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Defective opsonization

A common immunity deficiency

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Soothill, J. F., and Harvey, B. A. M. (1976). *Archives of Disease in Childhood*, **51**, 91. **Defective opsonization: a common immunity deficiency.** Serum opsonization of yeasts for phagocytosis by normal polymorphonuclear leucocytes was defective in 11 of 43 children with unexplained frequent infections. The children had a range of infections, largely bacterial, and only 3 had diarrhoea and rash in infancy. A similar defect in at least 6 of the 9 mothers of these children (of either sex), with normal function in the fathers, suggests that the defect was primary and was transmitted by an unusual form of dominant inheritance.

Four of 72 healthy adults and 1 of 11 children with unrelated disease showed similar defective function, but the incidence of the defect in the patients with frequent infection was significantly greater than this. The defective function can be corrected, *in vitro* and *in vivo*, by normal plasma at concentrations too low to be effective alone. This suggests that there is a defective factor rather than an inhibitor, and that different factors are limiting in normal and in defective plasma. Sera from affected members of the same family do not correct each other, but defective sera from different families usually do.

The first immunodeficiency described was of opsonization. In 1904, Wright and Douglas reported that the serum of subjects with frequent staphylococcal infection failed to opsonize staphyloccus for phagocytosis by normal polymorphonuclear leucocytes (PMNs). They claimed improvement occurred after immunization. We are aware of no further work on individual variation on this, perhaps the most important mechanism for specific antibacterial immunity, until Miller *et al.* (1968) reported an association between a syndrome of infantile exfoliative dermatitis, diarrhoea, and frequent infections, and a defect of serum opsonization for PMN phagocytosis of killed bakers' yeast, a system described by Brandt (1967). Miller *et al.* (1968) reported that both symptoms and function could be improved by plasma infusions. We reported a family with such a defect, affected members of which had symptoms ranging from death in infancy through chronic osteomyelitis to excellent health, so it is clear that such defects could underly a wider range of symptoms than those Miller described (Scott *et al.*, 1975). We have therefore studied children with frequent bacterial infections suggestive of a defect of the antibody-complement-phagocyte system (Soothill, 1975), for which no obvious explanation had been detected.

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Opsonization is a complex function, involving at least antibody and complement. Since yeast and the yeast extract zymosan activate complement directly through the alternative pathway, it is likely that the yeast assay will not be dependent on the antigenic experience. We have therefore used it rather than Wright's staphylococcal assay for diagnosis, though we have also made some observations with staphylococci. We have made similar studies of the parents to confirm a familial abnormality and so to exclude the possibility of a defect secondary to the infection. We report 11 families with such a defect.

Materials and methods

Opsonization was measured by counting heat-killed yeasts phagocytosed by normal leucocytes in the presence of the patient's or control serum (Miller *et al.*, 1968). Leucocytes were separated from heparinized blood by dextran sedimentation, centrifugation at 200 *g*, washing, and resuspension in normal saline at 5×10^6/ml. Bakers' yeast was suspended in normal saline, heated in a water bath at 100°C for 30 minutes, filtered through

gauze, and resuspended in saline at 1×10^9 particles/ml. Venous blood was allowed to clot at room temperature and serum was separated within 3 hours. If the test was not carried out within 8 hours of collection the sera were stored at −70°C.

Serum was diluted 1 in 10 in saline. To 0·1 ml of this, in a 2·5 ml stoppered plastic tube, 0·2 ml of the leucocyte suspension and then 0·1 ml yeast suspension were added. The tubes were rotated on a Matburn mixer in a 37° incubator for 30 minutes. The tubes were centrifuged for 5 minutes at 50 *g*, the supernatant was removed, and the deposit was resuspended in one drop of saline. A spread drop was dried on a slide and stained by the May-Grünwald/Giemsa method. 50 neutrophil polymorphonuclear leucocytes were identified in areas where they were close, but not clumped. The number of yeasts in each was recorded (0–7 or >7), and the phagocytosis index—the mean (and median) number of yeast particles per polymorph—was determined. Duplicate tubes were set up from each of which duplicate slides were prepared (with rare exceptions) and serum from a healthy subject was run as a positive control with each batch.

