Hypocomplementemia caused by C3 nephritic factors (C3 NeF): clinical findings and the coincidence of C3 NeF type II with anti-C1q autoantibodies.

Skattum, Lillemor Melander; Mårtensson, Ulla; Sjöholm, Anders

Published in:
Journal of Internal Medicine

1997

Citation for published version (APA):
Hypocomplementaemia caused by C3 nephritic factors (C3 NeF): clinical findings and the coincidence of C3 NeF type II with anti-C1q autoantibodies

L. SKATTUM, U. MÄRTENSSON & A. G. SJÖHOLM
From the Department of Medical Microbiology, Clinical Immunology Section, Lund University, Lund, Sweden


Objectives. The main purposes were to document manifestations associated with prolonged or clinically unexplained C3 deficiency and to approximate how often hypocomplementaemia of this kind is caused by C3 nephritic factors (C3 NeF), i.e. autoantibodies to alternative pathway C3 convertases. We also wished to distinguish between C3 NeF types I and II and to assess coincident autoantibody responses to the collagen-like region of C1q (C1qCLR).
Setting. The investigation was based on serum samples referred to a specialized laboratory for complement analysis in the course of several years.
Subjects. Twenty-five persons with C3 concentrations lower than 0.43 g L⁻¹, a third of the normal, were included in the study.

Results. Analysis using three methods provided evidence of C3 NeF in 20 persons with equal frequencies of C3 NeF types I and II. We also gave evidence of antibody specificity differences for the two types of C3 NeF. Six patients with C3 NeF type II showed antibodies to C1qCLR. Membranoproliferative glomerulonephritis was the predominant diagnosis and two patients had partial lipodystrophy reflecting the well-known association between these diseases and C3 NeF. Anaphylactoid purpura, systemic lupus erythematosus, and severe infection, mainly meningococcal disease, were also observed.

Conclusions. The study group was probably fairly representative of C3 deficiency syndromes as encountered in clinical practice. The findings emphasize the heterogeneity of C3 NeF, and that acquired C3 deficiency syndromes caused by C3 NeF should perhaps be considered more often in diagnostic work.

Keywords: autoimmunity, complement 3 nephritic factors, glomerulonephritis, meningococcal infections.

Introduction

Inherited complement deficiencies predispose to immune complex diseases and to invasive infections, illustrating the protective importance of the complement system [1–3]. Acquired deficiencies such as C3 deficiency caused by C3 nephritic factors (C3 NeF) may have similar pathogenetic consequences. C3 NeF are autoantibodies against alternative pathway C3 convertases [4–6], and are functionally defined by their capacity to stabilize these enzyme complexes against spontaneous decay [7] and to interfere with the regulatory actions of factors I and H [8,9]. C3 NeF produce hypocomplementaemia with low C3 concentrations in vivo due to the combined effects of C3 hypercatabolism and reduced C3 synthesis [10]. Hypocomplementaemia caused by C3 NeF is most clearly associated with membranoproliferative glomerulonephritis (MPGN) [11] and partial lipodystrophy (PLD) [12], but has also been reported in patients with meningococcal disease [1,3], systemic lupus erythematosus (SLE) combined with PLD [13] and in apparently healthy persons [14–16].

Heterogeneity of C3 NeF with regard to complement activating properties was first recognized by Ng & Peters [17]. It was found that all C3 NeF stabilized cell-bound C3 convertase, whereas efficient fluid phase C3 cleavage was supported by some C3 NeF...
(C3 NeF type I), but not by other C3 NeF (C3 NeF type II). Moreover, patients with C3 NeF type II had markedly low C5 concentrations indicating in vivo recruitment of a C3/C5 convertase. The capacity of C3 NeF type II to activate C5 and the terminal complement pathway was demonstrated in a separate study [18]. C3 NeF with properties similar to those described for C3 NeF type II have also been reported as properdin-dependent C3 NeF [19, 20].

In the present study, we wanted to document the spectrum of clinical manifestations and the prevalence of C3 NeF amongst 25 persons in which the finding of low C3 concentrations prompted further investigation with regard to the reason for the hypocomplementaemia. Complement protein concentrations in serum were determined and methods based on different principles were used for the detection of C3 NeF. With regard to autoantibody responses to other complement proteins, antibodies directed against the collagen-like region of Clq (anti-C1qCLR antibodies) are interesting diagnostic markers in SLE [21] and the hypocomplementaemic urticarial vasculitis syndrome [22], and have also been reported in MPGN [23, 24]. Measurement of anti-C1qCLR antibodies was performed on the assumption that coincidence of anti-C1qCLR antibodies and C3NeF might not be a random event.

