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Defective yeast opsonization and C2 deficiency in atopic patients

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SUMMARY

Twenty-seven per cent of atopic patients initially presenting with infantile eczema or hay fever were defective for yeast opsonization and 18% had low levels of C2; these deficiencies were mutually exclusive, suggesting that they are primary. Both defects were associated with each of four different atopic syndromes, some of which were related to certain HLA haplotypes.

INTRODUCTION

Miller *et al.* (1968) described patients in two families whose sera failed to opsonize yeasts for phagocytosis by normal human polymorphonuclear leucocytes. One of the clinical features noted in these patients was dermatitis (probably atopic eczema) and this was also common in a series of children with frequent infections who had this serum defect (Soothill & Harvey, 1976). A high incidence of allergy, especially eczema, has also been reported in individuals and families with heterozygous C2 deficiency (Mowbray, 1976).

In this report we describe yeast opsonization, C2 activity and HLA type in patients with different atopic syndromes.

MATERIALS AND METHODS

The patients, who fall into two main groups, have been described previously (Turner *et al.*, 1977). Forty patients from a paediatric dermatology clinic (mean age 8·2 years), presented with atopic eczema before 2 years of age; twenty-six of them subsequently developed other manifestations of allergy (asthma and/or hay fever). Forty patients from an allergy clinic (mean age 23·7 years) presented with hay fever (allergic rhinitis); nineteen subsequently developed asthma but none gave a history of eczema. An allergic history was obtained from each patient and prick skin tests done to eighteen common antigens (Turner *et al.*, 1977).

Data from three control groups are reported. That for yeast opsonization comprised forty-three young adult friends of parents of patients with leukaemia, and twenty-nine young adult members of the staff of the Institute of Child Health; the subgroups gave values similar to each other and to eleven control children (Soothill & Harvey, 1976). Controls for C2 activity were eighty young adult members of the St. Mary's Hospital Medical School, and sixty-four healthy schoolchildren aged between 11 and 13, kindly provided by Dr M. A. Preece and Dr J. O. Warner.

Tissue typing was done within 24 hr of bleeding and serum samples for opsonization and complement studies were aliquoted and stored at −70°C until required. Yeast opsonization was measured as previously described (Soothill & Harvey, 1976) on seventy-seven patients' sera and results expressed as an opsonization index (mean number of yeast/polymorph—Y/P). C2 activity was measured in seventy-seven patients' sera, seventy-five of which were also used in the yeast opsonization test. 2 µl of the patient's serum were added to 20 µl of homozygous C2 deficient serum (patient ED). 100 µl of a 3% suspension of sheep cells optimally sensitized with rabbit antibody were added and the mixture incubated at 37°C for 15 min. The samples were diluted with 1 ml of cold barbitone buffered isotonic saline, centrifuged (1500 *g*) at 4°C and the released haemoglobin estimated in a Gilford spectrophotometer (E_{550nm}). Values for control tubes without serum were subtracted from each determination. Standard curves were constructed by the addition of functionally purified C2 (Lachmann, Hobart

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& Aston, 1973) to the deficient serum. Aliquots of the C2 preparation were stored at −70°C and each used only once. The C2 deficient serum used was also mildly deficient in C4 and Factor B (60% and 65% of normal respectively). However, the addition of functionally purified C3 to the assay system did not produce haemolysis and a large excess of serum ED over the test serum was used to compensate for the C4 deficiency. Sera from healthy controls were included with each batch of unknowns. Results, expressed as a percentage of a reference preparation (a pool of twenty normal adult sera) were the mean of four determinations (duplicates repeated on different days).

C3 was measured in most sera by single radial immunodiffusion (Mancini, Carbonara & Heremans, 1965). The control sera used for the C2 comparisons were also used for the C3 comparisons. The values were log normally distributed and so the normal range was defined by mean ± 2 s.d. on log transformed data.

Statistical analyses were by Fisher's exact test. HLA typing was performed as described previously (Turner *et al.*, 1977).

RESULTS

Serum yeast opsonization

The distribution curves of yeast opsonization indices for the seventy-seven atopic patients and for seventy-two healthy adults are plotted in Fig. 1. Previous studies suggest that there is no change with

