

Antibodies in different immunoglobulin classes to *Candida albicans* in allergic respiratory disease

G. EDGE and J. PEPYS

Department of Clinical Immunology,
Cardiothoracic Institute, London

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Summary

Measurements by radiometric assay of specific IgE, IgA, IgM, IgD and IgG antibodies, in fifty-six patients with asthma, fifty-six with rhinitis and twenty with allergic bronchopulmonary aspergillosis (ABPA), showed differences in the patterns of antibody response between the three patient groups, both in terms of levels of class specific antibodies and the proportion of patients giving positive results.

Specific IgE antibody values were similar in all three groups and positive results were found in three-quarters of the patients in each group. No other class of antibody to *Candida albicans* gave values which were similar in all three patient groups.

Specific IgG and IgA antibody values were highest in ABPA and lowest in rhinitis; specific IgM antibody values were highest in asthma and lowest in ABPA; specific IgD antibody values were higher in rhinitis than asthma and very low in ABPA.

Specific IgG1 and IgG2 antibodies to *C. albicans* were higher in rhinitis than in asthma, whereas specific IgG3 and IgG4 values were significantly higher in asthma than in rhinitis.

There was a greater number of positive results for IgG1, IgG2 and IgG4 in patients with ABPA than in the other two patient groups where values for IgG1 and IgG2 antibody to *C. albicans* were mainly negative. Positive IgG3 antibody results occurred with greatest frequency in patients with asthma, half of them giving values greater than the mean + 2 s.d. of the control value; positive specific IgG4 antibody values occurred more frequently in asthma than in rhinitis.

Introduction

Candida albicans is a common commensal against which there is almost universal sensitization with the production of one or more classes of antibody. In addition, infection with this organism exhibits a varied spectrum of disease from local inflamma-

tory changes in 'thrust' to deep seated, fulminating disease in severely compromised patients. Precipitins to *C. albicans* antigens may be demonstrated in sera from healthy persons (Taschdjian, Seelig & Kozinn, 1973) and serological methods for the diagnosis of deep seated lesions, using the precipitin principle, are often unreliable and show little correlation between laboratories, possibly due to the differences in the antigen preparation (Faux *et al.*, 1975; Axelsen, 1976). The problems of these differences can be overcome to a great extent when the whole organism is used, as in agglutination and fluorescent antibody tests (Taschdjian *et al.*, 1973). Both agglutination (Muller, 1974) and fluorescent antibody (Lehner, 1970) systems can be adapted to give information about the class of antibody produced, but again raised titres which are apparently clinically insignificant cause confusion in interpretation.

The radiometric assay described here, was developed to measure specific antibodies in different immunoglobulin classes, to provide a more comprehensive basis for assessing the possible role of this organism in allergic respiratory disease (Longbottom *et al.*, 1976). Whole *C. albicans* yeast cells, the form in which *Candida albicans* may be met in the body, were used for the measurement of specific IgE and IgA antibodies in preliminary studies (Edge *et al.*, 1977). This work has now been extended and specific IgE, IgA, IgM, IgD and 'total' specific IgG as well as specific IgG1, IgG2, IgG3 and IgG4 antibodies to *C. albicans* yeast cells were measured in groups of patients with asthma, rhinitis and allergic bronchopulmonary aspergillosis. In this latter group antibodies to *C. albicans* are common, and they also give type I and type III reactions to intracutaneous injections of the mannan and extracts of *C. albicans* (Pepys *et al.*, 1968).

Patient and control groups

Sera were tested from fifty-six patients with asthma on oral corticosteroids, of whom thirty-three were atopic; from fifty-six patients with rhinitis, none of whom were on oral corticosteroids, of whom thirty-six were atopic, and from twenty patients with allergic bronchopulmonary aspergillosis (ABPA), all of whom were on oral corticosteroids. Control sera were included in each run and comprised equal numbers of cord blood and blood bank sera; no fewer than ten and no more than twenty control sera were included in any run.