**Materials and methods**

**Patients and inclusion criteria**

With few exceptions the sera investigated were originally referred to the laboratory for diagnostic complement analysis. The referral was sometimes based on a previous kidney biopsy diagnosis of MPGN or the finding of a low C3 concentration. C3 concentrations that were lower than a third of the normal (<0.43 g L⁻¹) were required for inclusion in the study. The sera were collected between 1973 and 1996 and were stored at −80°C. For most patients several sera were analysed. Patients with diseases characterized by variable C3 concentrations such as SLE [25] were excluded, with the exception of one patient, who showed a selective and persistent decrease of C3 that could not be accounted for by the clinical findings. Patients with acute poststreptococcal glomerulonephritis, as suggested by transiently low C3 concentrations returning to normal within 10 weeks [26] or by low C3 concentrations in conjunction with antibody responses to streptolysin O or DNase B, were not included in the study. As far as possible, records were reviewed in collaboration with the patient’s physician. In some cases (patients 4, 8 and 14), follow-up data were not available. Clinical data are summarized in Table 1.

Patients 10 and 11, 14 and 15, and 17, 22 and 25 were treated in the Department of Nephrology, University Hospital, Lund. Sera and clinical information were provided by Drs P. Bygren, H. Thysell, B. Lindergård, K. Westman and other colleagues. Patients 12, 20 and 24 were in the care of Dr S Hansson, East Hospital, and the serum of patient 5 was sent by Dr P.-O. Attman, Sahlgrenska Hospital, Gothenburg. Two patients (2 and 3) were investigated within the framework of a Danish study (GLOCY project) for which complement analysis was performed in our laboratory, and were included with the kind consent of Professor I. Lorenzen, Copenhagen. Patients 7 and 23 were first treated in Växjö Hospital and then in the University Hospital of Lund, with findings that were discussed with Dr N. Greberg, Växjö. Sera and clinical information concerning patients 4 and 13, who were treated in the University Hospital of Linköping, were provided by Dr G. Tibblin and Dr L. Öhman, respectively. Patient 6 was previously reported [13] by Dr A. Karstorp, Stockholm. Patient 8 was treated in St Göran’s Hospital, Stockholm. For the remaining patients, sera and clinical information were provided by Dr K.-O. Nilsson, University Hospital, Malmö (patient 9). Dr D. Ekholm, Kristianstad Hospital (patient 16), Dr K.-G. Prütz, Helsingborg Hospital (patient 18), Dr L. Sköldstam, Kalmar Hospital (patient 19), and by Dr T. Bergström, Borås Hospital (patient 21). We are indebted to Dr B. Jeglinsky, Vaasa Hospital, Finland, for arranging the follow-up investigation of patient 1.

**C3 NeF assays**

The following three methods based on different principles were used: an alternative pathway-dependent sheep erythrocyte (ShE) haemolytic assay [27], an assay for fluid-phase C3 cleavage as assessed by crossed immunoelectrophoresis (CIE) [11, 26], and isoelectric focusing (IEF) combined with alternative pathway-mediated haemolysis of guinea-pig erythrocytes (GpE) [4]. Ethylene diamine tetra-acetic acid (EDTA) was used in buffers for blocking of complement activation, and ethyleneglycol tetra-acetic acid (Sigma Chemical Co., St Louis, MO, USA) with Mg²⁺.
Table 1  Basic data in the 25 persons investigated