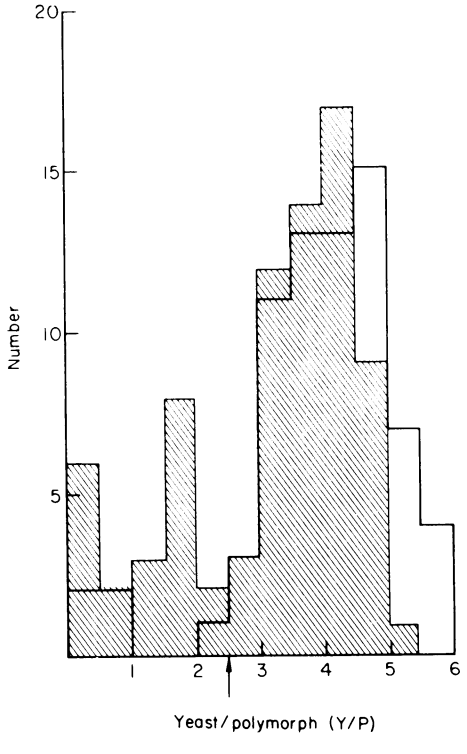

FIG. 1. Distribution curves of yeast opsonization by normal polymorphs in the presence of sera from seventy-two healthy laboratory workers and friends of parents of leukaemic children ( ), or sera from seventy-seven patients with atopy (▨). Results are expressed as numbers of yeast particles per polymorph.

age in the normal range reported here (Soothill & Harvey, 1976). The data for the patients form three peaks. Fifty-six patients (73%) gave values consistent with the major peak of the control population and above our laboratory diagnostic limit of 2·5 Y/P. Six individuals gave very low values (< 0.5 Y/P) and fifteen gave values between 0·5 and 2·5 Y/P.

The allergic illnesses in the twenty-one patients with defective opsonization are shown in Table 1. The percentage of defective sera in each of the four subgroups was greater than the 5% observed in the healthy population (Soothill & Harvey, 1976), and ranged from 18% in the patients with eczema com-

TABLE 1. Defective yeast oposinization, low C2 and HLA antigens in seventy-nine patients*

Syndrome	Defective yeast opsonization (< 2·5 yeast/polymorph)			Low C2 (< mean − 2·3 s.d. of controls)			Yeast opsonization and C2 both normal		
	Number	Number with A1 + B8	Number with A3 + B7	Number	Number with A1 + B8	Number with A3 + B7	Number	Number with A1 + B8	Number with A3 + B7
Eczema	5	1	0	2	1	0	6	1	0
Eczema plus asthma/hay fever	5	3	0	4	0	0	19	7	2
Hay fever and asthma	7	1	3	4	0	1	9	2	1
Hay fever only	4	1	0	4	0	2	11	1	0
Total	21†			14			45		

* Seventy-five patients were tested for both yeast opsonization and C2
† Three patients with defective yeast opsonization also had low C2 values

plicated by asthma and/or hay fever, to 45% of patients with eczema alone. We have already reported that HLA A1 + B8 was common in the patients of this series who had eczema plus asthma and/or hay fever, and HLA A3 + B7 was common in patients with hay fever and asthma (Turner *et al.*, 1977). The limited data suggest that these same relationships may apply to the patients with defective yeast opsonization as well as the group as a whole (Table 1). The frequency of HLA Bw35 in the whole series of seventy-seven patients (11%) was consistent with the expected value, but Bw35 was found in three of the six patients with very low opsonization (< 0·5 Y/P). This was significantly more frequent than in the other patients ($P < 0{\cdot}05$, without correction for the number of antibodies studied). Patients with each one of the four syndromes were observed in both the very low (< 0·5 Y/P) peak of yeast opsonization and in the intermediate peak (0·5–2·5 Y/P). The criteria for diagnosis of hay fever included positive

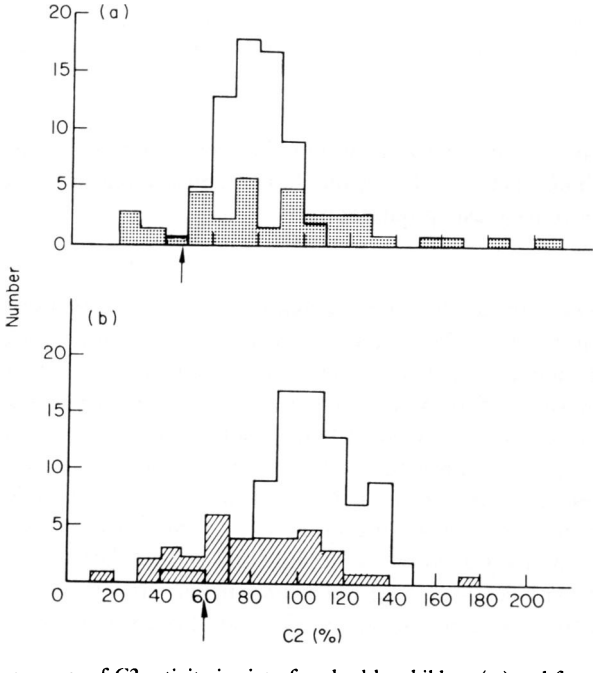

FIG. 2. (a) Distribution curves of C2 activity in sixty-four healthy children (□) and forty children presenting with infantile eczema (▨). (b) Distribution curves of C2 activity in eighty healthy medical students (□) and thirty-seven patients presenting with hay fever (▨). The diagnostic limit for each normal group (mean − 2·3 s.d.—see text) is indicated by arrows.

