Clin. exp. Immunol. (1978) 33, 425–429.

Identification of nephritic factor as an immunoglobulin

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(*Received* 13 *March* 1978)

SUMMARY

C3 nephritic factor (C3NeF) activity in sera from three patients with mesangiocapillary glomerulonephritis, one of whom had partial lipodystrophy, was found on chromatography to be associated with fractions containing IgG and no other detectable proteins. Immunoadsorption of IgG from these fractions with a highly purified anti-IgG removed the C3NeF, and the IgG, eluted after combination with the anti-IgG, retained C3NeF activity.

In each case the isolated IgG with C3NeF activity was found to contain more than one subclass of IgG and both kappa and lambda chains, indicating that the immunoglobulin comprising C3NeF in these patients is heterogeneous and not monoclonal.

The identification of C3NeF as an immunoglobulin suggests that it may be an autoantibody against antigenic determinants of complement components present in the C3 convertase of the alternative pathway.

INTRODUCTION

Nephritic factor (C3NeF), recognized by its ability to break down C3 in normal human serum *in vitro*, was originally detected in the serum of patients with hypocomplementaemic mesangiocapillary glomerulonephritis (MCGN) (Spitzer *et al.*, 1969). It has since been found in patients with partial lipodystrophy (PLD) alone (Peters *et al.*, 1973; Thompson & White, 1973; Sissons *et al.*, 1976), PLD and glomerulonephritis (Peters *et al.*, 1973; Sissons *et al.*, 1976), and PLD with recurrent infections (Alper, Bloch & Rosen, 1973; Sissons *et al.*, 1976). A similar factor has also been detected in one virtually normal person who had none of these conditions, but presented with a minor arthralgia of the thumbs (Karstorp, 1976).

C3NeF cannot therefore be defined precisely by its occurrence in any one disease. Indeed, the term 'nephritic factor', although its use is continued, is now a misnomer as it is found in patients without nephritis. Its only accepted definition has been functional—as a serum factor which breaks down C3 via the alternative pathway of complement activation. Attempts to define it immunochemically have led to two contrary answers; the first report showed C3NeF activity to be associated with IgG (Thompson, 1972), whereas subsequent work described C3NeF as a distinct serum protein separable from IgG (Vallota *et al.*, 1974).

In this paper we describe the isolation of C3NeF from the sera of three patients with MCGN, one of whom also had PLD, and its characterization as IgG.

MATERIALS AND METHODS

Patients. Each patient had MCGN of dense deposit type demonstrated histologically. One of them had partial lipodystrophy affecting the face.

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0099-9104/78/0900-0425$02.00

Normals. Sera for control observations were obtained from two healthy adults.

Sera. Sera were stored at −70°C with sodium azide 0·1%.

Antisera. Commercial antisera to C3, C1q, factor B, IgG, κ chains, λ chains, IgM, whole human serum (Behringwerke) and to IgG_1, IgG_2, IgG_3 and IgG_4 (Nordic) were used. Antiproperdin antiserum was the gift of Professor D. K. Peters, Royal Postgraduate Medical School, London.

Anti-IgG for immunoadsorption. Human IgG for the preparation of monospecific anti-human anti-IgG in large quantities was made by fractionation of Cohn fraction II on DEAE 52 (Whatman) using 0·02 M Tris/HCl buffer pH 7·0 and then on carboxymethyl (CM) Sephadex C50 (Pharmacia) using 0·01 M phosphate buffer pH 6·0. The purified IgG gave single lines on double immunodiffusion against anti-IgG and anti-whole antisera, and no detectable lines against anti-IgM.

Antiserum to the purified IgG was raised in rabbits, precipitated with 50% ammonium sulphate, redissolved and dialysed against sodium bicarbonate 0·1 M pH 8·3 with 0·5 M NaCl. On double immunodiffusion, the final preparation produced only a single line against normal human serum in varying concentrations and against commercial IgG (Behringwerke).

Chromatography of patients' and controls' sera. After dialysis against 0·02 M Tris/HCl buffer pH 7·0, 10–15 ml of serum were applied to a DEAE 52 column 2·5 × 50 cm equilibrated with the same buffer. Following elution with NaCl of increasing molarity (0·05 M to 0·5 M), fractions were concentrated (Amicon 52; filter PM 10) and tested for C3NeF activity. Pooled fractions with detectable activity were dialysed against 0·01 M phosphate buffer pH 6·0 and applied to a CM Sephadex C50 column 30 × 2·5 cm equilibrated with the same buffer. Elution was performed with the starting buffer with increasing concentrations of NaCl from 0·03 M to 0·33 M. The fractions comprising separate peaks were pooled, concentrated, dialysed against phosphate buffered saline (PBS) and examined for C3NeF activity. Active fractions were concentrated and underwent molecular sieve chromatography on Sephadex G200 (Pharmacia) using 0·2 M PBS pH 7·4.