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>First analysis (year/age of patient)</th>
<th>Initial C3 level (Reference area 0.94–1.77 g L(^{-1}))</th>
<th>Time between first and last sample</th>
<th>Clinical findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1972/29</td>
<td>0.20</td>
<td>12 years</td>
<td>Healthy</td>
</tr>
<tr>
<td>2</td>
<td>1973/nk</td>
<td>0.17</td>
<td>6 months</td>
<td>MPGN</td>
</tr>
<tr>
<td>3</td>
<td>1973/nk</td>
<td>0.08</td>
<td>6 months</td>
<td>MPGN</td>
</tr>
<tr>
<td>4</td>
<td>1973/30</td>
<td>0.03</td>
<td>–</td>
<td>PLD</td>
</tr>
<tr>
<td>5</td>
<td>1973/22</td>
<td>0.18</td>
<td>6 months</td>
<td>MPGN</td>
</tr>
<tr>
<td>6</td>
<td>1974/35</td>
<td>0.07</td>
<td>1 month</td>
<td>Healthy</td>
</tr>
<tr>
<td>7</td>
<td>1974/12</td>
<td>0.34</td>
<td>6 months</td>
<td>MPGN</td>
</tr>
<tr>
<td>8</td>
<td>1975/5</td>
<td>0.13</td>
<td>–</td>
<td>PLD, haematuria</td>
</tr>
<tr>
<td>9</td>
<td>1977/9</td>
<td>0.33</td>
<td>8 years</td>
<td>Suspected glomerulonephritis</td>
</tr>
<tr>
<td>10</td>
<td>1977/10</td>
<td>0.13</td>
<td>15 years</td>
<td>MPGN</td>
</tr>
<tr>
<td>11</td>
<td>1983/22</td>
<td>0.20</td>
<td>12 years</td>
<td>MPGN</td>
</tr>
<tr>
<td>12</td>
<td>1983/7</td>
<td>0.20</td>
<td>11 years</td>
<td>MPGN, meningococcal meningitis</td>
</tr>
<tr>
<td>13</td>
<td>1985/20</td>
<td>0.24</td>
<td>8 years</td>
<td>DNI</td>
</tr>
<tr>
<td>14</td>
<td>1986/59</td>
<td>0.13</td>
<td>–</td>
<td>Extracapillary glomerulonephritis</td>
</tr>
<tr>
<td>15</td>
<td>1987/17</td>
<td>0.12</td>
<td>1 month</td>
<td>Glomerulonephritis</td>
</tr>
<tr>
<td>16</td>
<td>1988/52</td>
<td>0.23</td>
<td>7 years</td>
<td>Chronic bronchitis</td>
</tr>
<tr>
<td>17</td>
<td>1989/24</td>
<td>0.25</td>
<td>4 years</td>
<td>MPGN</td>
</tr>
<tr>
<td>18</td>
<td>1990/35</td>
<td>0.13</td>
<td>3 years</td>
<td>Anaphylactoid purpura</td>
</tr>
<tr>
<td>19</td>
<td>1990/30</td>
<td>0.05</td>
<td>4 years</td>
<td>SLE, pneumonia</td>
</tr>
<tr>
<td>20</td>
<td>1990/12</td>
<td>0.09</td>
<td>4 years</td>
<td>MPGN</td>
</tr>
<tr>
<td>21</td>
<td>1992/11</td>
<td>0.03</td>
<td>4 years</td>
<td>MPGN, group C streptococcal septicaemia, meningococcal meningitis</td>
</tr>
<tr>
<td>22</td>
<td>1992/22</td>
<td>0.42</td>
<td>4 years</td>
<td>MPGN</td>
</tr>
<tr>
<td>23</td>
<td>1993/23</td>
<td>0.42</td>
<td>3 years</td>
<td>Glomerulonephritis</td>
</tr>
<tr>
<td>24</td>
<td>1995/11</td>
<td>0.34</td>
<td>1 year</td>
<td>MPGN</td>
</tr>
<tr>
<td>25</td>
<td>1996/19</td>
<td>0.05</td>
<td>7 months</td>
<td>MPGN</td>
</tr>
</tbody>
</table>

*MPGN, membranoproliferative glomerulonephritis; PLD, partial lipodystrophy; SLE, systemic lupus erythematosus; DNI, disseminated neisserial infections.*

added (Mg\(^{2+}\) EGTA) for selective blocking of classical pathway activation. ShE and GpE were purchased from the Veterinary University, Stockholm, Sweden. Cells from selected sheep had to be used for avoidance of background lysis by normal human sera in the ShE haemolytic assay.