Miller and Nilsson (1970) described a modification of this method, using Hanks's solution instead of saline and preincubation of yeasts with diluted serum before adding the polymorphs. We have compared the two methods and found that the latter gives higher values, but they ranked similarly. As we had used the original method for the control series, we have used it throughout this study. In some experiments reconstitution (mixing 0·05 ml of each of two 1 in 10 diluted sera), or addition (0·05 ml of 1 in 5 dilution of each serum) was done, and opsonization measured as above. Opsonization of staphylococci was measured by the method of Quie *et al.* (1966) using normal polymorphs prepared as above. PMNs, bacteria, and test or control sera were incubated for 20 minutes. The PMNs were centrifuged, washed three times, disrupted, and the bacteria were counted by the pour plate method.

Immunoglobulins G, A, and M, C3, and Factor B were measured by a modification of the radial diffusion method of Mancini, Carbonara, and Heremans (1965) using pooled sera from healthy adults as secondary standard, calibrated for immunoglobulins with the international reference preparation. Antisera to IgG, A, and M were from Wellcome Reagents Ltd, to C3 and GBG from Hoechst Pharmaceuticals Ltd. IgE was measured by a modification of the double antibody procedure (Gleich, Averbeck, and Swedlund, 1971) and results expressed as IU/ml. Total haemolytic complement, and the complement components were measured by the method of Lachmann, Hobart, and Aston (1973).

Results

Fig. 1 shows the yeast phagocytosis index for sera from 72 healthy adults, 35 females and 37 males. They were 29 laboratory workers and 43 friends of parents of children with leukaemia (Till *et al.*, 1975). The distribution of results is surprising: 4 (all well, 3 female including 2 laboratory workers) gave very low values. The distribution of the rest was consistent with a Gaussian one, and there is a gap between them and the 4 low ones. Repeated samples from these 2 laboratory workers gave consistently low values on repeated determinations. Omitting these 4, the female values were lower than the male ones, but the difference was not significant ($t=1{\cdot}86$, $P>0{\cdot}5$, 2-tail test). Means and standard deviations (SD) for the group (excluding the 4 low values) are shown at Fig. 1. We use mean −2 SD from these values, i.e. 2·5, as our lower limit of 'normal' for diagnostic purposes. Values for 11 children, ages ranging from 1 to 15, in hospital for noninfective surgical diseases, showed a similar distribution with one value less than 2·5 (0·14); the mean omitting this value was 3·91. Serum from Dr. Miller's patient: 2·02, 2·18.

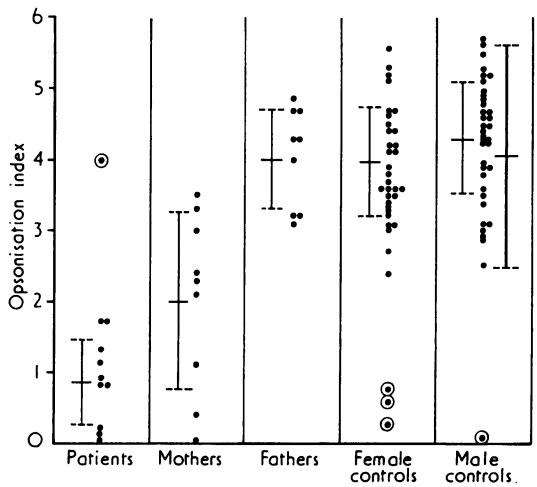

FIG. 1.—*Opsonization indices (mean yeasts/polymorph) for the 11 children with frequent infection and defective opsonization (Case 1 ⊙ had received plasma infusions before testing), 9 of their mothers, 9 fathers, and 72 healthy adults, 35 women and 37 men. Mean ±SD for each group is shown (excluding the values plotted ⊙, i.e. Case 1, and the 4 healthy adults with very low values). Mean ±2SD for the 68 healthy adults, after similar exclusions, are plotted on the right.*

We have measured yeast opsonization on sera from 43 children with frequent infections not explained by conventional tests. 10 gave values lower than this range, and one, who gave a value slightly above this limit (though well below the parallel control) is also included because she had already received plasma infusions, and 3 other members of her family gave abnormal values (Scott *et al.*, 1975). The incidence of the defect

is greater in the infected children than in either the healthy adults ($\chi^2=7\cdot8$, $P<0\cdot01$) or the children with surgical diseases ($\chi^2=0\cdot78$, $P<0\cdot5$).

