and therefore the choice of *A. fumigatus* source material is critical for the preparation of standardized and potent allergenic extracts (17). Their immunological and physico-chemical properties have been investigated. We identified

18 antigens acting as allergens by crossed radio-immunoelectrophoresis (CRIE); two major allergens Ag-10, Ag-40), 10 intermediate and six minor allergens (17). CRIE was also used by Longbottom (7, 9) on human ABPA serum and she found at least 12 antigens of *A. fumigatus* reacting with both IgE- and IgG-type antibodies.

The purpose of this study was to use the CRIE method for analysis of the individual IgE and IgG antibody patterns in the various *Aspergillus*-related diseases in order to investigate whether characteristic patterns could be identified, as well as the response to the major allergens Ag-10 and Ag-40.

MATERIAL AND METHODS

Mould extracts

Ten different strains of *A. fumigatus* were grown on synthetic culture medium under controlled and well-defined conditions (17). Equal parts of freeze-dried material from each strain were mixed resulting in a 10-strain mix. The mould extract was prepared as a 1:20 (w/v) preparation in 0.125 M ammoniumbicarbonate buffer (pH 7.8) under stirring at +4°–+8°C overnight. The extract was cleared by filtration and freeze-dried.

Rabbit antibody

Antiserum was raised by multiple intracutaneous immunizations with a crude 10-strain mix extract of *A. fumigatus* in complete Freund's adjuvant according to the method described by Harboe & Ingild (3). The antiserum used in this study was a pool of several bleedings from six rabbits.

Patient sera

The patients with *Aspergillus* asthma were selected from consecutive patients referred for allergological investigation due to asthma and/

or rhinitis. In these patients sensitization to *A. fumigatus* antigens was established on the basis of a history with mould allergy, skin test and positive RAST (mean 6.9 PRU/ml) to *A. fumigatus*.

ABPA and aspergilloma patients were selected from the department of lung diseases at the Karolinska Hospital (Stockholm). The diagnosis of aspergilloma was mainly based on X-ray findings and confirmed at operation or by cultivation of fungus from sputum. The diagnostic criteria for ABPA were chronic obstructive lung disease and associated X-ray changes with changing infiltrates, blood eosinophilia or fibrosis with signs of bronchial wall thickening. The diagnosis was in all cases supported by a raised serum IgE concentration and positive RAST (0.35–17.5 PRU/ml) and skin test with *A. fumigatus* antigens. Seven individuals fulfilled the diagnostic criteria for aspergilloma, six for ABPA and 25 for *Aspergillus* allergy.

Crossed immunoelectrophoresis and crossed radio-immunoelectrophoresis

Crossed immunoelectrophoresis (CIE) analyses were performed as previously described (15, 17) with an extract prepared from equal parts of 10 different strains of *A. fumigatus*, containing both spore and mycelium components as antigen.

CRIE (IgE-CRIE, IgG-CRIE) was conducted according to the method previously published (11, 15, 17). The CIE-plates were incubated with patient sera (Aspergilloma, ABPA and *Aspergillus* asthma) and isotopes (¹²⁵I-anti-IgE or ¹²⁵I-anti-IgG, 72000 cpm/ml, respectively). The autoradiographic exposure was performed in a cassette at low temperature (–70°C) under defined time periods, 1 h to 27 h, corresponding to CRIE class A to G, respectively. CRIE class A indicates dense radiostaining while CRIE class G corresponds to weak but specific radiostaining. All CRIE experiments were performed under identical experimental conditions to be able to compare the radiostaining patterns of the different patient sera. The data were evaluated independently by two persons familiar with the reading of CRIE reference system.

RESULTS

CRIE was applied as a serological test for differentiation of *Aspergillus*-related antibodies in patients with various types of *Aspergillus*-induced disorders. Fig. 1a-1c demonstrate IgE- and IgG-antibody binding patterns for patients with aspergilloma, ABPA or *Aspergillus* asthma. The position of the major allergens Ag-10 and Ag-40 are indicated by arrows in the figures.