The ShE haemolytic assay was performed in two steps as previously described [27, 28]. In the first step, 40 µL of patient serum was incubated with 40 µL of normal serum and 120 µL of ShE (6 × 10⁷ cells) at 30°C for 10 min in the presence of Mg\(^{2+}\) EGTA allowing formation of C3 NeF-stabilized C3 convertase on the cell surface. The cells were washed five times in EDTA-containing buffer. In the second step, the cells were incubated with 0.2 mL of rat serum (dilution 1/10) at 37°C for 60 min in the presence of EDTA for development of convertase sites. Values were given as percentage haemolysis of the cells with 15% haemolysis as the normal limit. As observed by López-Trascasa et al. [28], some C3 NeF-containing sera produced substantial haemolysis by recruitment of human C5-C9 during the first step of the assay. For this reason, results were given as the sum of haemolysis in the two steps of the assay.

In the assay for C3 cleavage, unchelated patient serum was incubated with an equal volume of normal serum for 1 h at 37°C. EDTA at 10 mmol L\(^{-1}\) was added after 10 min of the incubation time in order to reduce background C3 cleavage [11, 26]. Patient serum and normal serum that were mixed after incubation served as controls. Results were evaluated by planimetry with measurement of the total area covered by immunoprecipitate and the area representing cleaved C3, mainly C3c. Values were given as percentage C3 cleavage after correction for C3 cleavage in each control. C3 cleavage values that were lower...
than 10% were considered normal. Assays were repeated with Mg$^{2+}$-EGTA chelation of the sera during the first 10 min of the incubation time.

The IEF assay combined with C3 NeF detection by haemolysis of GpE [4] was modified by performing the first step in agarose gel and by using properdin-deficient serum [2] as a source of complement in the second step. Thus, serum samples were subjected to IEF in 1% (w/v) agarose (Isogel, FMC BioProducts, Rockland, ME, USA), sucrose 0.2 mol L$^{-1}$, and 5% (v/v) Ampholine pH 3.5–10.0 (Pharmacia LKB, Uppsala, Sweden). Stained protein standard pl-markers (pl Calibration Kit Electran, range 4.7–10.6, 44270 2G, BDH, UK) were used. IEF was performed in a 2117 Multiphor apparatus (LKB, Bromma, Sweden) at 50 V cm$^{-1}$ for about 1 h and at 80 V cm$^{-1}$ for 10 min. After the IEF step the gel was covered by a 1% agarose gel (SeaKem ME agarose, FMC BioProducts) with 2% (v/v) GpE and 5% (v/v) properdin-deficient serum or normal serum in Mg$^{2+}$-EGTA-containing buffer. The gels were incubated in a humid chamber at 37°C for 1-3 h for development of haemolytic banding patterns. The use of properdin-deficient serum in the second step markedly reduced background haemolysis of GpE, but did not change results as compared with those obtained with normal serum as a source of complement in the gel.

**Complement proteins**

Serum concentrations of the complement proteins Clq, C3, C4, C5, factor B and properdin were determined by electroimmunoassay. Concentrations of factors I and H, and C4-binding protein (C4 bp) were measured in sera that did not show evidence of C3 NeF. The reference areas for complement proteins were those reported by Johnson et al. [29]. The reference area for C4 bp was based on determinations in 25 healthy adults. Values were given in weight units based on the assumption that the pooled reference serum used contained each protein at a concentration stated in the literature [30].

**Antibody specificities of C3 NeF**

An enzyme-linked immunosorbent assay (ELISA) using microtitre plates coated with combinations of purified C3, factor B, factor D and properdin in the presence of Ni$^{2+}$ [31] was employed for comparison of C3NeF in different sera. The purified proteins were available in the laboratory [32]. The plates were first coated with 2 µg of C3 per well, and were then blocked with 1% human serum albumin (Sigma). Factor B was added (5 µg per well) to yield C3bB-like complexes and factor B together with factor D (50 ng per well) to yield C3bBb complexes in the solid phase. Inclusion of properdin (1 µg per well) was made to generate C3bBbP complexes. For control purposes plates were also coated with properdin alone. Sera were applied at a 1/50 dilution in EDTA-containing buffer. Binding of IgG to the solid phase antigens was detected with rabbit F(ab')2, antihuman Fcγ (Cappel, Organon Teknika, Durham, NC, USA) conjugated with alkaline phosphatase (Type VII-S, Sigma Chemical Co.) according to Voller [33]. For each serum, background absorption was assessed by the consistent inclusion of a blocked microtitre plate well without complement proteins.