Neither parent of Case 7 (Table I) was available for study; nor was the father of Case 11, or the mother of Case 6. The values for the 9 fathers studied did not differ from those of the male controls ($t=0\cdot96$, $P>0\cdot15$, one-tailed test) or from the control sera run in parallel ($t=0\cdot263$, $P>0\cdot49$, paired t-test, one-tail), and all were above the diagnostic value. The 9 mothers gave values significantly lower than the female control series ($t=5\cdot8$, $P<0\cdot001$, one-tail test); 6 were below the diagnostic level and 2 more were considerably lower than the control samples studied in parallel. The mothers gave values significantly lower than the fathers ($t=4\cdot24$, $P<0\cdot001$, two-tail test), and not significantly different from the affected children ($t=2\cdot59$, $P>0\cdot35$, two-tail test).

The main clinical features of these 11 children are shown in Table I. The infections were found mainly in the skin and the respiratory tract, and staphylococci were most frequently cultured. Other features included generalized rash 5, diarrhoea 5, mental defect 2, fits 3, and bone infection 2. Case 1 died with histiocytosis (Scott *et al.*, 1975), and Case 10 with intestinal perforation and epidermolysis bullosa, which was probably coincidental; the perforation and the defect were diagnosed rapidly on admission, but he remained too ill for laparotomy in spite of plasma infusions, etc. The mothers were well, except the mother of Case 3 had a possible increase of skin infection, occurring when her son had infections, and the mother of Case 8 had very severe and persistent local reactions to wasp stings.

Sera of 5 of our children, 3 of the mothers, and 1 of the healthy laboratory workers with defective yeast opsonization, and serum of Dr. Miller's patient were used for opsonization of staphylococci. The mean number of organisms counted in normal polymorphs after opsonization with these sera was $1\cdot28\times10^5$ (range $1\cdot08\times10^5$ — $1\cdot56\times10^5$) similar to that for the parallel healthy control serum ($1\cdot3\times10^5$), and higher than the mean of ten sera from healthy donors done in other batches ($9\cdot5\times10^4$).

Serum from a healthy subject with normal yeast opsonization, from one of the patient's mothers, and from a healthy laboratory worker giving low values, were studied in doubling dilutions from neat serum (10 times the usual amount) to 1:128 (Fig. 2). Little or no opsonizing activity was detected in any sera at 1:128, but above this the difference was observed throughout the range of dilutions.

Miller *et al.* (1968) described correction of the defect *in vitro* by addition of normal serum, even at a dilution which had no opsonization function itself. We have confirmed this (Table II). Little opsonization occurred with either 10 μl of the defective serum or 1 μl of normal serum; 5 μl of defective serum and 1 μl of normal serum gave considerably more opsonization, suggesting that different factors are limiting in these different preparations. Similar reconstitutions were done to all the defective sera (5 μl) by adding 5 μl of normal serum. Values for these mixtures did not differ significantly ($t=0\cdot8189$, $P>0\cdot2$, paired t-

TABLE I

Clinical findings

Case no.	Sex	Age of onset	Rash	Diarrhoea	Infection site	Organisms	Miscellaneous
1	F	4 w	+	+	Skin, bone, etc.	*Staph. aureus*	Died, histiocytosis
2	F	2 d	+	±	Skin, lungs, mouth, nails	*Staph. aureus* *C. albicans*	Mucocutaneous candidiasis
3	M	8 w	−	−	Ears, meninges	*Staph. aureus* *Strep. pneumoniae*	Mental defect, fits
4	M	2 yr	−	−	Skin, mouth	*Strep. aureus*	Brother has transient ID
5	F	6 m	−	−	Ears, lungs	—	Fits, allergy
6	M	3 yr	−	−	Skin	*Strep. pyogenes*	Calcification
7	M	4 m	+	+	Ears, lungs	*H. influenzae*	Died, heart failure
8	M	2 m	−	+	Ears, skin, lungs	*Ps. aeruginosa*	Mental defect, fits, poor growth
9	F	1 m	+	−	Bone	—	—
10	F	2 d	+	+	Skin, lungs	*Esch. coli* *Ps. aeruginosa* *Kl. spec.*	Died, epidermolysis bullosa, perforated duodenum
11	M	3 w	−	+	Lungs	*Strep. pyogenes*	Poor growth