The typical CRIE pattern of the aspergilloma groups is shown in Fig. 1a. No visible radiostaining could be detected in the IgE-CRIE pattern, but there was a pronounced binding of specific IgG antibodies, both as to number of components and degree of specific IgG-binding. All aspergilloma patients showed a similar IgG-binding pattern. Fig. 1b demonstrates a typical IgE- and IgG-CRIE pattern of a subject from the ABPA group. The CRIE analysis of this patient serum shows a mixed pattern characterized by specific IgE- and IgG-binding to many different

A. fumigatus antigens. Numerous visible radiostaining immunoprecipitates of varying degree could be detected in both IgE- and IgG-CRIE patterns. Further, patients with ABPA showed IgG-antibody binding to most of the components to which IgE-antibody binding was induced.

IgE- and IgG-CRIE patterns of a patient from the *Aspergillus* allergy group are shown in Fig. 1c. There was a strong binding of specific IgE antibodies but the number of components was few compared with those found in ABPA patients (Fig. 1b). The IgG-titers for *Aspergillus*-allergy patients are very low as no visible radiostaining of ¹²⁵I-anti-IgG could be seen in any of the IgG-CRIE patterns.

Table 1 summarizes the average numbers of visible radiostained precipitates in the IgE- and IgG-CRIE patterns for the three patient groups. Sera of patients with aspergilloma demonstrated a high degree of specific IgG-binding, and these antibodies recognized many different *A. fumigatus* antigens (mean value 12, range 4-16). *Aspergillus* asthma patients showed a wide range of specific IgE antibodies (average 5 different spe-

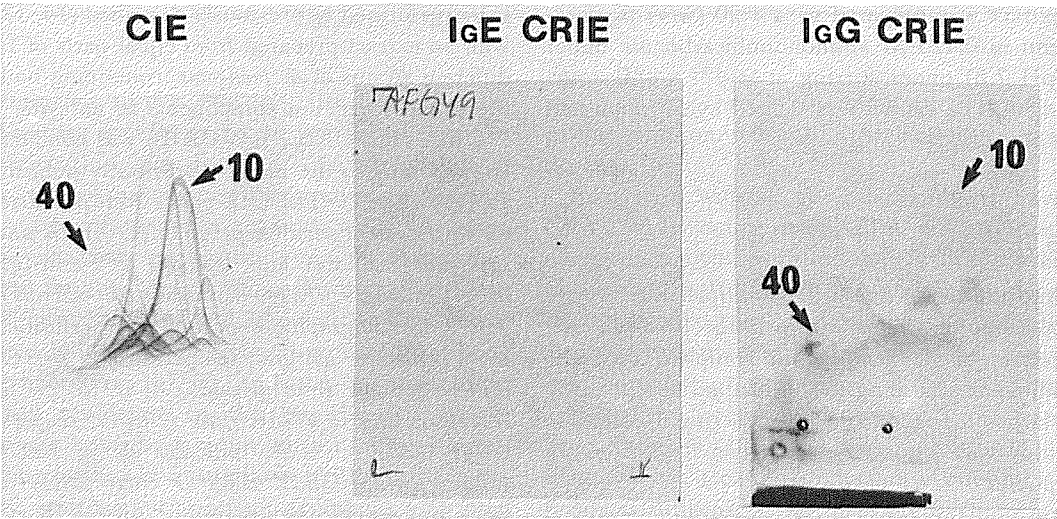


Fig. 1a. Crossed immunoelectrophoresis of *A. fumigatus* against hyperimmune rabbit antisera, after protein staining with Coomassie Brilliant Blue. 0.2 mg of a 10-strain mixture was analysed in agarose electrophoresis (pH 8.6) with 10 V/cm at 15°C for 35 min, followed by electrophoresis perpendicular to the first dimension with 2 V/cm for 18 h in agarose containing rabbit antiserum (10 µl/cm²). Anode to the right in the first dimension and at top in the second dimension electrophoresis. X-ray films from IgE-CRIE and IgG-CRIE experiments illustrating the radiostaining pattern of a typical aspergilloma patient. Exposure time corresponding to CRIE class G. Precipitates containing the identified allergens, Ag 10 and Ag 40, are indicated by arrows.