**Autoantibodies to C1qCLR**

Anti-C1qCLR were determined by ELISA and immunoblot analysis as previously described [22].

**Results**

**Detection of C3 NeF**

The ShE haemolytic assay gave evidence of C3 NeF in 19 of the 25 persons investigated with values ranging between 21 and 100% haemolysis (Fig. 1). After the first step of the assay, haemolysis was almost always less than 10%. However, increased haemolysis in the first step was repeatedly found with the sera of patient 21 (100% haemolysis), patient 25 (83% haemolysis), patient 7 (59% haemolysis), patient 20 (28% haemolysis), and patient 11 (21% haemolysis). Increased fluid phase C3 cleavage in unchelated sera was found by CIE in 16 patients. The values ranged between 15% and >90%. Two of the patients with increased C3 cleaving activity were negative in the ShE haemolytic assay. There was no correlation between results apart from the fact that four sera were negative in both the C3 cleavage and the ShE haemolytic assay (Fig. 1). Repetition of the C3 cleavage assay with Mg$^{2+}$-EGTA-chelated sera gave no evidence of classical pathway activation.

Isoelectric focussing of serum followed by detection of C3 NeF by alternative pathway-mediated haemolysis of GpE reproducibly gave clear banding patterns, indicating polyclonal or oligoclonal antibody responses in the sera of 10 persons (Fig. 1).
Fig. 1 Fluid phase C3 cleavage for assessment of C3 NeF (C3 cleavage) as compared with results of a sheep erythrocyte haemolytic assay for C3 NeF (ShE lysis) in 25 sera investigated for the reason of hypocomplementaemia with low C3 concentrations. Closed circles indicate sera showing C3 NeF by isoelectric focusing (IEF) combined with haemolysis of guinea-pig erythrocytes. Open symbols indicate sera that were negative in the IEF assay. The five sera considered not to contain C3 NeF are indicated with open triangles.

Complement proteins

Eleven of the 25 persons with low C3 values had essentially normal concentrations of the other complement proteins. In another 12 persons the hypocomplementaemia included low C5 concentrations with or without low concentrations of other complement proteins. Low factor B concentrations were observed in seven persons: all of these had C5 values that were low or in the low normal range (Fig. 2). Moderately low properdin concentrations (about 40% of the normal) were found in two MPGN patients, who did not show evidence of C3 NeF (patients 10 and 22). Nineteen persons showed normal concentrations of C1q and C4, indicating that classical pathway activation was not a principal cause of hypocomplementaemia in the patients. In five persons the concentrations of C1q or C4 were modestly low. One patient (14) showed markedly low C4, C3, C5, and factor B concentrations.

The five patients considered not to have C3 NeF (see below) were investigated with regard to the concentrations of factor I, factor H, and C4 bp. Values were found to be normal or moderately high.

Evaluation of findings

Differentiation between C3 NeF type I and C3NeF type II depends on a combination of findings [17], and no specific assays for each of the two C3 NeF types have been previously reported. When we compared findings with regard to fluid-phase C3 cleavage and ShE haemolysis with complement protein levels in the sera, two principal groups of C3 NeF-positive patients could be distinguished. The first group showed normal C5 concentrations in combination with increased C3 cleavage and ShE haemolysis consistent with criteria for C3 NeF type I. In the second group, the presence of C3 NeF type II was suggested by findings of low C5 concentrations in combination with increased ShE haemolysis and weak or absent C3 cleaving activity. However, the two groups were only partly distinct (Figs 1 and 2).

Results of the IEF assay proved to be of considerable interest. It was found that IEF haemolytic banding patterns only occurred in sera showing increased C3 cleavage (Fig. 1). Furthermore, positivity of the IEF assay was associated with normal concentrations of C5 (Fig. 2). The implication that the IEF assay specifically detects C3 NeF type I was critical for our
Table 2 Evaluation of findings with regard to C3 NeF in the hypocomplementaemic sera with low C3 concentrations