Method

Candida albicans A (B311) (Mycological Reference Laboratory, London School of Hygiene and Tropical Medicine) was grown in 2-4 l batch size, in Sabouraud broth medium, with continuous stirring at room temperature for 48 hr, to produce a culture of cells in the blastospore form. The culture was examined for sterility and killed by the addition of sodium azide (0.5% w/v). Cultures were harvested by centrifugation and washed with phosphate buffered saline (pH 7.4) by centrifugation (2000 rev/min for 10 min) and resuspension, in four changes of PBS. Cells were stored at 4°C in PBS, containing 0.1% sodium azide and 2% normal, inactivated goat serum. Normal inactivated rabbit serum was used as the serum diluent, in cases where the radiolabelled antiserum was a rabbit antiserum. The cells were used within 2 days, or rewashed and used within a week.

With the exception of the antihuman IgE serum, all antisera used were commercially available and supplied as the gamma globulin fraction. The protein concentration was adjusted to give 1.0 mg/ml with PBS and 50 λ volumes of the diluted antisera

were labelled with 1.0 mCi of ^{125} Iodine (IMS 30, RadioChemical Centre, Amersham) using the chloramine-T method of Hunter & Greenwood (1962). The radiolabelled protein was separated from low molecular weight material by passage through a column of Sephadex G25 fine (Pharmacia) and eluted with PBS. Fractions (0.5 ml) containing over 90% protein-bound radio- ^{125} Iodine were pooled. After the addition of an equal volume of normal goat or rabbit serum, to prevent release of free iodine (Bogdanove & Strash, 1975) the labelled monospecific anti-human immunoglobulin antisera were frozen in aliquots at -70°C until required. Labelled antibody preparations were used within one half-life (60 days) of preparation. After thawing, the activity of the labelled antiserum was diluted to give 100,000 ct/min in 50 λ . Non-specific uptake of label in the assay was reduced by absorbing the preparation with *C. albicans* cells (1.0 ml packed cell volume) before use.

Goat antihuman IgE was obtained as a gift from Professor Arbesman, University of Buffalo, New York. Rabbit antihuman IgG (lot 025), antihuman IgA (lot 095) and antihuman IgM (lot 027) were obtained from Dako-immunoglobulins, Copenhagen. Goat antihuman IgD (lot 1019) was obtained from Seward Laboratories, London. Sheep antisera to human IgG1-4 subclasses were obtained from the Department of Experimental Pathology at the University of Birmingham. The specificity of the anti-IgG1 and IgG3 sera is as high. There are differences of opinion regarding the anti-IgG2 and IgG4 antisera when depending upon mode of testing may show cross-reactivity.

The assay procedure was based on the RAST (Wide *et al.*, 1967) and was essentially a 'sandwich' method. All assays were carried out on duplicate volumes (50 λ) of sera in disposable, rigid polystyrene tubes. Serum samples were incubated overnight at room temperature, with 500 λ of a suspension of 10% packed *C. albicans* cell volume, the cells being maintained in suspension whilst being dispensed by means of a magnetic stirrer. After incubation, cells were washed by centrifugation and resuspension (on a vortex mixer) in four changes of PBS. After the final wash had been decanted, the absorbed ^{125} Iodine-labelled antisera was added to each tube using 50 λ per tube. After further overnight incubation at room temperature the test materials were washed in six changes of PBS and the residual radioactivity was counted on a gamma-counter.

Statistical analysis

Cord blood and bloodbank sera were used in all assays as controls. No significant difference in specific antibody values was found for these two groups and their values were therefore combined to give the criterion of positivity, test values greater than the mean $+2$ s.d. of these control values were considered positive. Where assays for the three patient groups were carried out at different times, statistical analysis was carried out the mean value of duplicate tests as a ratio of the mean control value for the particular test run. Thus the results of class specific antibody assays were analysed in two ways; by direct comparison between patient groups (Mann Whitney Test), and in terms of the prevalence of positive values within patient groups (χ^2 test). Within individual patient groups, correlations between antibody values in the different immunoglobulin classes were carried out (Spearman rank correlation).

