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IMMUNOCONGLUTININ AND COMPLEMENT CHANGES IN CHILDREN WITH ACUTE NEPHRITIS

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SUMMARY

Immunoconglutinin and electrophoretically altered forms of complement are detectable only after the fall in complement levels in acute nephritis, and may occur even when the fall is not noted. The delay between the postulated initiating streptococcal infection and the development of immunoconglutinin is longer than would be expected. The immunopathological significance of these findings is discussed.

INTRODUCTION

There is considerable evidence for an immunopathogenic basis for many forms of glomerulonephritis (Unanue & Dixon, 1967). The fall in serum haemolytic complement (C′) activity which occurs frequently in acute glomerulonephritis (Gunn, 1914; Lange *et al.*, 1951a,b; Walton & Ellis, 1958; Lange, Wasserman & Slobody, 1960; Gewurz *et al.*, 1968) and the observation of altered forms of the complement component C′3 (antigenic designation—β_{1C}-globulin) on immunoelectrophoretic analysis of sera from such patients (Soothill, 1965, 1967; West *et al.*, 1967) suggest the participation of C′ in an immunological reaction but the mechanism of pathogenesis of acute nephritis following streptococcal infection has not been precisely established. Alper & Rosen (1967) have found increased catabolic rates of β_{1C} in acute nephritis, which is consistent with this interpretation of complement data, though they have shown normal complement decay in some forms of nephritis with low serum complement. The involvement of the complement system in nephritis is obviously complex.

Abnormally raised levels of immunoconglutinin (Ik), autoantibody to hidden antigenic determinants of C′3 (Lachman & Coombs, 1965) and C′4 (Lachmann, 1966) which are revealed when they react with immune complexes, has been found in the serum of patients with acute nephritis (Coombs, Coombs & Ingram, 1961), providing further evidence for an immunopathogenic basis for this disease, but these previous studies have not reported precise information on the types of cases studied or on the time relations of this abnormality to the other changes.

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We report here the findings of a study of five children with the acute nephritis syndrome (four of them probably of streptococcal aetiology) in whom sequential determinations were made of haemolytic C′, antigenic determinations of β_{1C} (C′3) and immunoelectrophoretic qualitative demonstration of altered forms of β_{1C} and Ik estimation, from the acute phase through to convalescence.

MATERIALS AND METHODS

The five children studied (of whom T.A. and T.S. were siblings) had some or all of the following features: preceding sore throat, oliguria, oedema, hypertension, haematuria, proteinuria and azotaemia (Table 1). Group A β-haemolytic streptococci were isolated from three and an elevated anti-streptolysin O titre was observed in three, providing evidence of

TABLE 1. Clinical features of acute nephritis syndrome

Patient	Age	Sex	Preceding sore throat	Oliguria	Oedema	Maximum BP	Haematuria	Proteinuria	Highest blood urea	Throat swab	Highest ASO
T.S.	7¼	M	0	+	+	160/110	+	+	76	+	600–800
T.A.	5¾	M	+	+	+	190/100	+	+	53	+	Not recorded
D.M.	6	M	+	++	++	120/90	++	++	288	+	>800
C.G.	10	M	+	−	+	150/88	+	++	56	0	50–200
M.R.	7¼	F	0	+	+	130/90	++	++	256	0	333

ASO, Anti-streptolysin O (u/ml).

streptococcal aetiology in four of the five; no such evidence was obtained in patient C.G. All patients received standard conservative therapy including penicillin. Four patients recovered clinically, but C.G. had persistent haematuria and heavy proteinuria 3 months after the initial illness.

Control sera obtained from nineteen healthy children aged 2–12 years undergoing minor operations, such as repair of uncomplicated hernias, were also studied for C′, β_{1C} and Ik, and sera from a further twenty-eight older school children, selected at random, were estimated for β_{1C} and Ik.

Haemolytic complement assay

Haemolytic complement activity (C′) was estimated by the method of Mayer (1961) on serum from clotted venous blood, on the day of collection or, after storage at −20°C, on the following day, using sheep red blood corpuscles, stored at 4°C in Alsever's Solution (Wellcome Reagents Ltd) and rabbit haemolytic serum (Wellcome Reagents Ltd). Sensitized sheep cells and test serum were incubated for 90 min.

