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Measles infection

INVOLVEMENT OF THE COMPLEMENT SYSTEM

J. A. CHARLESWORTH, B. A. PUSSELL, L. P. ROY, M. R. ROBERTSON & J. BEVERIDGE
Departments of Medicine and Paediatrics, Prince Henry Hospital and University of New South Wales, and Department of Medicine, Royal Alexandra Hospital for Children, Sydney, Australia

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SUMMARY

The complement system was examined in fifty patients with acute, apparently uncomplicated measles; forty-six were children less than 10 years old. Twenty showed evidence of pathological complement activation. In thirteen of these the pattern was consistent with activation of the classical pathway while in the other seven data suggested utilization of an alternative pathway. An additional eleven patients had isolated reduction in C1q without alteration in concentration of other components; these were excluded from the classical pathway group. No patient had detectable immune complexes or C3 splitting activity in serum; however, it is suggested that the abnormal complement patterns observed are likely to indicate the presence of circulating immune complexes in a high percentage of patients with this infection.

INTRODUCTION

The measles virus has been implicated in the immunopathogenesis of a number of human diseases (Connolly *et al.*, 1967; Dayan *et al.*, 1967; Adams & Imagawa, 1962; Brown *et al.*, 1971; Peters *et al.*, 1973). There is now substantial evidence for its involvement in the aetiology of subacute sclerosing panencephalitis (SSPE) (Connolly *et al.*, 1967; Dayan *et al.*, 1967; Katz, Oyanagi & Koprowski, 1969; ter Meulen, Katz & Muller, 1972; Dayan & Stokes, 1972; Vandvik & Norrby, 1973) which is considered the prototype of prolonged 'slow' virus infection in man. There are diagnostically high titres of measles antibody in the serum of patients with SSPE (Connolly *et al.*, 1967) and Vandvik & Norrby (1973) have demonstrated a measles-specific oligoclonal IgG antibody in the cerebrospinal fluid (CSF) of such patients; morphological evidence also suggests the aetiological agent to be the measles virus (Johnson & Byington, 1971; ter Meulen *et al.*, 1972; Thormar *et al.*, 1973). However, it remains unclear whether the primary abnormality lies in the host or the infecting virus; indeed, both mechanisms have been implicated by certain authors (Lischner, Sharma & Grover, 1972; Sell *et al.*, 1973).

There is also increasing evidence of a relationship between measles and multiple sclerosis (MS) (Adams & Imagawa, 1962; Brody *et al.*, 1972; Salmi *et al.*, 1973). Patients with MS have elevated titres of measles antibodies in the serum and CSF and these serum antibodies have been shown to react with different structural components of the measles virus (Salmi *et al.*, 1973); similar reactivity has been demonstrated for the CSF antibodies in a smaller percentage of patients. Recent discoveries of an increased incidence of histocompatibility antigens (HL-A) 3 and 7 (Jersild, Svejgaard & Fog, 1972) and a single determinant (LD-7a) on mixed lymphocyte culture (MLC) (Jersild *et al.*, 1973) have raised the question of altered host immune-responsiveness to viral antigens in MS.

Peters *et al.* (1973) reported an association between an acute attack of measles and the clinical onset of partial lipodystrophy (PLD), a rare disorder of subcutaneous tissue known to be related to hypo-

Correspondence: Dr J. A. Charlesworth, Renal Unit, Prince Henry Hospital, Little Bay, New South Wales 2036, Australia.

complementaemic mesangiocapillary glomerulonephritis (MCGN) (Williams, Peters & Scopes, 1972). Dr Peters proposed that prolonged complement depletion, a feature of PLD, may predispose to subsequent immune tissue injury. In this study, we examined the complement system in fifty patients suffering from an acute, apparently uncomplicated attack of measles. There was evidence of pathological complement activation in twenty patients with involvement of the classical pathway in thirteen of these, while the remainder showed patterns consistent with utilization of an alternative pathway.