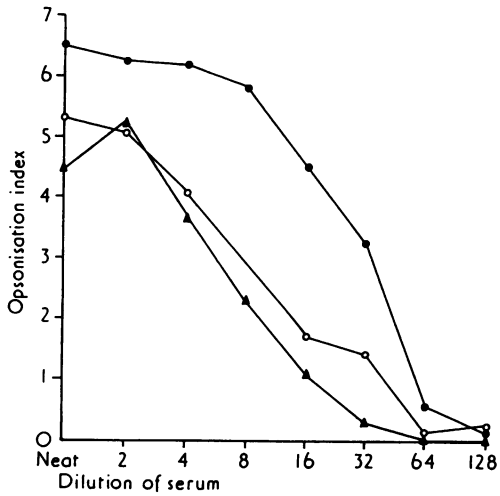

FIG. 2.—*Opsonization index (mean yeasts/polymorph) for neat and diluted serum from a healthy subject ●, the mother of Case 3 ○, and a healthy laboratory worker with the defect ▲.*

TABLE II

Correction by normal serum

Normal serum (μl)	Patient's serum (μl)	Opsonization index
10	0	4·34
0	10	0·42
1	0	0·05
1	5	1·58

test, one-tail) from those obtained from normal sera run in parallel; they are expressed as % of their parallel control in Table III. There was therefore no evidence of an inhibitory factor in any of the patients' sera, providing further evidence that the limiting factor in the patients' sera is different from that in the normal sera.

When sera were mixed in this way, between affected members of the same family, such correction usually did not occur; i.e. 6 of the 10 values are less than the expected (the mean of the values for the two mixed sera) and only 3 of the 10 combinations were >20% units greater. When sera

TABLE

Values of opsonization indices expressed as % of parallel normal control

Case no.	1 GF	1 GGM	1 M	2	2 M	3	3 M	4	5	5 M
1 GF	42	32/45	28/43		95/33	93/40	86/26	98/32		89/29
1 GGM		46	30/45		103/35	100/42	100/28	99/34		78/31
1 M			43		76/33	89/41	98/27	79/33		95/30
2				25	14/24					
2 M					23	95/31	89/17	93/23		80/20
3						38	30/24	96/30		91/27
3 M							10	85/16		54/13
4								22		88/19
5									48	19/32
5 M										16
10										
10 M										
8										
8 M										
7										
9										
9 M										
11										
11 M										
X										
Control										

The Table comprises 9 symptomatic children (see Table I) and their and other's relatives are indicated by the symbols M=mother, GF=grandfather, and GGM=great grandmother, an affected laboratory worker (X), and a healthy control.

Values are also given of mixtures of sera (upper figure) and the mean of the values for the two mixed sera (lower figure). Mixtures of 2 family members are underlined.

from different families, including one of the healthy adults with this defect, were mixed, correction occurred; all 83 mixtures gave greater opsonization than the mean values of the unmixed sera, and only 5 gave values <20% greater; all of these 5 involved the serum of one boy whose parents were not studied, and a girl and her mother. The simplest explanation of this is that most of these families have different defects, but alternative explanations are possible (see below).

Such correction by normal plasma occurs *in vivo* too, as described by Miller *et al.* (1968). This was the probable explanation of the relatively high values in our first patient (Scott *et al.*, 1975), who had already had plasma infusions when first studied, and values increased further when more plasma was infused. Only 2 of the other patients were ill enough to warrant plasma infusions. One died so quickly that effects could not be assessed; the other (Case 8, aged 2 years 5 months) had had a discharging mastoid wound for many months which stopped discharging after infusion of 3 units fresh frozen plasma, though discharge recurred with a diarrhoeal illness a few days after the last infusion, so any benefit was very brief. Opsonization tests on his serum showed definite and cumulative increase with the infusions (Fig. 3). The decay of the activity was consistent with a