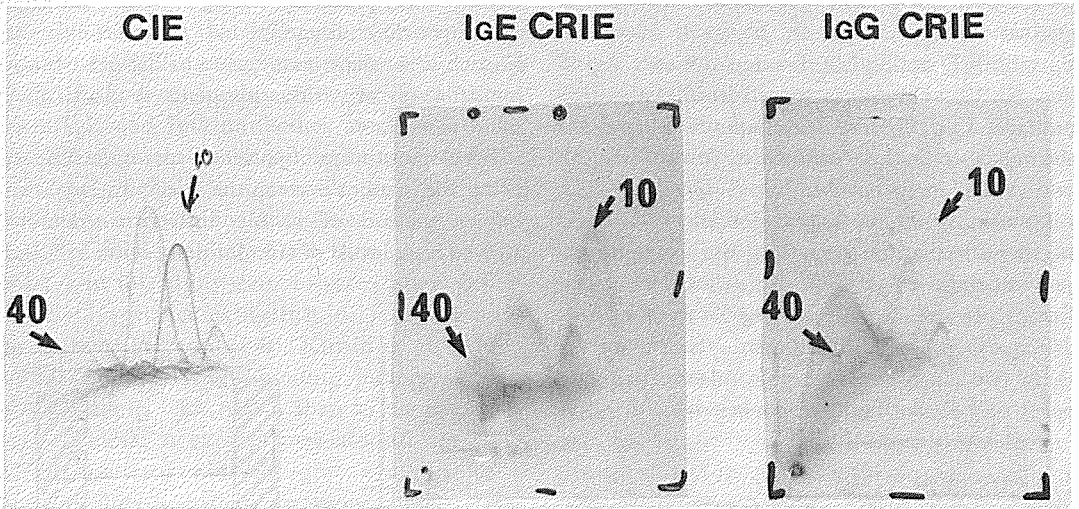


Fig. 1b. X-ray films from IgE-CRIE and IgG-CRIE experiments illustrating the radiostaining pattern of a typical ABPA patient. Exposure corresponding to CRIE class G. Experimental conditions for CIE-CRIE analysis as described in Fig. 1a.

cificities, range 4–16) directed towards *A. fumigatus* antigens. Sera of patients with ABPA showed a high number of specific IgE and IgG antibodies (average 8 components, range 4–17, and 9 components, range 4–16, respectively) directed towards different *A. fumigatus* antigens.

The number of patients in the various groups with IgE and IgG antibodies directed against the major *A. fumigatus* antigens, Ag-10 and Ag-40, is shown in Fig. 2. *Aspergillus* allergy patients showed specific IgE binding towards Ag-10 (58%) and Ag-40 (80%), while these