Anti-complementary activity was studied in all sera with C′ < 20 units/ml in both heated (56°C for 30 min) and unheated serum in the following way:

Test serum was added to serum from a healthy subject in the proportions 1, 10, 20 and

50%, and these, with saline controls, were incubated at 4°C for 1 hr and then titrated for C′ activity. Anti-complementary activity was regarded as present if the complement value was reduced by more than 20% of the value expected from the volume of complement-containing serum used. In the two sera in which such activity was detected, it could be detected at the 10% tube.

Immunoelectrophoresis

Blood (venous or capillary) was taken straight into bottles containing EDTA. Immunoelectrophoresis of the plasma was carried out in 'Ionagar' No. 2 (Oxoid Ltd, London) with barbitone buffer, pH 8·6, within 30 min of taking the blood, using a rather long electrophoretic run to accentuate the electrophoretic separation in the β region. Fresh normal plasma collected similarly was run in parallel in every case. The antiserum was raised in rabbits by a modification of the method of Ellis & Gell (1958) using *Salmonella typhosus*, and certain contaminating antibodies were removed by absorption with the 'exclusion' peak material from Sephadex G-200 ultrafiltration of normal human serum.

β_{1C}-Globulin estimation

β_{1C} and derivatives (hereafter referred to as β_{1C}) were estimated by the single gel diffusion precipitin method (Mancini, Carbonara & Heremans, 1965) using the same antiserum. The values are expressed as a percentage of a reference serum.

Immunoconglutinin titration

The conglutination reaction was performed by the method of Coombs *et al.* (1961)—Method IIb. Serial dilutions of the test sera, in dilute inactivated horse serum, were examined for their ability to clump sheep red blood corpuscles sensitized with a suitable bovine serum containing naturally occurring Forssman antibody and alexinated with horse complement. The following controls were included in each run:

(a) *Positive control*: first peak material from Sephadex G-200 ultrafiltration of hyperimmune rabbit serum (rabbit injected with human serum albumin).

(b) *Negative control*: known human serum with no demonstrable Ik activity.

(c) *Specificity control*: sheep red cells were exposed to heat inactivated (56°C for 30 min) horse serum and anti-sheep red cell antibody. The conglutinating reaction was performed with these cells and the test serum, at the highest concentration used in the conglutinin titration, to control for clumping action other than conglutination.

RESULTS

As is shown in Fig. 1, there is no obvious trend of β_{1C} values of control subjects with age, and their distribution appears to be logarithmic. Hence log transformation of data has been used for statistical calculation. The lowest values obtained from the patients are also plotted; these are clearly low in four and normal in one. Haemolytic C′ values of controls are also apparently log distributed with no age trend in the controls (Fig. 2). Hypocomplementaemia is seen in four (the same subjects who had low β_{1C}), with a normal C′ level in one. In one (T.S.), no evidence of complement activity was detected, and he was the only patient with anti-complementary activity which was detected in the last two sera studied