MATERIALS AND METHODS

Patients. Fifty patients were studied. All but four of these were children between the ages of 1 and 10 years who were admitted to the infectious paediatric ward. All had the classical clinical features of measles and, in some, diagnosis was substantiated by measurement of serum antibody titres.

Serum and plasma samples. Blood was collected by a single venipuncture within 48 hr of admission. Serum was separated and stored at −60°C with sodium azide (0·01%) as a preservative. Plasma was collected in ethylenediaminetetraacetic acid (EDTA).

Complement studies. Measurements of individual complement components were expressed as a percentage of a pool of serum from normal, age-matched controls. C1q, C1s, C1 esterase inhibitor, C4, C3, factor B (glycine-rich beta glycoprotein), Properdin (P), and C5 were measured by radial immunodiffusion (Mancini, Carbonara & Heremans, 1965) using monospecific antisera. Antiserum to human properdin was a gift from Dr A. F. Michael and was prepared according to the method of Pensky *et al.* (1968). Cobra venom convertase (CoFC) and C7 were measured by haemolytic assays in agarose as described previously by Lachmann, Hobart & Aston (1973).

C3 splitting activity (C3 nephritic factor, C3 Nef) was assayed by crossed antibody electrophoresis (Laurell, 1965) as described by Peters *et al.* (1972). C3 breakdown products were detected in fresh plasma in EDTA (0·02 M) by single-stage immunoelectrophoresis (IEP) using antibody specific to all three determinants of C3 (Charlesworth *et al.*, 1974).

Immune complexes. Each serum was assayed for immune complexes by the C1q precipitation test (Agnello, Winchester & Kunkel, 1970) using either purified C1q or fresh normal serum as a source of C1q.

Immunoglobulins G, A and M were measured by radial immunodiffusion.

RESULTS

Complement data are summarized in Fig. 1. A small percentage of patients did not have measurements of P, CoFC and C7. Serum concentrations of C1q were reduced in 48% of patients, C4 in 12%, C3 in 26% and C5 in 16%. There was evidence of activation of the classical pathway in thirteen patients. Ten of these had reduction in at least two of the components C1q, C4 and C3 and eight out of ten had C3d detectable in fresh plasma (see Fig. 2). The remaining three patients in this group had C1q values below 40%, positive C3d and normal serum concentrations of IgG. Twelve other patients had low C1q without reduction in C4; eleven of this group had normal levels of all other complement components (the other patient is described below) and only one had detectable C3d. Four had serum IgG values below 700 mg%.

In seven patients complement profiles were consistent with activation of an alternative pathway; four had reduced concentration of C3 and the others had C3 levels at the lower limit of normal with significant reduction in C5. C3d was positive in four of this group. All had normal concentrations of C4 and six out of seven had normal C1q. The other patient had mild reduction of C1q (54%) which was attributed to hypogammaglobulinaemia (serum IgG 660 mg%); his C4 level was 190%.

The complement profiles of the two subgroups showing pathological complement activation are contrasted in Fig. 3 and Table 1 summarizes details of C1q, C4, C3, P and C5 in each subgroup. Mean values for C1q, C4, C3 and P were reduced significantly for the entire group and for the subgroup showing classical pathway consumption. C3, P and C5 were reduced in the patients with alternative pathway utilization. There was significant correlation between levels of C1q and C4 for the whole group ($P<0·05$); however, there was no significant correlation between other pairs of components (i.e. $P>0·05$).