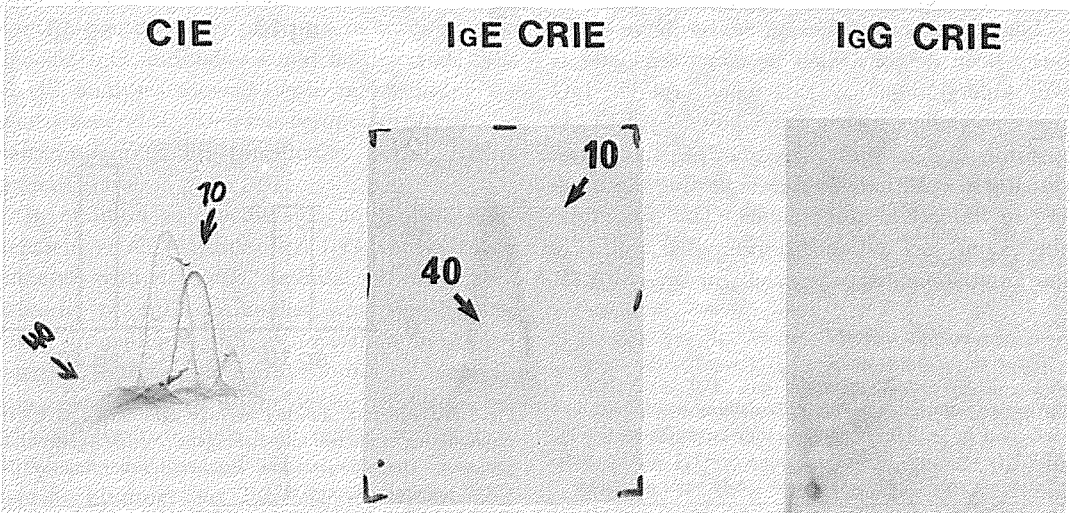


Fig. 1c. X-ray films from IgE-CRIE and IgG-CRIE experiments illustrating the radiostaining pattern of a typical *Aspergillus* asthma patient. Exposure time corresponding to CRIE class G. Experimental conditions for CIE/CRIE analysis as described in Fig. 1a.

patients did not exhibit IgG-CRIE radiostaining against the two major *A. fumigatus* allergens. Specific IgE antibodies directed towards Ag-10 and Ag-40 were demonstrated in all six ABPA patients. Further, in ABPA patients 2/6 and 4/6 showed specific IgG antibodies towards Ag-10 and Ag-40, respectively. No visible IgE-CRIE radiostaining for the two major allergens could be identified in the sera from the aspergilloma patients. However, IgG antibodies with specificities towards Ag-10 and Ag-40 were seen in 7/7 and 6/7 of aspergilloma patients. Obviously, these two allergens are highly relevant with respect to IgG antibody responses as well.

DISCUSSION

In a previous study using IgE-CRIE analyses of 26 individuals with suspected allergy to *A. fumigatus* (17), we could identify 18 *A. fumigatus* antigens which recognized specific IgE antibodies in the patient sera analysed. This study has now been extended to include two other groups of patients with *Aspergillus*-related disorders: aspergilloma and ABPA. As both IgE and IgG antibodies are thought to play important pathogenic roles in ABPA and the occurrence of IgG antibodies is of diagnostic importance in aspergilloma, the IgE-CRIE analysis was complemented with IgG-CRIE.

Large amounts of IgG antibodies have previously been demonstrated in sera from patients with aspergilloma (5, 13). The serologic IgG titers nowadays mostly determined by ELISA, give the total amount of specific IgG and consequently are of limited value regarding the binding to individual components. The total IgG-titer alone has a low discriminative value regarding patients with aspergilloma or ABPA (5,

13). Further, problems can be expected with the use of poorly characterized *A. fumigatus* extracts in such a sensitive assay, due to different binding affinities of various antigens on the ELISA microtiter plate.

Serologic studies of patients' sera with ABPA, using the patient's serum as precipitating antibodies instead of rabbit antiserum, demonstrated high levels of circulating specific IgE and