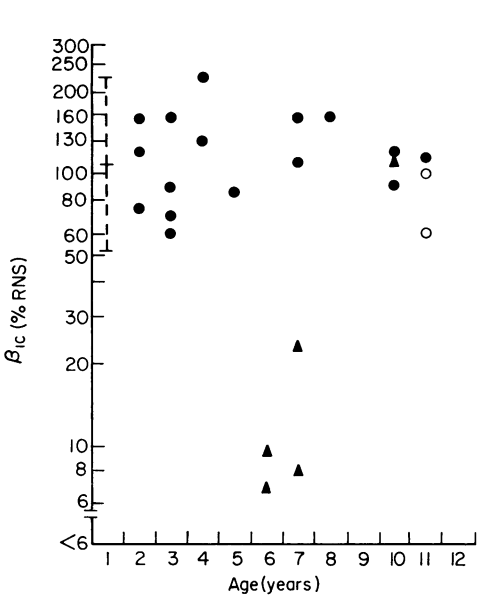

FIG. 1

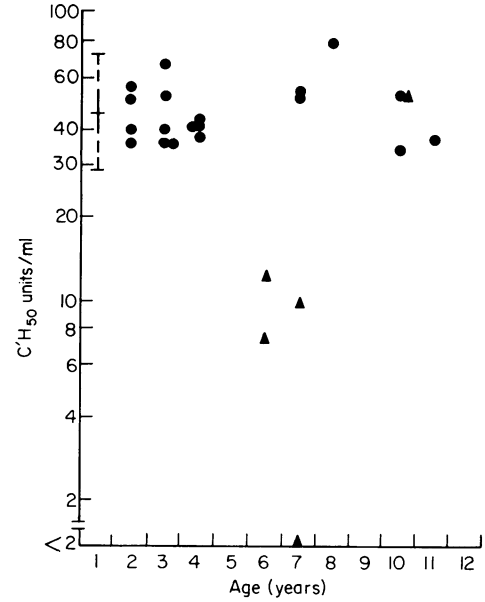

FIG. 2

FIG. 1. Concentration of β_{1C} in serum from healthy children (● = hospital controls; ○ = school controls) and the lowest values detected in five children with acute nephritis (▲). Broken vertical line, geometric mean; ±2 SD calculated from logged data.

FIG. 2. Serum haemolytic complement values plotted as in Fig. 1.

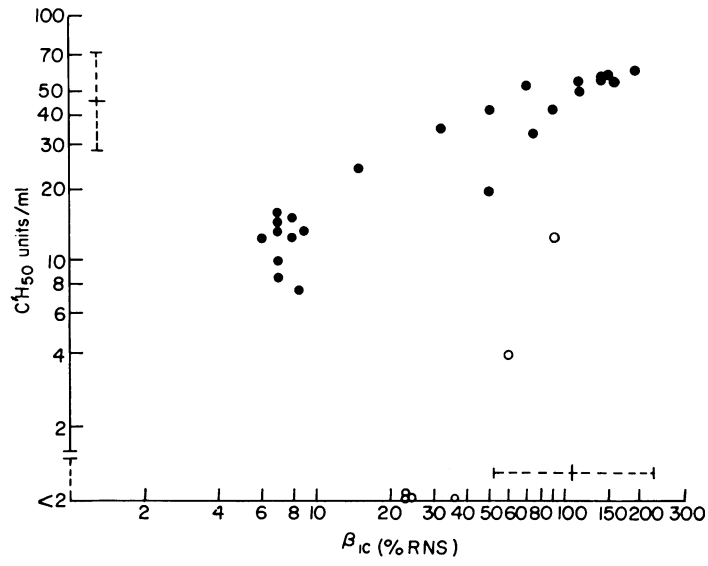

FIG. 3. The relationship between serum haemolytic complement and β_{1C} in the patients with acute nephritis. The values for the patient T.S. who had anti-complementary activity at the recovery phase, are plotted (○). Geometric means and 2 SD for the control population are plotted (broken lines).

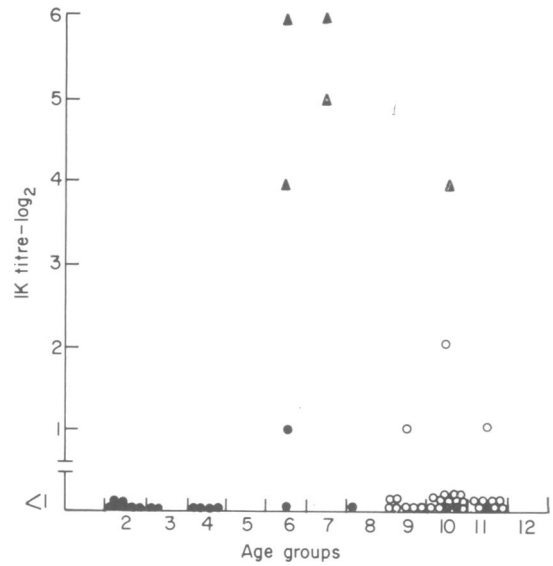

FIG. 4. Serum immunoconglutinin values plotted as in Fig. 1.