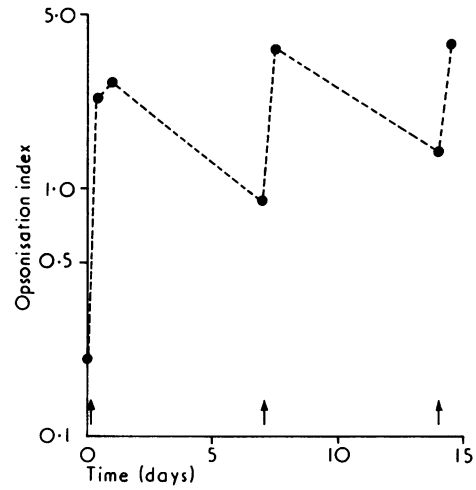

FIG. 3.—*Case 8, aged 2½ years. Opsonization index (mean yeasts/polymorph) before and after infusions (↑) of 3 units fresh frozen plasma.*

III

(bottom figure in each column) for 20 subjects defective for yeast opsonization

10	10 m	8	8 M	7	9	9 M	11	11 M	X	Control
	87/43	99/32		42/24	64/22	71/50		93/60	58/27	99/71
	102/45									107/73
	98/43					55/51		92/61		89/72
	80/33	76/23		36/14	48/13	67/41		75/51	85/18	95/62
	93/41					86/48				96/69
	84/27	79/16		39/8	41/6			80/44	76/11	90/55
	88/33	55/22		66/14	33/12			88/50	66/17	95/61
	92/30	71/19		49/11	73/9	88/37		86/47	77/14	82/74
28										
	43	70/30		35/24	32/23	92/51		99/61	65/28	80/72
		22	33/44	47/14	83/12	90/40			64/17	103/61
			66					98/72		95/83
				5	10/4	59/32		85/42	59/9	98/53
					2	68/30		85/40	31/7	79/51
						58	94/54		67/35	105/79
							50	110/64		
								78	94/45	111/89
									12	97/56
										100

half-life of 4 or 5 days. The values immediately after infusion were near to those of the normal serum run in parallel. This interesting finding may be related to the high values obtained from the *in vitro* mixtures and may have the same explanation, i.e. that the factor defective in the patient's serum is not the limiting one in normal serum.

Opsonization is usually a function of antibody and complement. Immunoglobulins were present at normal or high concentration in the sera of these children, except that IgA was not detected in the first sample for Case 5. An IgG antibody (ASO) was present in 4 of the 5 samples studied, and an IgM antibody (isoagglutinin) in all 4 studied (Table IV). Serum complement components were measured functionally, by Professor P. J. Lachmann, in 4 of these sera (Table V). All gave values within the normal range, with the possible exception of C4 in Case 7. He was very ill at the time, and this could well have been an effect of his illness. The younger brother of Case 4 also had frequent infections; he had normal opsonization and low levels of IgG, IgA, and IgM; his infections stopped without replacement treatment. Opsonization was normal in sera from 9 patients with various immunoglobulin deficiencies (Table VI).

Discussion

These 11 children confirm the deduction, drawn from our first family (Scott *et al.*, 1975), that defective yeast opsonization can be associated with frequent infection, without neonatal exfoliative dermatitis and diarrhoea, the syndrome described by Miller *et al.* (1968), and the symptoms need not be severe. We have not encountered other forms of neoplasm besides the histiocytosis seen in our first child (it is not certain that this is a neoplastic process) or chronic infections in adult members of the families besides the chronic osteomyelitis leading to amputation in her affected grandmother.

The high incidence (4 in 72) of similarly defective function in healthy adults and in children with irrelevant illnesses suggests that either the incidence in the children with recurrent infection was a chance one, or the defect is important in common disease; since the incidence was significantly greater