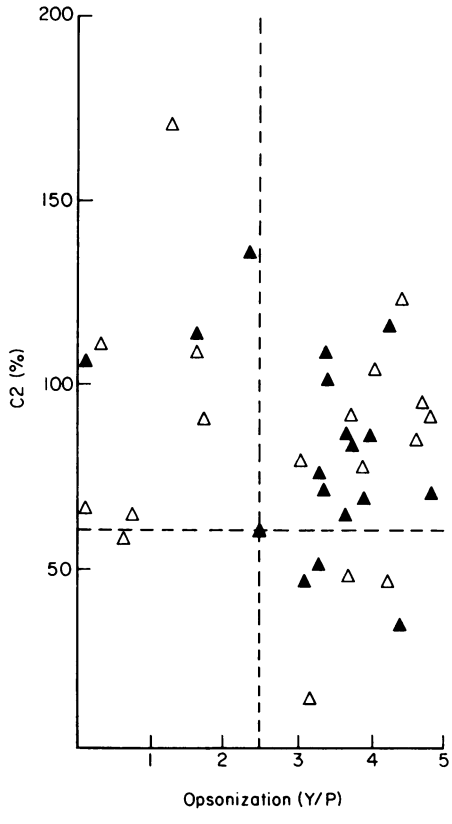

FIG. 3. The relationship between yeast opsonization and C2 activity in thirty-six patients who initially presented with allergic rhinitis (hay fever). The vertical dashed line indicates our laboratory diagnostic limit for yeast opsonization and the horizontal line is the mean − 2·3 s.d. value for adult C2 levels. (▲) = Hay fever; (△) = hay fever plus asthma.

skin tests, but this was not so for the eczema group. The nine patients with eczema and negative skin tests were evenly distributed between the uncomplicated and complicated eczema groups and among those with normal and defective yeast opsonization.

Serum C2 activity

The distribution curves of the adult and child control groups are plotted in Fig. 2. Values are rather higher for the adults than for the children; the former match the hay fever patients well for age and the latter match the eczema patients; they have therefore been analysed separately. Both patient distribution curves were more widely spread than were the controls; there were more low values in both, and more high values in children with eczema. The distribution plots of both patient groups are compatible with the existence of separate peaks comprising the lowest values, but this is far from clear and does not define a cut-off for presumed heterozygotes for C2 deficiency. In 509 subjects, Glass *et al.* (1976) reported six values presumed to be heterozygous, i.e. 1·2%; this corresponds to values less than mean −2·3 s.d., and we have applied this to the adult and child control data to define diagnostic levels. By these criteria six patients with eczema (15%) and eight patients with hay fever (22%) had low C2 levels. Patients with low C2 activity occurred in each of the four atopic syndrome groups (Table 1). Among the small number in each subgroup, the distribution of A3 + B7 is apparently similar to that of the group as a whole, but none of the ten patients with complicated eczema and HLA1 + B8 had low C2 activity. There was an insignificant trend for association of C2 deficiency with Bw40 (23% of low C2 patients compared with 11% expected), but no association with A10, B18 or Bw35 in this series.

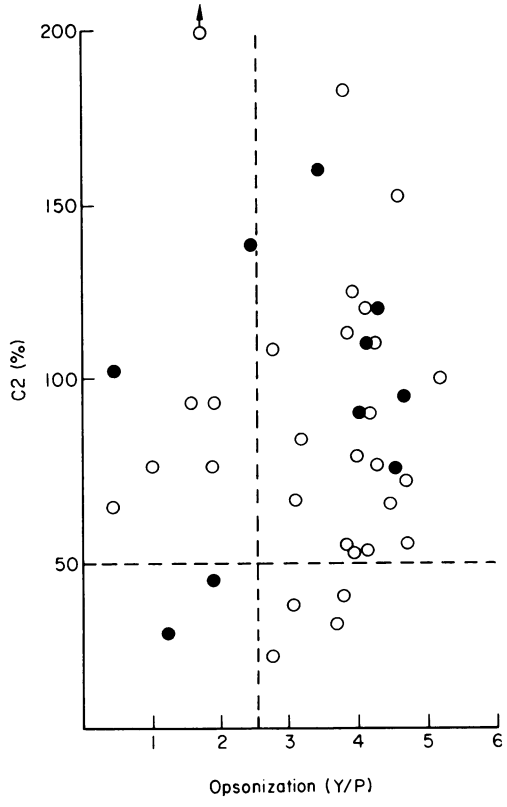

FIG. 4. Yeast opsonization and C2 activity in thirty-nine patients who initially presented with infantile eczema, plotted as in Fig. 3, with mean − 2·3 s.d. for control children indicated for C2. (●) = Eczema; (○) = eczema plus asthma and/or hay fever.