(Fig. 7). This was detectable when about 10% of the patient's serum was added to complement-containing serum, but no such activity was detected in the earlier sera of this child, even at 50% mixture with the complement-containing serum. The anti-complementary activity was thermolabile. There was a good correlation between serum C′ and β_{1C} values (Fig. 3) in the results obtained from all patients except T.S., in whom C′ was relatively lower than β_{1C}. This can readily be explained for the last two sera from this child because of their observed anti-complementary activity, and the lack of correlation between β_{1C}

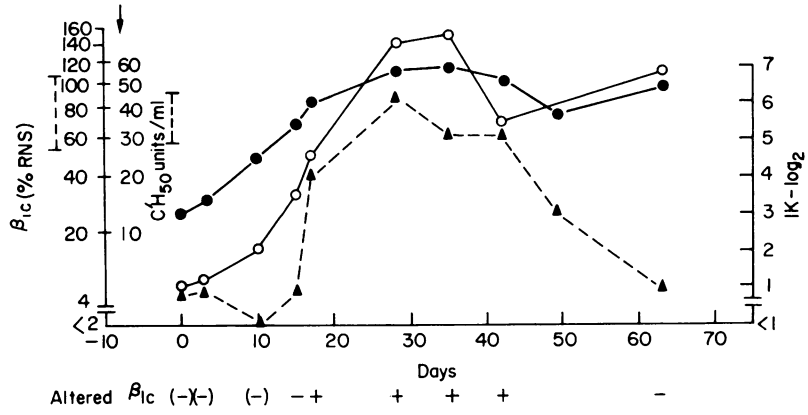

FIG. 5. Serum immunoconglutinin (▲), haemolytic complement (●) and β_{1C} (○) in acute nephritis (D.M., boy, 6 years) following streptococcal infection; mean and −2 SD for the healthy population indicated (vertical broken line). Immunoelectrophoretically determined altered β_{1C} indicated + or −, or (−) if the β_{1C} level was too low for detectability of the altered forms. Onset of symptoms indicated by arrow. Day 0 is date of admission.

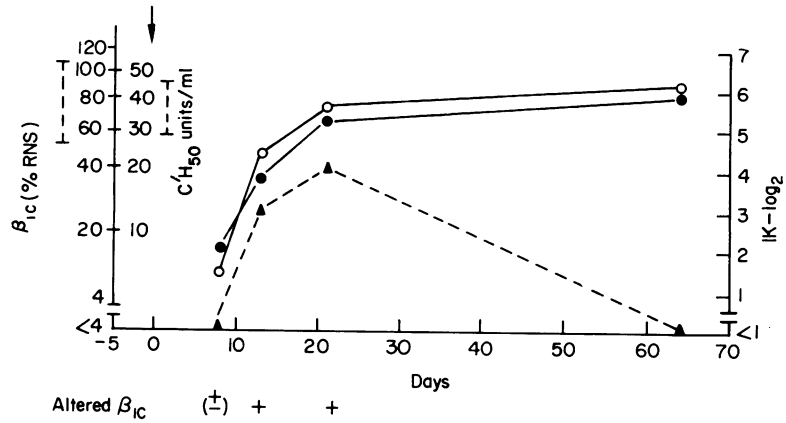

FIG. 6. Serum immunoconglutinin and complement changes in post-streptococcal acute nephritis (patient T.A., boy, 6 years) plotted as in Fig. 5.

and C′ in the other sera from this child suggests that this is a more sensitive method of detecting anti-complementary activity than was the titration we used.

Immunoconglutinin of $\frac{1}{2}$ or greater titre was detected in only four of the forty-seven control children studied. The positive sera were from older children, but their number is too small to constitute a trend with age (Fig. 4). The highest value obtained from each of the five patients is strikingly elevated.