Results

Differences in the levels of specific antibody to *C. albicans* between the three patient groups were found in all immunoglobulin classes except IgE; direct comparison of

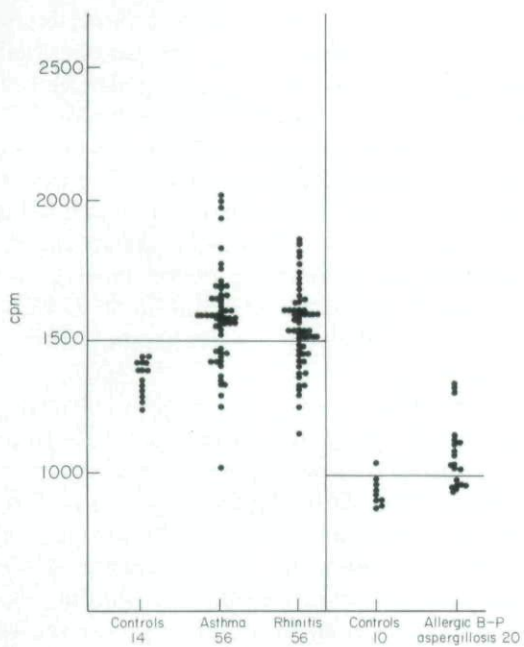


Fig. 1. Specific IgE antibody to *C. albicans* yeast cells.

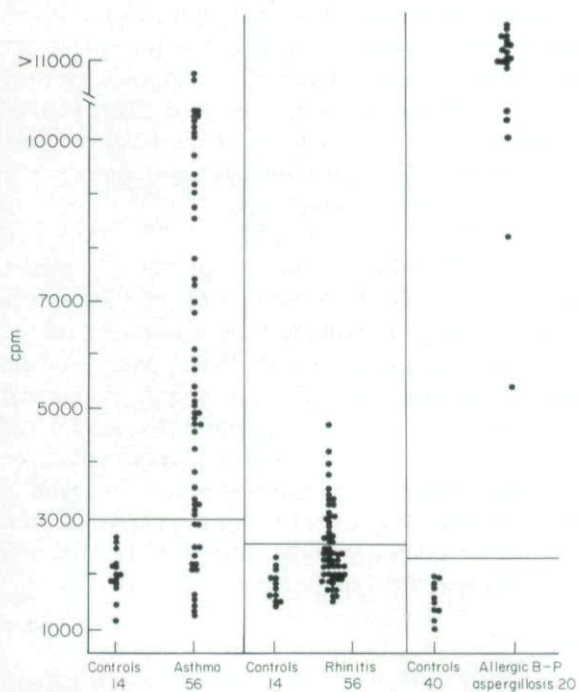


Fig. 2. Specific IgA antibody to *C. albicans* yeast cells.

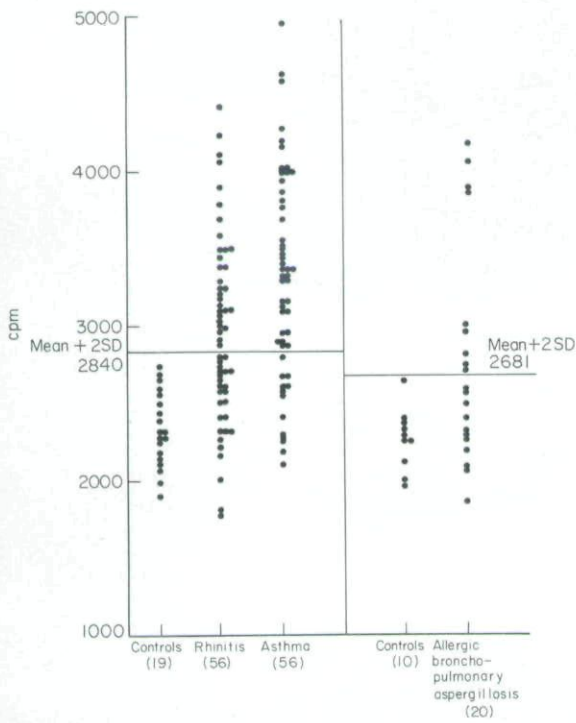


Fig. 3. Specific IgM antibody to *C. albicans* yeast cells.