TABLE IV

Immunoglobulins and antibodies

Case no.	Age	IgG	IgA	IgM	IgE	ASO	Isoagglutinin	C3
		(IU/ml)				Units	Titre	% Standard
1	3 m	64	13	132	800	—	—	140
2	5 yr	224	120	328	800	16	Anti B $\frac{1}{32}$	—
3	14 m	100	30	112	11·2	ND	Anti A $\frac{1}{32}$	112
							Anti B $\frac{1}{4}$	
4	4 yr	148	28	112	48	256	—	—
5	16 m	120	ND*	152	—	—	—	88
6	6 yr	139	87	106	—	—	—	—
7	8 yr	160	94	376	2	—	—	—
8	2 yr	176	63	193	74	128	Anti A $\frac{1}{2}$	188
							Anti B $\frac{1}{2}$	
9	6 yr	135	61	106	14·4	512	—	120
10	3 m	80	36	94	—	—	—	76
11	19 m	254	226	193	—	—	Anti B $\frac{1}{2}$	480

*IgA was detected at rising concentrations in subsequent samples.
ASO, antistreptolysin O; ND, not detected; —, not studied.

TABLE V

Haemolytic complement (CH_{50}), complement components, and C3 proactivator (C3PA)

Case no.	CH^{50}*	C2†	C3†	C4†	C5†	C6†	C7†	C3PA†
10 M	1600	95		70	110	56	100	110
7	880	80	>50	32	62	113	240	70
8	1480	100		70	120	100	97	115
8M	1120	100		60	120	100	48	115

M, mother.
*units; † % pooled normal sera.

TABLE VI

Opsonization by hypo-γ-globulinaemia sera

Age (yr)	Sex	IgG	IgA	IgM	Opsonization index
		(IU/ml)			
3	M	15	3	9	4·80
11	M	3	3	5	3·94
4	F	40	18	60	3·06
10	M	20	3	5	4·98
5	M	13	3	9	2·50
7	M	6	0	0	3·02
4	M	12	32	16	4·14
3	F	136	20	152	4·32
18	M	115	0	172	3·94

in the children with unexplained frequent infection than in the control population, the first possibility is unlikely. Further work is therefore needed to investigate the significance of this heterogeneity in the aetiology of common infection, immunopathology (including cerebral disease), and neoplasia. We failed to detect the defect in families of children with leukaemia (Till *et al.*, 1975). It is another example of presumed genetically determined variation of immunity function, of potential disease significance, occurring within the conventional 'normal range', as we have shown for IgA deficiency and atopy (Taylor *et al.*, 1973).

The observation of similarly defective function in the mothers, who were mainly healthy, supports the view that the abnormality is the cause and not an effect of the infections, and that it is genetically determined. The affected children of either sex apparently got it only from their mothers since the fathers were all normal. This indicates a dominant inheritance defect of an unusual kind. A possible mechanism for the failure to detect children acquiring it from their fathers will be presented later, but that fathers can transmit this defect was shown in our first family (Scott *et al.*, 1975), since the maternal grandfather apparently transmitted the defect to his symptomless daughter, the patient's mother. Miller also detected abnormality in the mothers only of his 2 patients (Miller *et al.*, 1968; Jacobs and Miller, 1972), though he reported low values in other relatives on both sides of the family, which is even more difficult to interpret.

The infections these children had were mainly bacterial, especially staphylococcal; they usually involved the respiratory tract and skin, as in other defects of the antibody-complement-phagocyte pathway. Bone infection, meningitis, mental defect, and fits also occurred. 3 patients died, but the rest seem to get less infection as they get older. Wright's demonstration of defective staphylococcal phagocytosis in common staphylococcal skin infections (Wright and Douglas, 1904) is apparently similar, but we failed to detect defective staphylococcal opsonization in our patients, using a different technique from his. The mothers were mainly symptomless, but the mother of Case 3 often had skin infections when her son did, which may have been effects of both environmental and genetic predisposition; the mother of Case 8 had severe persistent reactions to wasp stings. We expected that the frequent infections in the younger brother of Case 4 would be due either to a similar defect, or to a repeated contact with his brother's infections, and we were surprised to find hypo-γ-globulinaemia. His symptoms have cleared, and though the immunoglobulins remain low, we suspect that he may have transient immunodeficiency. This may be a coincidence, but an aetiological link is possible (see later).