Altered forms of β_{1C} were seen in four of the patients studied. It was detectable only in sera with β_{1C} level high enough for clear delineation of the precipitin arcs, which apparently became limiting when the β_{1C} was about 20% of the standard. In one patient (M.R.), in whom levels of β_{1C} were persistently less than this, the altered β_{1C} was not detected. A range

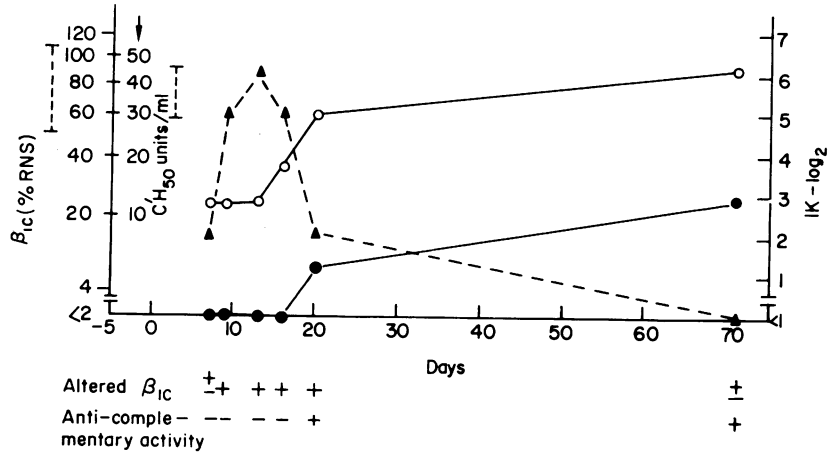

FIG. 7. Serum immunoconglutinin and complement changes in post-streptococcal acute nephritis, plotted as in Fig. 5 (patient T.S., boy, 7 years). This patient had anti-complementary activity in the recovery phase.

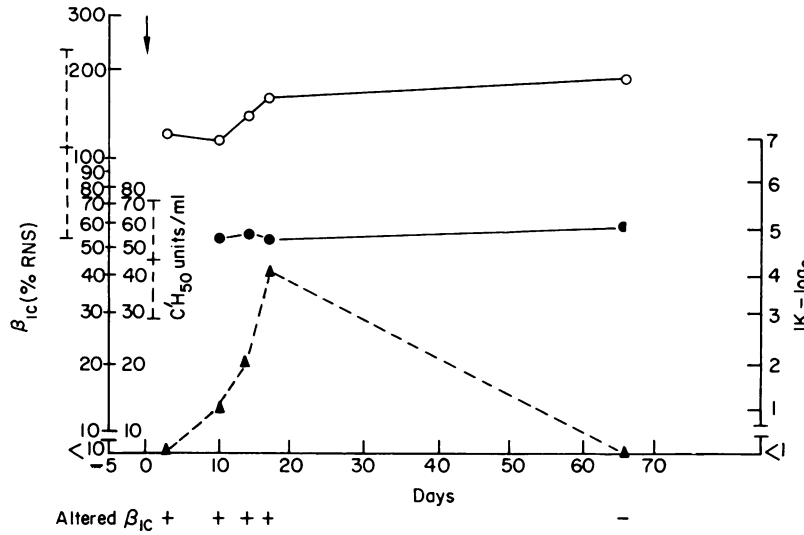

FIG. 8. Serum Ik and complement changes in the nephritis syndrome (in patient C.G., boy, 10 years) in whom there was no evidence of streptococcal aetiology or fall in serum complement, plotted as in Fig. 5.

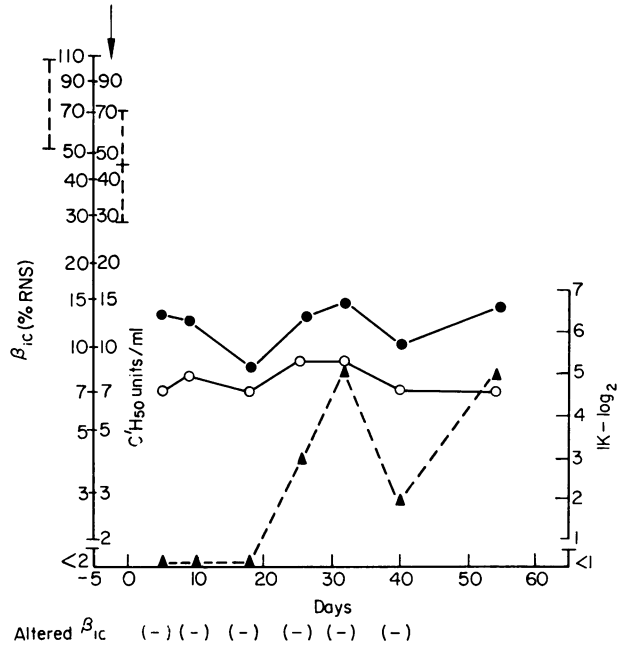

FIG. 9. Serum immunoconglutinin and complement changes in acute nephritis following streptococcal infection, plotted as in Fig. 5, in a patient (M.R., girl, 7 years) in whom the β_{1C} level was too low for detectability of the altered forms by immunoelectrophoresis, indicated (−). The serum complement was persistently low throughout the illness.