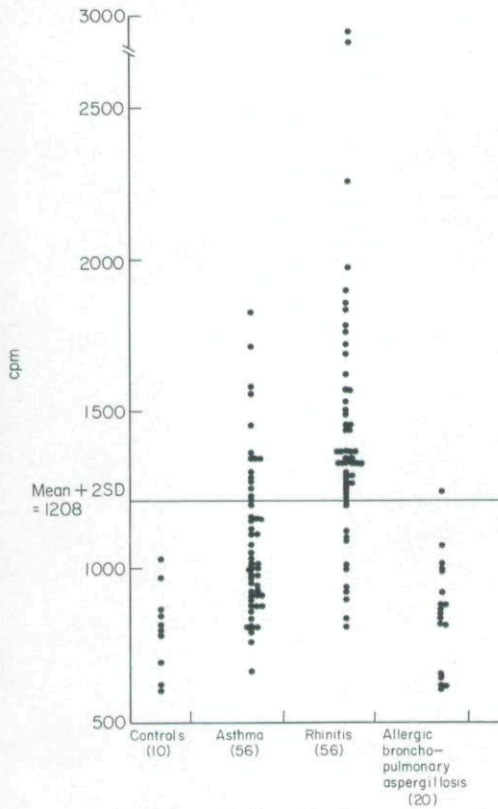


Fig. 4. Specific IgD antibody to *C. albicans* yeast cells.

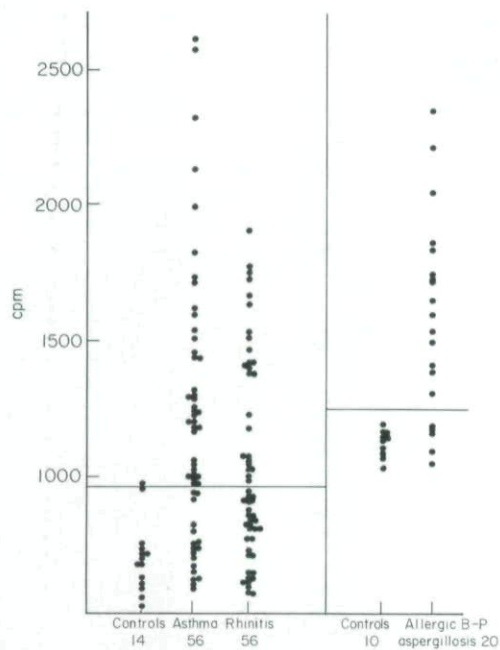


Fig. 5. Specific IgG antibody to *C. albicans* yeast cells.

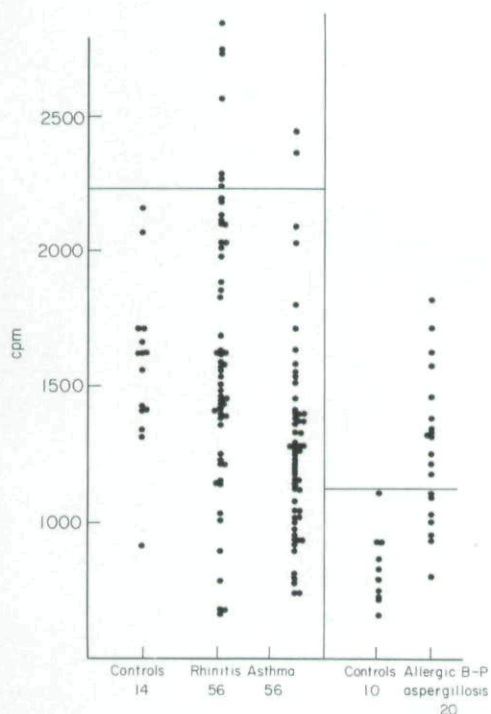


Fig. 6. Specific IgG₁ antibody to *C. albicans* yeast cells.

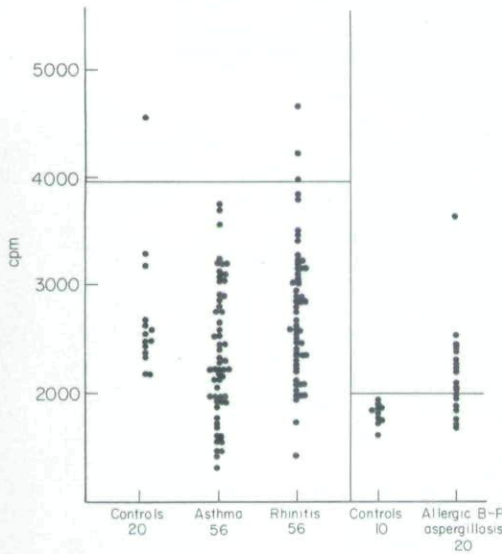


Fig. 7. Specific IgG₂ antibody to *C. albicans* yeast cells.

specific IgE antibody values showed them to be similar in all three groups. The numbers of patients in each group who gave positive results for IgE antibody were also similar (Table 1).