The nature of the defect is still unknown. Miller and Nilsson (1970) suggested that it was a functional abnormality of C5 in spite of normal haemolytic complement values, as occurred in our patients, because they found that purified C5 could correct the defect *in vitro*, as would normal mouse serum but not C5-deficient mouse serum. Since then homozygous C5 deficiency has been reported in man, and yeast opsonization by such serum is normal (Rosenfeld and Leddy, 1974). We chose the yeast assay because yeasts activate the alternative pathway of complement directly (Gütze and Muller-Eberhard, 1971), so variation of antigen contact would be relatively unimportant. We suspect that the defect lies in the alternative pathway of complement (we have some unpublished circumstantial evidence for this), but that the assay detects both alternate pathway and classical pathway activity. The correction of the defect by normal serum preparations too dilute to opsonize themselves, also shown by Miller and Nilsson

(1970), points strongly to the defect being a missing factor and not an inhibitor, and that different factors are limiting in the two preparations. This can explain why infusions of a relatively small volume of plasma can have such a large effect on the plasma opsonization activity. This, and the reasonable half-life of 4 or 5 days (this is falsely short since dilution into extravascular space will be occurring during the time of study) support the claim of value for plasma therapy reported by Miller *et al.* (1968). It should certainly be considered in acute infections in such children, but the fact that the children in this series have either died, or been in reasonable health has meant that prolonged prophylactic infusion has not been indicated, in our view. Symptomatic children with this defect will probably benefit from the increased awareness and general measures appropriate for management of immunodeficient children (Soothill, 1975) and this may well reduce the incidence of permanent disability at the stage when they largely grow out of their infections.

The strangest of our results are the inheritance, and the intercorrection between (but not within) families. The latter superficially suggests that we are studying 12 different defects, but the other similarities, and the limited number of known opsonizing factors makes this unlikely. An alternative hypothesis, which might explain both of these observations, could have important preventive implications. It is likely that the assay system can detect opsonization by antibody and classical pathway, as well as by the alternative pathway. Antibody is heterogeneous, even to simple antigens, and there are considerable individual differences in the antibody response to a single antigen, some of them genetically determined; these are likely to be especially marked in the antibody to a whole organism, and especially to bakers' yeast, which are probably mainly cross-reacting antibodies. There is evidence that complement may have a role in the afferent limb of the antibody response (Feldman and Pepys, 1974) and that, besides its well known negative effect, passively transferred maternal antibody may also have a positive role in the initiation of the antibody response (Segre and Myers, 1964). Perhaps the defective yeast phagocytosis is due to a primary abnormality in the alternative pathway of complement, which is detectable by the yeast phagocytosis test because the antibody to yeast in each affected individual is too limited to activate adequately the classical pathway; perhaps the combination of different antibodies in serum mixtures is adequate for this so the primary defect is bypassed. If maternal IgG antibody and complement have a positive role in the development of the child's immune response, it is possible that something similar happens in the initiation of the immune response in infants who acquire this gene from affected mothers. Those, like the mother of our first patient, who receive the gene from their fathers, are perfectly well because the defective alternative pathway of complement can be covered adequately (in our hygienic environment) by the classical pathway activated by a full and heterogeneous antibody response: this would occur because the passively transferred maternal functions (perhaps IgG antibody and possibly complement itself) contributing to the development of the child's response had been normal. If the child gets the gene from his mother, perhaps he gets defective initiation of his own antibody response, as well as the defective alternative pathway function; then he will be liable to infections. If this were so, it is possible that injection in such children of pooled IgG or plasma at birth could prevent symptoms permanently. The tendency for infections to improve in these children with age may represent a gradual development of the antibody response, so that a wide range of antibodies become available to activate the classical pathway. Perhaps the alternative pathway is specially important for immunity in the period of physiological hypo-γ-globulinaemia, when maternal antibody has decayed, and before the child has been primed to a wide range of infections. An alternative explanation for the inter-family correction is that antiallotype systems in the different serum samples is leading to sufficient classic pathway activation so that sufficient C3 is deposited on the yeasts as innocent bystanders, that they can be opsonized through C3 feedback pathway.

Most of the affected children had normal or high serum immunoglobulin concentrations and the opsonization test was normal in children with immunoglobulin deficiency. The observation of transient immunoglobulin deficiency in one of these children (Case 5) and the brother of another (Case 4), and possibly the thymus abnormality of Case 1, supports the view that there is some link between the defect and the development of the specific immune response.