<table>
<thead>
<tr>
<th>Patient nos.</th>
<th>IEF</th>
<th>CIE (%)</th>
<th>ShE (%)</th>
<th>Complement proteins</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, 6, 8, 11, 12, 16, 19</td>
<td>+</td>
<td>15–&gt;90</td>
<td>21–70</td>
<td>C1q, C4, C5, B, and properdin normal or close to normal</td>
<td>C3 NeF type I</td>
</tr>
<tr>
<td>2, 4</td>
<td>&gt;90</td>
<td>47–81</td>
<td>Low factor B and C5 or low factor B</td>
<td>C3 NeF type I</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>+</td>
<td>57</td>
<td>&lt;15</td>
<td>C1q, C4, C5, factor B, and properdin normal</td>
<td>C3 NeF type I</td>
</tr>
<tr>
<td>5, 7, 15, 20, 21</td>
<td>-</td>
<td>&lt;10–17</td>
<td>37–100</td>
<td>Low C5, Modestly low C1q or C4 in 3 patients</td>
<td>C3 NeF type II</td>
</tr>
<tr>
<td>17, 25</td>
<td>-</td>
<td>&lt;10–13</td>
<td>52–95</td>
<td>Low C5 and factor B</td>
<td>C3 NeF type II</td>
</tr>
<tr>
<td>14</td>
<td>-</td>
<td>38</td>
<td>33</td>
<td>Low C4, C5 and factor B</td>
<td>Probable C3 NeF type II</td>
</tr>
<tr>
<td>13, 24</td>
<td>&lt;10–18</td>
<td>39–90</td>
<td>C1q, C4, C5, factor B, and properdin normal</td>
<td>Probable C3 NeF type II</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>&gt;90</td>
<td>&lt;15</td>
<td>Low C5 and factor B</td>
<td>Fluid phase C3 cleavage due to other reason than C3 NeF</td>
<td></td>
</tr>
<tr>
<td>10, 22</td>
<td>-</td>
<td>&lt;10</td>
<td>&lt;15</td>
<td>Low properdin, low or borderline C5</td>
<td>No evidence of C3 NeF</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
<td>&lt;10</td>
<td>&lt;15</td>
<td>Low C5 and factor B</td>
<td>No evidence of C3 NeF</td>
</tr>
<tr>
<td>23</td>
<td>-</td>
<td>&lt;10</td>
<td>&lt;15</td>
<td>C1q, C4, C5, factor B, and properdin normal</td>
<td>No evidence of C3 NeF</td>
</tr>
</tbody>
</table>

*Results are summarized for isoelectric focusing of serum combined with haemolysis of guinea-pig erythrocytes (IEF), fluid phase C3 cleavage as assessed by crossed immuno-electrophoresis (CIE), a sheep erythrocyte haemolytic assay (ShE), and for complement protein concentrations.

With regard to atypical findings amongst the 10 patients considered to have C3 NeF type I, two patients had moderately low factor B concentrations, and one of these had a low C5 concentration as well. It is also noteworthy that one patient with clear evidence of C3 NeF type I was negative in the ShE haemolytic assay, which shows that this assay is not altogether reliable for detection of C3 NeF.

Sera from seven patients fulfilled the criteria for C3 NeF type II, i.e. low concentrations of C3 and C5, weak or undetectable C3 cleavage in the CIE assay, pronounced haemolysis in the ShE assay and absence of a banding pattern in the IEF assay (Table 2). In another two patients the discrepancy between weak C3 cleavage and marked haemolysis in the ShE assay suggested the presence of C3 NeF type II, even if C5 concentrations were normal. One patient showed C3 cleavage and low C4 concentrations. C3 NeF type II were probably present despite these atypical findings. Simultaneous presence of the two types of C3 NeF might be considered. To address this question, a specific assay for C3 NeF type II would have been required.

The persistent hypocomplementaemia of five patients could not be ascribed to C3 NeF. One of these showed very pronounced C3 cleavage, but was negative in the ShE and IEF assays. Two MPGN patients in the C3 NeF-negative group were the only patients in the study to show low properdin concentrations.

Antibody specificities of C3 NeF

Four sera with C3 NeF type I (2, 11 and 8 and 12) and four sera with C3 NeF type II (5, 17 and 20 and 21) were compared with regard to IgG binding to solid-phase antigens composed of alternative pathway convertase components (Fig. 3). The findings provided evidence of IgG binding to C3bBb in sera containing C3NeF type I. By contrast, sera with C3NeF type II did not show more IgG binding to C3bBb than to wells coated with C3 or C3 and factor B. With one of the C3NeF type II sera, clear IgG binding to C3bBbP was obtained. No reactivity was detected in controls with properdin alone. This suggested that C3NeF type I and C3NeF type II have distinct antibody specificities, and that at least some C3NeF type II recognize the C3bBbP complex.