Specific IgA antibody values were significantly higher in ABPA than in asthma ($P > 0.001$) and higher in asthma than in rhinitis ($P < 0.01$). All patients with ABPA had positive IgA values; this was significantly greater than the number of asthmatic patients giving positive IgA results ($P < 0.01$). The rhinitis patients had fewer positive values than the other two groups of patients ($P < 0.001$).

Values for specific IgM antibody to *C. albicans* were significantly higher in asthma than in rhinitis ($P < 0.02$) and ABPA ($P < 0.02$); there was no difference in specific IgM values between the rhinitis and ABPA patients. The numbers of patients who had

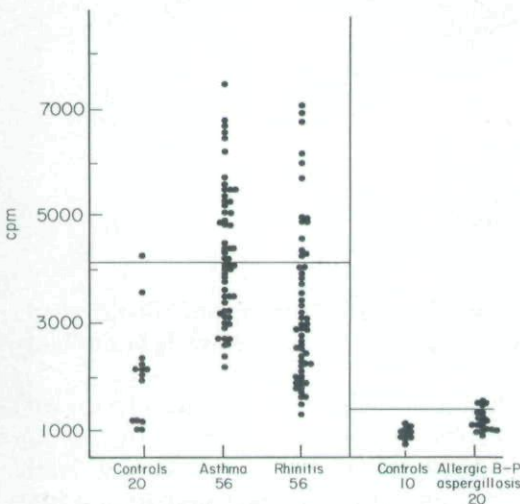


Fig. 8. Specific IgG₃ antibody to *C. albicans* yeast cells.

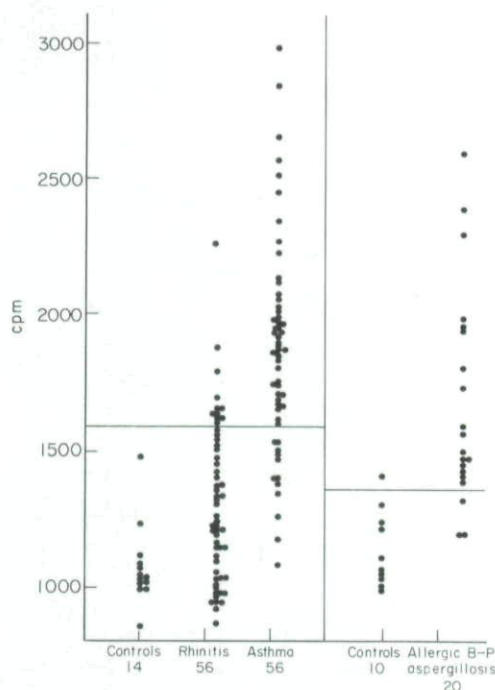


Fig. 9. Distribution of specific IgG₄ to *C. albicans* yeast cells.

Table 1. Number of patients giving positive values for specific antibodies to *C. albicans* yeast cells

Antibody class	Asthma (n=56)	Rhinitis (n=56)	Allergic broncho-pulmonary aspergillosis (n=20)
IgE	40 (71%)	38 (68%)	13 (65%)
IgA	42 (75%)	22 (39%)	20 (100%)
IgM	43 (77%)	30 (54%)	9 (45%)
IgD	15 (27%)	45 (80%)	1 (5%)
IgG	37 (66%)	26 (46%)	15 (75%)
IgG ₁	2 (4%)	7 (13%)	13 (65%)
IgG ₂	0	3 (5%)	14 (70%)
IgG ₃	28 (50%)	14 (25%)	4 (20%)
IgG ₄	44 (78%)	11 (20%)	17 (85%)

positive IgM antibody levels was significantly higher in asthma than rhinitis ($P < 0.01$) and ABPA ($P < 0.01$); similar proportions of patients gave positive IgM antibody results in the latter two groups.

Specific IgD values were significantly higher in rhinitis than in asthma ($P < 0.001$) and values for the asthma group were significantly higher than the ABPA group ($P < 0.01$). The number of patients with positive IgD antibody results was significantly higher in rhinitis than in asthma ($P < 0.001$) and greater in asthma than in ABPA ($P < 0.02$), where only one patient had a positive result.