A simple test has shown a common variation of human immunity function, which is sometimes important in health. This may be similar to that described by Wright and Douglas 70 years ago. Clinical value can be immediately gained from these results, but the full practical and theoretical possibilities deduced from it depend on identification of the defect and its experimental investigation.

We are grateful to colleagues who referred patients for study; to Dr. M. E. Miller for the serum of his patient; to Professor P. J. Lachmann for estimations of haemolytic complement; and to Mr. J. O'Shea for estimating the serum immunoglobulins, etc.

REFERENCES

Brandt, L. (1967). Studies on the phagocytic activity of neutrophilic leukocytes. *Scandinavian Journal of Haematology*, **Supp. 2.**

Feldmann, M., and Pepys, M. (1974). Role of C3 in *'in vitro'* lymphocyte co-operation. *Nature*, **249,** 159.

Gütze, O., and Muller-Eberhard, H. J. (1971). The C3-activator system: an alternative pathway of complement activation. *Journal of Experimental Medicine*, **134,** 905.

Gleich, G. J., Averbeck, A. K., and Swedlund, H. A. (1971). Measurement of IgE in normal and allergic serum by radioimmunoassay. *Journal of Laboratory and Clinical Medicine*, **77,** 690.

Jacobs, J. C., and Miller, M. E. (1972). Fatal familial Leiner's disease: deficiency of the opsonic activity of serum complement. *Pediatrics*, **49,** 225.

Lachmann, P. J., Hobart, M. J., and Aston, W. P. (1973). Complement technology. *Experimental Immunology*, Chapter 5. Ed. by D. M. Weir. Blackwell, Oxford.

Mancini, G., Carbonara, A. O., and Heremans, J. F. (1965). Immuno-chemical quantitation of antigens by single radial immunodiffusion. *Immunochemistry*, **2,** 235.

Miller, M. E., and Nilsson, U. R. (1970). A familial deficiency of the phagocytosis-enhancing activity of serum related to a dysfunction of the fifth component of complement (C5). *New England Journal of Medicine*, **282,** 354.

Miller, M. E., Seals, J., Kaye, R., and Levitsky, L. C. (1968). A familial plasma-associated defect of phagocytosis: a new cause of recurrent bacterial infections. *Lancet*, **2,** 60.

Quie, P. G., White, J. G., Holmes, B., and Good, R. A. (1966). Decreased bactericidal activity of polymorphonuclear leucocytes in children with chronic granulomatous disease. *Journal of Clinical Investigation*, **45,** 1058.

Rosenfeld, S. I., and Leddy, J. P. (1974). Hereditary deficiency of fifth component of complement (C5) in man. (Abst.) *Journal of Clinical Investigation*, **53,** 67a.

Scott, H., Moynahan, E. J., Risdon, R. A., Harvey, B. A. M., and Soothill, J. F. (1975). Familial opsonization defect associated with fatal infantile dermatitis, infections, and histocytosis. *Archives of Disease in Childhood*, **50,** 311.

Segre, D., and Myers, W. L. (1964). Antibody production in baby pigs. *American Journal of Veterinary Research*, **25,** 413.

Soothill, J. F. (1975). Immunity deficiency states. *Clinical Aspects of Immunology*, p. 649. Ed. by P. G. H. Gell, R. R. A. Coombs, and P. J. Lachmann. Blackwell, Oxford.

Taylor, B., Norman, A. P., Orgel, H. A., Stokes, C. R., Turner, M. W., and Soothill, J. F. (1973). Transient IgA deficiency and the pathogenesis of infantile atopy. *Lancet*, **2,** 111.

Till, M. M., Jones, L. H., Pentycross, C. R., Hardisty, R. M., Lawler, S. D., Harvey, B. A. M., and Soothill, J. F. (1975). Leukaemia in children and their grandparents: studies of immune function in six families. *British Journal of Haematology*, **29,** 575.

Wright, A. E., and Douglas, S. R. (1904). On the action exerted upon the staphylococcus pyogenes by human blood fluids and on the elaboration of protective elements in the human organism in response to inoculations of a staphylococcus vaccine. *Proceedings of the Royal Society*, **74,** 147.

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