Serum levels of C1s, C1 esterase inhibitor, factor B and C7 were essentially normal. Values for CoFC varied widely, four patients failing to generate significant convertase activity (i.e. less than 10% of normal). C3 splitting activity was negative in all sera and immune complexes were never detected. IgG

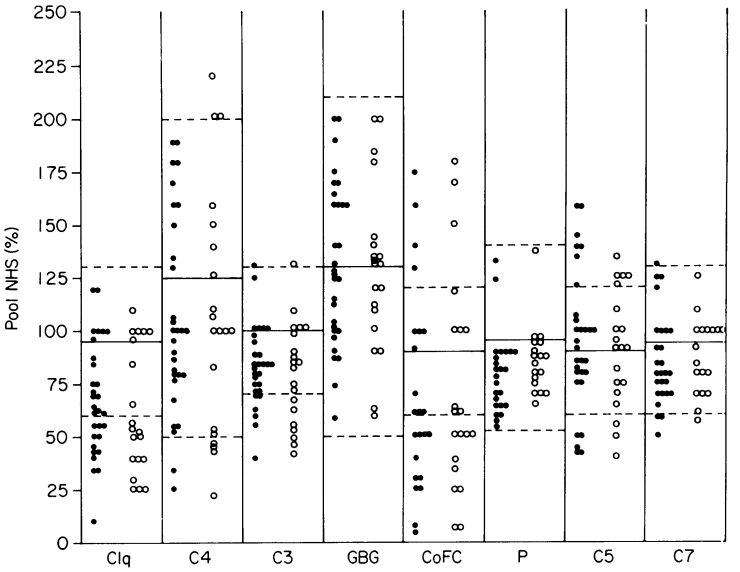

FIG. 1. Complement components in measles with serum concentrations expressed as a percentage of pooled normal serum (NHS). Means$\pm$2 s.d. are shown by solid and interrupted lines. ●, Negative C3d; ○, positive C3d.

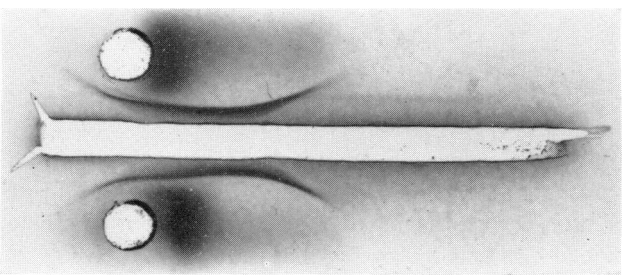

FIG. 2. Immunoelectrophoresis of fresh measles plasma showing presence of C3d (top well). Fresh normal plasma is in lower well. The antibody has reactivity to C4 as well as the A, B and D determinants of C3.

TABLE 1. Summary of complement components* for measles subgroups

		Clq	C4	C3	P	C5
All patients (N=50)	Range	10–120	22–220	40–130	54–137	41–160
	Mean	65·5	107·4	81·9	81·2	93·7
Patients with classical pathway activation (N=13)	Range	10–100	21–109	48–110	64–94	43–120
	Mean	40·9	62·7	79·6	75·8	84·9
Patients with alternative pathway activation (N=7)	Range	54–120	50–200	40–74	60–94	41–125
	Mean	90·3	146·4	58·6	74·6	65·6
Normal	Range	60–130	50–200	70–130	55–140	60–120
	Mean	95	125	100	96	90

* All values expressed as percentage of pooled normal serum.

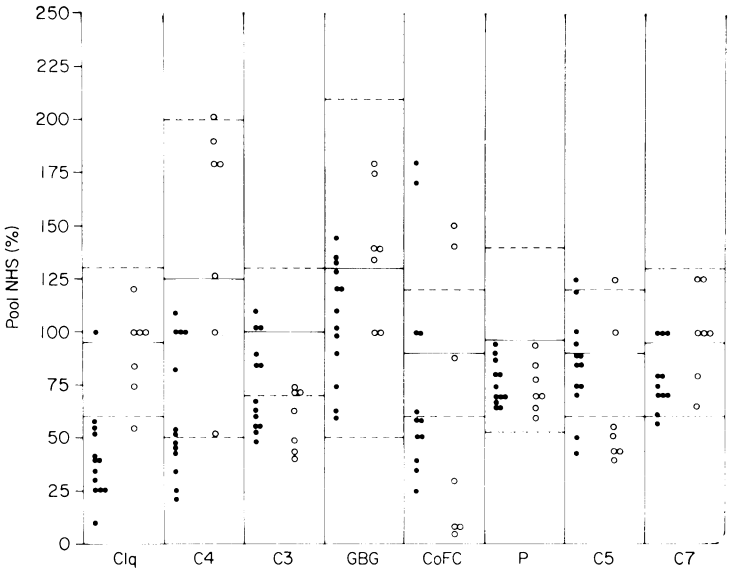

FIG. 3. Comparison of complement profiles for patients showing pathological complement utilization ●, Classical pathway activation; ○, alternative pathway activation.