Values for 'total' specific IgG antibodies to *C. albicans* masked differences which occurred in the specific IgG1-4 subclass values. 'Total' specific IgG antibody values were higher in asthma than in rhinitis and ABPA ($P < 0.05$) where values were similar. The number of patients giving positive 'total' specific IgG results was similar in asthma and ABPA, but significantly lower in rhinitis ($P < 0.02$).

The values for specific antibodies to *C. albicans* in IgG1 and IgG2 subclasses were highest in ABPA and higher in rhinitis than in asthma ($P < 0.001$ for both subclasses). The number of patients with positive specific IgG1 and IgG2 antibody results was greatest in ABPA and results were predominantly negative in asthma and rhinitis.

Values for specific IgG3 antibody were higher in asthma than in rhinitis ($P < 0.001$)

Table 2. Correlation between different classes of specific antibody to *C. albicans* in patients with allergic broncho-pulmonary aspergillosis

	IgD	IgA	IgM	IgG	IgG1	IgG2	IgG3	IgG4
IgE	$P < 0.001$ ($r = 0.710$)	NS	NS	NS				
IgD		NS	$P < 0.05$ ($r = 0.50$)	NS				
IgA			NS	$P < 0.02$ ($r = 0.66$)				
IgM				$P < 0.02$ ($r = 0.51$)				
IgG					$P < 0.001$ ($r = 0.782$)	$P < 0.001$ ($r = 0.724$)	$P < 0.001$ ($r = 0.801$)	$P < 0.001$ ($r = 0.746$)
IgG1						$P < 0.001$ ($r = 0.714$)	$P < 0.01$ ($r = 0.643$)	$P < 0.01$ ($r = 0.631$)
IgG2							$P < 0.001$ ($r = 0.795$)	$P < 0.001$ ($r = 0.720$)
IgG3								$P < 0.01$ ($r = 0.652$)

Table 3. Correlation between different classes of specific antibody to *C. albicans* in patients with asthma

	IgD	IgA	IgM	IgG	IgG1	IgG2	IgG3	IgG4
IgE	NS	NS	NS	NS				
IgD		NS	$P < 0.02$ ($r = 0.27$)	$P < 0.02$ ($r = 0.28$)				
IgA			$P < 0.01$ ($r = 0.32$)	$P < 0.01$ ($r = 0.519$)				
IgM				$P < 0.001$ ($r = 0.447$)				
IgG					$P < 0.001$ ($r = 0.624$)	NS	NS	NS
IgG1						NS	NS	NS
IgG2							NS	NS
IgG3								NS

Table 4. Correlation between different classes of specific antibody to *C. albicans* in patients with rhinitis

	IgD	IgA	IgM	IgG	IgG1	IgG2	IgG3	IgG4
IgE	$P < 0.02$ ($r = 0.72$)	NS	NS	NS				
IgD		NS	NS	NS				
IgA			NS	NS				
IgM				NS				
IgG					NS	$P < 0.01$	$P < 0.02$ ($r = 0.45$)	$P < 0.001$ ($r = 0.347$)
($r = 0.674$)								
IgG1						$P < 0.02$ ($r = 0.34$)	NS	NS
IgG2							$P < 0.01$ ($r = 0.412$)	NS
IgG3								NS

and lowest in ABPA. The number of patients with positive IgG3 antibody results to *C. albicans* was significantly higher in asthma ($P < 0.01$) than in the other two groups, where the number of patients giving positive results was similar.

Specific IgG4 antibody values were highest in ABPA and significantly higher in asthma than in rhinitis ($P < 0.01$). The number of patients giving a positive result for IgG4 antibody to *C. albicans* was similar in asthma and ABPA; the proportion of positive results was higher in these two groups than in the rhinitis group ($P < 0.001$).

Correlations between the levels of specific antibody to *C. albicans* in the different immunoglobulin classes, within each patient group, can be seen in Tables 2–4. There was no correlation between specific IgE and 'total' specific IgG values in any patient group. The patterns of correlation between different classes of antibody are different for each patient group. Values for specific IgA and IgG antibody to *C. albicans*, and for IgM and 'total' specific IgG antibodies, correlated in patients with asthma and in patients with ABPA, but not in patients with rhinitis.