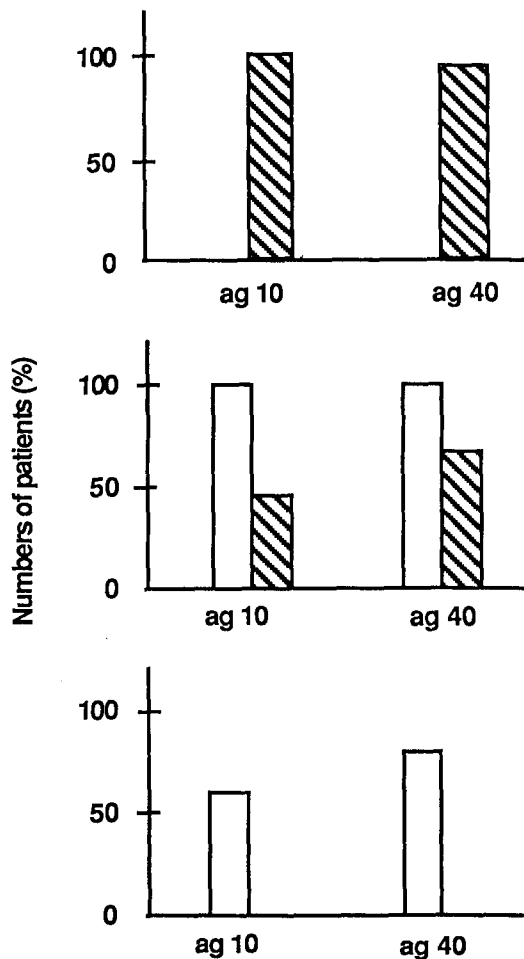


Fig. 2. Allergogram showing average IgE- and IgG-CRIE radiostaining of the two major allergens (Ag-10, Ag-40) in *A. fumigatus*. The allergens are numbered according to the CRIE reference pattern (17).

Radiostaining corresponding to CRIE class A-G was evaluated. Open bars symbolize specific IgE-binding and hatched bars specific IgG-binding. a) Aspergilloma ($n = 7$); b) ABPA ($n = 6$); c) *Aspergillus* allergy ($n = 25$).

Table 1.

Average number of visible radiostaining precipitates in IgE- and IgG-CRIE in patients with aspergilloma ($n = 7$), ABPA ($n = 6$) and *Aspergillus* asthma ($n = 25$). CRIE radiostaining corresponding to classes A-G were evaluated

	Aspergilloma	ABPA	Aspergillus asthma
IgE	0	8	5
IgG	12	9	0

IgG (7, 9). Using this technique Longbottom (7, 8) has shown that IgE antibodies have affinity for *Aspergillus* antigens recognized by human IgG antibodies. In this study we have shown that IgE and IgG antibodies are produced by humans against the same important *Aspergillus* antigens when the antigens were precipitated by rabbit anti-*aspergillus* antibodies.

IgG-CRIE analyses of sera from patients with ABPA in this study demonstrated many different IgG specificities against *A. fumigatus* antigens (average number 9). Kurup et al. (6) detected a broader IgG-binding pattern in sera from patients with ABPA than in patients with aspergilloma. A careful inspection of our IgG-CRIE patterns did not give evidence of a broader IgG specificity or more intense IgG-binding in ABPA patient sera compared with aspergilloma patient sera. According to Greenberger et al. (2), however, significantly higher IgE- and IgG-titers were found in patients with *A. fumigatus*-induced asthma, not fulfilling the diagnostic criteria for ABPA. In our study, the CRIE analysis as described here for sera of aspergilloma, ABPA and *Aspergillus* asthma could not discriminate between the various *Aspergillus*-related disorders from the total amount of circulating antibodies, but rather from the specificities (Table 1).

Aspergillus asthma patient sera differed significantly from the other patient sera studied as no specific IgG antibodies could be detected. IgE-CRIE analyses showed dense radiostaining towards several *A. fumigatus* components with the same range of IgE-specificities as for ABPA patient sera.

In addition to specific IgE antibodies, Katzenstein et al. (4) identified IgG antibodies in patients with *Aspergillus* asthma. However, the amount of IgE antibodies was significantly lower in patients with asthma than in ABPA patients, but similar to that found in aspergilloma patients. This might be due to differences between the two trials with regard to test performance, e.g. cut-off limits, degree of non-specific binding, etc. Further, it might reflect diagnostic difficulties, some patients with ABPA may in later stages develop aspergilloma and their reactivity may diminish with age.

The presence of IgE and IgG antibodies towards *A. fumigatus* specific antigens was only investigated in sera from patients with three different clinical diseases caused by *A. fumigatus*. The sensitivity and specificity of diagnostic tests should be investigated in a normal diagnostic situation, e.g. distinguishing a solid non-infectious lung tumor from an aspergilloma or other disseminated lung diseases with bronchial obstruction from ABPA. Further studies are also needed to investigate the possibility of utilizing such markers as IgG or IgE antibodies, with specificity towards Ag-10 or Ag-40, in the diagnosis of diseases caused by inhalation of or infection with *A. fumigatus*. Even in the future, the most appropriate tests for *Aspergillus* allergy will still be skin testing, RAST and bronchial challenge tests with properly standardized allergens.

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