of qualitative differences was noted in the immunoelectrophoretic appearances of the altered β_{1C}, as was described by Soothill (1967).

The sequential results in the five patients are indicated in Figs. 5–9. Certain similarities are noted. In all except patient C.G., who had normal complement levels throughout (he was studied from the day of onset of symptoms), C′ and β_{1C} were low on the 1st day of study. Rise in serum complement was noted in the period 12–20 days after onset of symptoms, and as the level of β_{1C} rose to about 20% the altered component became detectable. It was detectable throughout the disease period in the patient C.G.

Ik was usually undetectable at the onset of study, and rose after 10–36 days of symptoms, to reach a peak in about 7 days. It, and the altered β_{1C}, were the last abnormalities to disappear. Ik persisted in the child with persistent hypocomplementaemia. The behaviour in the child whose complement levels never fell was similar to that of those who did.

Follow-up of renal function is still at an early stage at the time of writing. Proteinuria and haematuria have virtually disappeared in all except patient C.G. who differed in not having a low complement, and having no evidence of streptococcal aetiology. Only a trace of proteinuria persisted in patient M.R. in spite of persistent hypocomplementaemia and elevated Ik.

DISCUSSION

The four phenomena studied are closely interrelated. There have been previous reports of parallelism of C′ and β_{1C} (Ellis & Gell, 1958) and between C′ and the functional measurement of β_{1C}–C′3 (Gewurz *et al.*, 1968) in acute nephritis. The latter showed some heterogeneity of the effect of different forms of nephritis on different complement components. The discrepancy resulting from anti-complementary activity in all sera of a patient, in only some of which such activity could be detected by titration, was interesting.

It is likely that the altered β_{1C} detected by immunoelectrophoresis arises as a result of involvement of β_{1C} in an immune reaction (Soothill, 1967). It is intelligible that there must be enough of the protein in one or other of its forms for it to be detectable at all, before the altered forms can be detected, so the detection of this only when the levels of β_{1C} rose is not surprising. Material detected in this way is probably not soluble antigen–antibody complex, but we do not know whether it has toxic effect on vessels, cells, etc.

Immunoconglutinin is antibody to reacted β_{1C} (C′3) and C′4, though its relationship to the substances defined by immunoelectrophoresis is not clear. The presence of both at the same time suggests that they are not simply antigen and antibody. Ik occurs 5–10 days after an antigen stimulus (Marks & Coombs, 1957) and it was detectable in the first few days of upper respiratory infections in humans (Ngu, Barratt & Soothill, 1970), so, since there is extensive evidence that acute nephritis follows streptococcal infection 12 or more days later (Earle & Jennings, 1959), it is surprising that the Ik in acute nephritis occurs so late—probably over 32 days after the infection in one case. One possible explanation is that there was so much reacted complement that the patients remained in a state of antigen excess for this system for some days. One must, therefore, consider whether Ik has a beneficial autoimmunity—or whether it contributes to the formation of toxic complexes.

The numbers are too small to recognize significant clinico-pathological relationships in this group, but it is worth noting that the only patient with persistence of significant renal function abnormality was the one patient without evidence of streptococcal aetiology who never had low serum complement. Two of the others still had minor urinary abnormality

2–3 months after onset of symptoms; previous experience suggests that this is not incompatible with full recovery. The syndromes were initially indistinguishable. The child with normal complement levels throughout differed in no way, clinically, from the rest. The altered β_{1C} was detected from the start in him. This phenomenon, with normal complement levels has been noted previously (Soothill, 1967). Of course, one cannot exclude a complement depression before the time of study, since symptoms of acute nephritis may be very mild at first.

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