Immunoadsorption. Anti-IgG was coupled to cyanogen bromide (CNBr) activated sepharose 4B (Pharmacia). The serum fractions containing C3NeF, which also contained IgG, were applied to a CNBr-anti-IgG column, and IgG subsequently eluted by cycling of (i) glycine-HCl 0·1 M pH 3·0 with 0·5 M NaCl and (ii) PBS 0·3 M pH 7·2 buffers. Acid eluates were brought to pH 7·0 with 0·01 M NaOH, the eluates combined, concentrated, dialysed against PBS and tested for C3NeF activity.

IgG was removed from the serum fractions with C3NeF activity by anti-IgG solidified with gluteraldehyde following the method of Avrameas & Ternynck (1969). Four ml of anti-IgG prepared in our own laboratory was stirred at room temperature and 1·5 ml of 2·5% gluteraldehyde in water was added dropwise with the formation of a precipitate. After repeated washing with PBS and centrifuging until the supernatant was protein-free (O.D. zero at 280nm), the precipitate was incubated at 4°C with a sample of IgG with C3NeF activity with constant stirring for 2–3 hr. The mixture was centrifuged and the supernatant examined for IgG content and C3NeF activity, after ultracentrifugation to remove endotoxins and IgG aggregates.

Double immunodiffusion. This was used against the appropriate monospecific antisera for the detection of IgG in C3NeF containing fractions of chromatographed serum, the exclusion of other detectable proteins, and the characterization with respect to IgG subclasses, κ and λ chains of IgG found to have C3NeF activity.

C3 splitting. This was detected by crossed immunoelectrophoresis (Peters *et al.*, 1972) and the requirement of the reaction for Ca^{++} and Mg^{++} examined by addition of EDTA 0·01 M and EGTA 0·01 M + Mg^{++} 0·08 M respectively.

RESULTS

Isolation of C3NeF

In each patient's serum C3 splitting activity independent of Ca^{++} but requiring Mg^{++} was detected in the first protein peak eluted from the DEAE column (1·71–2·00 mmhos at 4°C) and subsequently in the third protein peak obtained from the CM Sephadex column (5·16–5·97 mmhos at 4°C) (Figs 1 and 2). For each patient C3NeF activity from these concentrated fractions appeared in the first protein peak of the Sephadex G200 column. Following concentration, the C3NeF-containing pool from each patient was found to contain only IgG on double immunodiffusion against anti-IgG, anti-IgM, anti-C1q, anti-C3, anti-B and anti-properdin, and only one line was detected against anti-whole antiserum.

Immunoadsorption

Incubation of each of the three patients' IgG-containing C3NeF fractions with solidified anti-IgG produced a supernatant in which IgG was undetectable by double immunodiffusion against anti-IgG. Each supernatant was devoid of C3 converting activity. The IgG from the three C3NeF-containing fractions, which was eluted from the CNBr-anti-IgG column, had C3-converting activity requiring Mg^{++}, but not Ca^{++}, in each case.

Characterization of IgG with C3NeF activity

The IgG eluted from the CNBr-anti-IgG column was used for each patient. Analysis by double

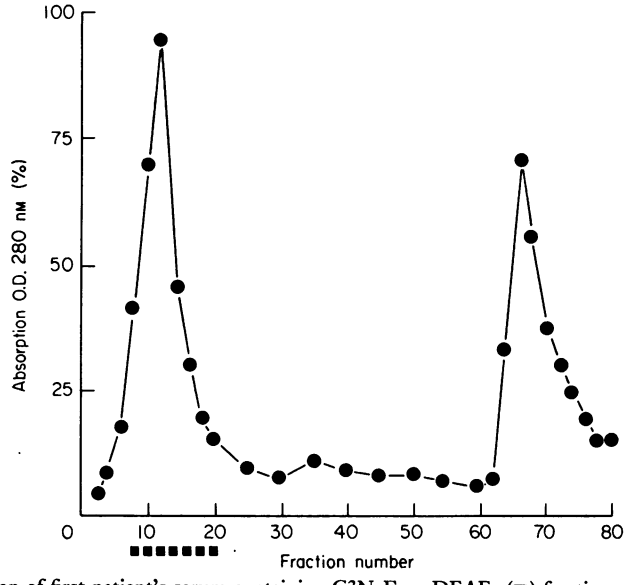

FIG. 1. Fractionation of first patient's serum containing C3NeF on DEAE; (■) fractions containing C3NeF activity. The C3NeF in the sera of the two other patients was found in equivalent fractions.

immunodiffusion of the IgG with C3NeF activity in each case showed it to contain IgG_1, IgG_3, κ and λ chains; IgG_2 and IgG_4 were not detected.

Controls

Chromatography, as described above, of normal human serum from two fit adults produced purified IgG with no detectable C3 splitting activity. Adsorption to and elution from the CNBr-anti-IgG column of these separate samples of IgG did not render them capable of converting C3.