Results of serial C3 and C3 NeF analysis

When repeated samples were analysed from patients that were followed for extended periods of time, the findings were mostly found to be stable. However, in four persons (1, 7 and 13, and 24) the C3 concentrations were normalized with time. One person (1) was an apparently healthy blood donor, whose hypocom-
Antibody specificities of C3NeF type I (closed circles, patients 2, 8, 11 and 12) as compared with C3 NeF type II (open circles, patients 5, 17, 20 and 21). The purified alternative pathway components C3, factor B (B), factor D (D), and properdin (P) were used for coating of microtitre plate wells in the presence of Ni2+. C3 and B were combined for generation of a C3bB-like complex, C3, B and D for generation of C3bBb, and C3, B, D and P for generation of C3bBbP in the solid phase. Binding of patient serum IgG to the complexes was detected using rabbit F(ab')2 antihuman Fcγ conjugated with alkaline phosphatase. Values were corrected for background. No IgG binding was found in normal control sera or when plates were coated with P alone (not shown).

Complementaemia was discovered incidentally during collection of samples for establishment of reference areas for complement proteins. C3 values remained low for at least 1 year, after which we lost contact with the donor, who moved abroad. A new sample was obtained 11 years later, and the C3 concentration was now entirely normal. Interestingly, the IEF assay showed the same oligoclonal C3NeF pattern as that observed in the first samples. Patient 7 had MPGN with normalization of C3 and disappearance of C3 NeF type II in the course of 3–5 months. Patient 13, who showed gonococcal septicaemia, meningococcal meningitis and recurrent throat infections, had low C3 concentrations and evidence of C3 NeF type II for more than 2 years. In serum obtained 6 years later the C3 concentration was in the low normal range and C3NeF were not found. Patient 24 had MPGN, C3 NeF type II and a low C3; 1 year later, C3 NeF had disappeared and C3 was normal.

Autoantibodies to C1qCLR

Anti-C1qCLR antibodies were found in six of the 10 patients with C3 NeF type II (Fig. 4). In the sera of patients 13, 17, 20, 21 and 25 the anti-C1qCLR antibodies were detectable by ELISA and not by immunoblot analysis. Patient 7 showed an unusual immunoblot pattern with selective staining of the polypeptide C chain of C1q.

Discussion

We believe that the patient group investigated was fairly representative of persistent or prolonged C3 deficiency as encountered in clinical and diagnostic practice. On the other hand, the design of the study implied possible selection effects. Most likely, the well-known association between MPGN and hypocomplementaemia favoured identification of patients with this disease as compared with patients showing other clinical manifestations. The fact that the two patients with PLD were diagnosed before 1976 may reflect a current interest in the condition at that time.

In contrast to previously described patients with SLE and C3 NeF [13], the SLE patient of the present study had no evidence of PLD. Anaphylactoid purpu-
ra has been reported in patients with deficiency of C4 or C2 [1], but not in C3 deficiency states. Interestingly, infection problems in the patients were not restricted to systemic neisserial disease, but also included streptococcal septicaemia, pneumonia and chronic bronchitis. The findings show that C3 deficiency syndromes with manifestations of immunological disease or severe infection are most often caused by C3 NeF. We wish to emphasize the variety of manifestations associated with C3 NeF and that these rare syndromes may be easily overlooked in diagnostic work.

The strong association between C3 NeF and MPGN suggests that C3 NeF might directly contribute to complement activation and inflammation in the kidney. On the other hand, MPGN has been observed in patients with primary complement deficiencies [1, 3]. This together with the recent description of regular MPGN development in pigs with factor H deficiency [34] makes it unnecessary to assume that C3 NeF have other pathogenetic functions than the capacity to produce acquired C3 deficiency.