Deficiency of both yeast opsonization and C2 activity was observed in only one hay fever patient, and two eczema patients (Figs 3 and 4).

C3 levels of all fifty-eight samples tested were within the normal limits defined by our control sera.

DISCUSSION

Eczema and other allergies commonly occur in patients presenting with frequent infection who are deficient in yeast opsonization (Soothill & Harvey, 1976). The latter appears to be a functional defect of the alternative pathway of complement (Soothill & Harvey, 1977; Yamamura, Dasilva & Valdimarsson, 1978). It has also been reported that patients with rather low (presumably heterozygous) levels of C2 frequently have an allergic disease (Mowbray, 1976). The present study has shown that these defects are common in the atopic population (27% and 18% respectively).

The observed associations could arise if the defects were either causes or effects of the disease, or alternatively, they could be genetically but not aetiologically linked. If they were effects it would most likely be through consumption, but the normal C3 levels provide some evidence against C2 consumption. The virtual mutual exclusion of the two defects, they co-existed in only three of seventy-five samples—rather less than the population frequency of 1 in 20 for the opsonization defect (Soothill & Harvey, 1976; Yamamura *et al.*, 1978), establishes that they are independent and supports the view that they are not the result of *in vivo* consumption. It has been established that a low value in these

assays may be familial in the healthy relatives of symptomatic patients, both those with the opsonizing defect and frequent infections (Soothill & Harvey, 1976) and those with low C2 and rheumatic diseases (Gibson *et al.*, 1976). We therefore regard a primary role for these defects as probable. The high levels of C2 in some children with eczema may have been the result of its known acute phase reactive properties; this was not found in the adults with hay fever.

It has been suggested that atopy is an effect of defective antigen handling due to primary immunodeficiency and the association of atopy with transient IgA deficiency, which was detectable before disease presentation, is strong evidence for an aetiological role of immunodeficiency (Soothill, 1976). The presence of complexed IgE in atopics (Brostoff, Johns & Stanworth, 1977) lends support to the idea that antigen handling may be defective in these patients, perhaps as a result of low affinity antibodies to inhaled or ingested allergens, but there is no direct evidence that the defects we describe are causative.

We do not know the molecular basis of the defect in those sera with poor yeast opsonization. The *in vitro* consumption of C3 in human sera incubated with allergens (Berrens & van Rijswijk-Verbeek, 1973) suggests that complement may be relevant for atopy, and there is a possible mechanism for the role of an alternative pathway defect in the pathogenesis of much allergic disease. IgE antibody is induced experimentally by administration of very small doses of antigen with a powerful adjuvant (Jarrett *et al.*, 1976). When antigens are first ingested they readily pass through the gut mucous membrane. *E. coli* endotoxin activation of complement in the sera of patients with low yeast opsonization is defective (Soothill & Harvey, 1977); this could result *in vivo* in the abnormal persistence of endotoxin, which could act as an adjuvant for normally ingested antigen and lead to an IgE response. The way in which C2 deficiency could lead to defective handling at mucous surfaces is less clear, but it could result in defective clearance of normally absorbed antigen which could then cause sensitization.

There have been few studies of the restoration of complement defects by plasma infusions, and none in C2 heterozygotes, but plasma does produce a dramatic effect in patients with defective yeast opsonization (Miller *et al.*, 1968; Soothill & Harvey, 1976), especially on their diarrhoea. The effect on atopy needs further study. It is possible that neonatal infusion might prevent the atopy, as well as other manifestations of immunodeficiency (Soothill & Harvey, 1976).

In general, the association of the tissue antigen combinations A1 + B8 with complicated eczema and A3 + B7 with hay fever was observed in atopics with the opsonization defect, in those with low C2 and also in those with neither defect. However, the patients with low C2 and complicated eczema did not have the expected high frequency of A1 + B8.

A weak association of C2 deficiency with Bw40 has been noted previously (Gibson *et al.*, 1976), but we did not find a corresponding excess of A10 or B18. The increased frequency of HLA Bw35 in the patients with very low yeast opsonization, not observed in the patients with intermediate values, suggests that there may be two different defects. This association is also consistent with the view that the defect may be inherited as a dominant characteristic (Soothill & Harvey, 1976), and may be associated with a gene product of the sixth chromosome.

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