Specific IgM and IgA antibody values showed significant correlation in the asthma group only. IgM and IgD antibody to *C. albicans* were significantly correlated in asthma and ABPA but not in rhinitis.

Specific IgE and IgD antibody values were correlated in rhinitis patients and in ABPA patients, but significant correlation between these two immunoglobulin classes was not seen in asthma.

Differences were also seen in the patterns of positive correlations between IgG subclass values in the three patient groups. 'Total' specific IgG antibody values correlated significantly with IgG antibodies in all four subclasses, in patients with ABPA; in patients with asthma significant correlation between 'total' specific IgG antibody and IgG1 was seen; in rhinitis significant correlation occurred between 'total' specific IgG and IgG2, IgG3 and IgG4 antibody values.

Significant correlation was seen between all the IgG subclass antibody values only in patients with ABPA.

Discussion

The assay described was suitable for detecting antibodies semiquantitatively in all classes of immunoglobulin; IgA, IgM, IgE, IgD and IgG, and in all four IgG subclasses.

In three-quarters of the patients with asthma, *C. albicans* cell specific antibodies were found in the three major immunoglobulin classes, IgG, IgA and IgM, and in the IgE class; specific IgD antibodies were found in one fourth of this group of patients.

The patients with rhinitis had the greatest number of positive IgD antibody results and these correlated with positive IgE antibody results in this patient group. Positive specific IgA, IgM and 'total' specific IgG results were found in approximately half the rhinitis patients.

All the patients with ABPA had positive specific IgA results, and all but 1 of this group had negative IgD antibody results to *C. albicans* cells. Positive IgE antibody and 'total' specific IgG antibody results were found in three-quarters of the ABPA patients.

Whilst significantly raised specific IgE antibody to *C. albicans* showed a similar prevalence in all three patient groups, overall specific IgE antibody values were not significantly higher in atopic than in non-atopic patients.

In the analysis of the IgG 1-4 subclass antibody results allowance must be made for the possible cross reactivity of the anti-IgG2 and IgG4 sera. The patients were however placed into well-defined groups, so that differences in the findings in terms of the different subclasses are regarded as of potential significance. Analysis of the specific IgG1-4 subclass values in the three groups of patients showed that, although the number of patients with positive 'total' specific IgG antibody results was similar in asthma and ABPA, and significantly lower in rhinitis, there were marked differences in IgG subclass values in each group. Thus in asthma and rhinitis, specific IgG1 and IgG2 antibody results were predominantly negative, whereas in ABPA, IgG1 and IgG2 antibody results were mainly positive. Specific IgG3 antibody to *C. albicans* was found to be positive in half the patients with asthma but only in one quarter of those with rhinitis and ABPA. Positive specific IgG4 results were found most frequently in ABPA and asthma, but in only 20% of the rhinitis patients. Thus it seems that the high number of asthmatic patients (66%) with positive 'total' specific IgG results is a reflection primarily of the IgG3 and IgG4 subclass antibodies whereas the high number of ABPA patients giving positive 'total' specific IgG antibody results is a reflection of the IgG1, IgG2 and IgG4 subclass antibody results. The lower proportion of rhinitis patients giving positive 'total' specific IgG antibody results is in agreement with the lower number of positive values in the IgG subclasses overall.

The patterns of specific antibody response to *C. albicans* cells seen in these three different groups of patients suggest that these differences in levels and prevalence of significantly raised values, are due to differences in the type of patient or variation in the antigenic stimulus. The alternative proposal, that they arise as a common consequence of antigen exposure, seems unlikely since the only differences then expected would perhaps be quantitative, in accordance with the length of time of antigen exposure. It seems that different responses to *C. albicans* antigens in the three disease groups examined, may have different consequences which may or may not be clinically relevant.

The presence of specific IgE antibodies to *Candida* cells in these three groups of patients with allergic respiratory disease means that a role for *C. albicans* in allergic respiratory disease merits consideration. It is possible that the finding of elevated levels

of cell-specific IgG4 antibodies, in patients with ABPA, may be associated with the presence of S-TS IgG antibodies as already reported to the *C. albicans* mannan in this group (Edge & Pepys, 1977). Evidence for larger amounts of IgG4 on the cells of patients with mould allergy, though not identified as specific mould antibody is another pointer in the same direction. (Vijay, Perelmutter & Bernstein, 1978).

Acknowledgments

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