The reason for the low C3 concentrations in the patients without C3 NeF remains to be clarified. Inherited C3 deficiencies are very rare, and include C3 hypercatabolism syndromes secondary to deficiency of the control proteins factors I or H, as well as primary C3 deficiency and dysfunction [1–3, 35]. There was no evidence of inherited C3 deficiencies in the present study, even though C3 hypercatabolism of the type discussed by Linshaw et al. [36] might possibly be considered in patient 9. With regard to acquired C3 deficiency syndromes other than those caused by C3 NeF, paraprotein immunoglobulin light chains reacting with factor H [37] could have been present in patient 3, who showed very pronounced C3 cleavage that was not caused by C3 NeF. Nephritic factors of the classical pathway, C4 NeF [38], also produce hypocomplementaemia with low C3 concentrations and could have been involved in the patients.

The ShE haemolytic assay used depends on the dual effect of C3 NeF on spontaneous and control protein-mediated decay of cell-bound C3/C5 convertase [27, 28]. Considering the findings of Ohi et al. [9], the assay may be more relevant for screening purposes than stabilization assays with purified components in which analysis is usually restricted to effects on the spontaneous decay of the enzyme. Studies of fluid phase C3 cleavage by CIE or other means is a classical, but not altogether specific approach for the demonstration of C3 NeF. The assay was of limited value for detection of C3 NeF type II, but provided important supplementary information when combined with the ShE haemolytic assay for C3 NeF.

A somewhat surprising result was that IEF of patient IgG followed by haemolytic detection with GpE as target cells [4] appeared to be a specific assay for C3NeF type I. On the other hand, the finding is in line with the proposed 'bystander' mechanism for haemolysis of GpE according to which haemolysis does not involve formation of cell-bound C3 convertase [39].

Little is known of events leading to production and persistence of C3 NeF. However, disappearance of C3 NeF following bilateral nephrectomy and transplantation has been described [40], which suggests antigen-driven autoantibody production. Anti-idiotypic responses to C3 NeF might also support important regulatory functions [41]. In the present study, a few patients showed spontaneous disappearance of C3 NeF and normalization of C3 with time. C3 was also found to be normalized in spite of persisting C3 NeF, which raises further questions with regard to regulatory mechanisms.

Anti-C1qCLR antibodies of the variety found in SLE [21, 22] were detected by ELISA in five of the 10 patients considered to have C3 NeF type II, but were not found in patients with C3 NeF type I. A sixth patient with C3 NeF type II (patient 7) showed immunoblot reactivity with the C chain of C1q. Anti-C1qCLR antibodies in SLE are immunoblot negative and probably recognize conformational epitopes of bound intact C1q molecules [22]. Other anti-C1qCLR antibodies recognize B and C chain epitopes of C1q as detected by immunoblot analysis [22]. The findings suggest an atypical anti-C1qCLR antibody in patient 7.

The coincidence of anti-C1qCLR antibodies with C3 NeF type II could imply that the immunizing antigens for this type of C3 NeF might be presented as parts of a complex containing at least C1q and a C3/C5 convertase. C1q antibodies and two variants of C3 NeF have been previously documented in patients with different histopathological types of MPGN [23, 24]. In contrast to our results, the presence of any one autoantibody was not found to be associated with the presence of any other autoantibody [24]. The discrepancy might partly be due to
methodological differences.

In conclusion, C3 NeF types I and II were the most common causes of C3 deficiency syndromes as encountered in clinical and diagnostic practice. Anti-C1qCLR antibodies were only found in patients with C3 NeF type II. The fairly broad spectrum of disease manifestations observed was consistent with findings in primary complement deficiencies. The results suggest that acquired C3 deficiency syndromes caused by C3 NeF should be considered more often in diagnostic work.

Acknowledgements

The study was supported by the Swedish Medical Research Council (Project no. 7921), the Swedish National Association against Rheumatism, King Gustav V’s 80th Birthday Fund, Greta och Johan Kock’s Foundation, Alfred Österlund’s Foundation, Crafoord’s Foundation, Ake Wiberg’s Foundation, Nanna Svartz’ Fund and Thelma Zoega’s Fund. The work was conducted within the framework of the Biomed. 2 project no. BMH4-CT96–1005.

References

29 Johnson U, Truesson L, Gustavii B. Complement components in 100 newborns and their mothers determined by electriom-

Received 16 January 1997; accepted 13 May 1997.

Correspondence: Dr Anders G. Sjöholm, Clinical Immunology Section, Department of Medical Microbiology, Sölvegatan 23, 223 62 Lund, Sweden (fax: +46 46 189117).