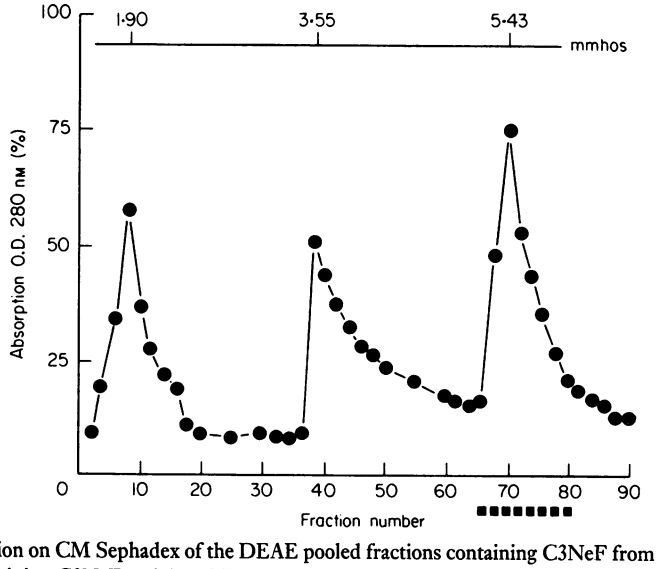

FIG. 2. Fractionation on CM Sephadex of the DEAE pooled fractions containing C3NeF from the first patient; (■) fractions containing C3NeF activity. C3NeF in the DEAE pooled fractions of the two other patients was found in equivalent fractions.

DISCUSSION

The chromatographic and immunoadsorption studies on the three patients' sera containing C3NeF have shown that in each case C3NeF was inseparable from IgG. These observations confirm and extend the original claim by Thompson (1972) that C3NeF is an immunoglobulin, and do not support the findings in three patients in whom C3NeF was found to be a protein immunochemically separable from IgG with γ electrophoretic mobility and a molecular weight of 150,000 daltons (Vallota *et al.*, 1974). The IgG's in our patients were found to contain more than one subclass of IgG and both κ and λ chains. This suggests that the IgG comprising C3NeF may be heterogeneous and oligoclonal in origin, although further purification experiments are needed to clarify this point.

Other work has recently been reported showing that C3NeF was immunochemically associated with IgG. Using anti-Fc or anti-Fab antisera to immunoadsorb C3NeF either in partially purified form or in native serum, Davis *et al.* (1977b) found that C3 activating ability was removed, and subsequently recovered on elution. These authors also found both κ and λ chains in two separated IgG's, but in other samples they detected either κ or λ chains alone.

Sissons *et al.* (1976) demonstrated that in the sera of two patients with PLD and without nephritis C3NeF activity following chromatography was associated with IgG and no other detectable proteins; immunoadsorption experiments were not performed.

Circumstantial evidence for C3NeF being an immunoglobulin has been provided by the study of a pregnant patient with PLD, when C3NeF activity was detected in the baby's serum for two weeks following birth, indicating transplacental transport (Davis *et al.*, 1977a). Electrophoresis of C3NeF-containing sera with development of haemolytic zones produced by C3NeF, and isoelectric focussing of the same sera with subsequent demonstration of bands of haemolysis have both produced evidence of heterogeneity consistent with C3NeF being an immunoglobulin (Davis *et al.*, 1977b).

The demonstration that C3NeF is an immunoglobulin does not directly further understanding either of its role in the pathogenesis of the diseases in which it occurs, or its function in the alternative pathway of complement activation. C3NeF binds to and acts as a stabiliser of the alternative pathway convertase $\overline{C3bBb}$, prolonging its half-life and therefore effective C3 activation (Daha, Fearon & Austen, 1976). Since C3NeF is an immunoglobulin, its binding to the alternative pathway convertase may be an expression of antibody activity against antigenic determinants revealed in the convertase. Thus, C3NeF could be considered to be an autoantibody which has evolved to increase the degree of activation of the alternative pathway. Autoantibodies reactive with complement components are already recognized. These are the immunoconglutinins, which are mostly of the IgM class (Henson, 1968), and are directed against determinants on C3b. Immunoconglutinins against C4 and $\overline{C567}$ have also been detected (Thompson & Lachmann, 1970), and thus there is a precedent for autoantibodies developing against components of the complement system.

Hypocomplementaemia in MCGN and PLD is due, in part, to C3 hypercatabolism through C3 activation, as well as to reduced synthesis (Charlesworth *et al.*, 1974). It has been suggested that the hypocomplementaemia in MCGN precedes the nephritis and predisposes to it by rendering the subject immunodeficient (Peters & Lachmann, 1974). C3eNF obviously plays an important role in this process, but it is still not clear whether it is the prime abnormality or secondary to another mechanism responsible for maintaining the continued complement activation.

The demonstration in several cases of MCGN, PLD, or both that C3NeF is an immunoglobulin does not exclude the possibility that other forms of C3 activator could occur in these diseases. Apart from the non-immunoglobulin protein already mentioned, a serum fraction containing IgG and IgA which activated the alternative pathway in a case of MCGN has been reported (Berthoux *et al.*, 1974). Since the molecular weight of this fraction was greater than 200,000, it may have been an immune complex. In another instance, a cryoprecipitate isolated from the serum of a patient with MCGN exhibited alternative pathway activation, but had no detectable immunoglobulins or complement components (Bartlow, Roberts & Lewis, 1977).

We thank the Medical Research Council for funding this work and the Wellcome Trust for support for Dr D. Gwyn Williams.

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