levels ranged between 530 mg% and 1810 mg% (mean 995 mg%). Concentrations of IgA and IgM were low or normal.

DISCUSSION

Pathological activation of the complement system was demonstrated in 40% of fifty patients with acute measles infection, concurring with the previous work of Ecker *et al.* (1946) who found 35% of such patients to have reduced titres of total haemolytic complement. The most striking feature of the study was the low concentration of C1q found in twenty-four patients. In twelve of these there was no other convincing evidence of classical pathway activation and four of this group had moderately reduced levels of serum IgG, a factor known to influence C1q concentration independently of immune mechanisms (Kohler & Müller-Eberhard, 1969, 1972); the remaining eight patients, with low C1q and normal levels of IgG, were excluded from the classical pathway group; however, it was not apparent why reduction in C1q should occur in such patients. Williams *et al.* (1974) emphasized the limitations of interpreting complement data from serum measurements alone, as it has been shown (Alper & Rosen, 1967; Charlesworth *et al.*, 1974) that variations in complement synthesis and catabolism may occur without producing alteration in serum concentration. It is possible, therefore, that certain patients had pathological complement consumption despite approximately normal serum profiles. Similarly, Schutte *et al.* (1975) have demonstrated the 'acute phase' reactivity of C1s, factor B and, to a lesser extent C4, C3 and C5. This phenomenon would tend to obscure the changes of abnormal complement activation by elevating serum concentrations of these components. It emphasizes the severity of the pathological process in those patients where such concentrations were reduced. The absence of correlation between individual components—apart from C1q and C4—presumably reflects the presence of at least three subgroups within the total population, each exhibiting a different pattern of complement reactivity.

While pathological complement consumption seems likely to reflect the presence of circulating immune complexes in a high percentage of patients with acute measles, serious long-term sequelae are rare. Such an observation supports the view that chronic 'slow' virus disease is dependent on altered immune competence of the host as well as possible modifications in the immunobiology of the infecting organism itself. The failure to detect immune complexes by the C1q precipitation test may well reflect the in-

sensitivity of the assay and Dixon and co-workers, using a highly sensitive cytolytic assay, have reported the detection of complexes in two out of four patients with SSPE (International Congress of Nephrology, 1975).

Joseph, Cooper & Oldstone (1975) reported complement consumption by the measles virus *in vitro*. They showed this to be via an alternative pathway when antibody was directed against virus-infected cells while the classical pathway was activated when such antibody reacted against the free virus. The reason for different patterns of activation is not understood although it seems conceivable that both pathways may be required for the effective clearance of virus material in the individual patient. Peters *et al.* (1973) have observed alternative pathway consumption in patients with PLD, with or without MCGN, and our findings of a group of patients with a similar pattern of complement reactivity supports the proposed relationship between PLD and measles. It seems possible that persistent intracellular measles infection, potentiated by a defect in clearance of the virus, may lead to prolonged complement activation and depletion with the subsequent occurrence of an immune complex disorder. We could not find evidence of C3 splitting activity (C3 Nef) in these patients although such a phenomenon is a frequent finding in PLD. This failure to detect C3 Nef is not surprising in view of the short duration of the disease and the fact that reduction in C3 concentration was not profound (Vallota *et al.* (1972) have emphasized the correlation between hypocomplementaemia and the presence of C3 Nef.) Perhaps more sensitive methods will detect this phenomenon in such patients and it is clear that follow-up studies may yield important observations on the potential role of the measles virus in human diseases.

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