Monomeric Complement-Activating IgG Paraproteins

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Monomeric Complement-Activating IgG Paraproteins

Marten Trendelenburg,2* Christoph Hess,* Mitsuko Kondo-Oestreicher,† Jean D. Tissot,‡ Peter Späth,§ and Jürg A. Schifferli*

Three patients presented a unique syndrome of recurrent panniculitis with an IgGκ paraprotein and depletion of the early components of the classical pathway of complement. The IgGκ paraproteins were monomers with a normal structure, and with no evidence for aggregation, as assessed by electron microscopy and ultracentrifugation. Both heavy and light chains were of normal molecular size (SDS-PAGE), and the paraproteins were not heavily glycosylated. However, the paraproteins from all three patients had unusual features that included abnormal behavior on gel filtration chromatography and a heavy chain of high pI. When analyzed by fast protein liquid chromatography (Superdex 200), elution of the paraproteins was retarded, particularly when the ionic strength was increased. This retardation was partially reversed in 20% alcohol, and fully reversed in 6 M guanidine-HCl. Neither anti-C1 inhibitor nor anti-C1q autoantibodies were found in any of the patients’ sera. However, the paraproteins bound to the globular heads of C1q at normal ionic strength. They activated C4 in normal human serum, but not in C1q-deficient serum. Activation led to the formation of C13-C1 complexes. Taken together, the data suggest that the unusual paraproteins have the capacity to bind C1q, which then leads to activation of C1. The ability of these paraproteins to activate C1, in spite of their being soluble monomers, is likely to be related to their unique physicochemical features. The Journal of Immunology, 1999, 163: 6924–6932.

R
current febrile and nodular panniculitis is known as
Weber-Christian syndrome (1–3). Different diseases may
lead to the clinical picture of nodular panniculitis (4–8).
Hypocomplementemia with paraproteinemia has been described in
a series of patients with panniculitis (3). Whether in such cases the
paraprotein was directly responsible for complement activation
and depletion has not been further analyzed. It is known that
autoantibodies directed to C1 inhibitor or C1q may be responsible
for low levels of complement components of the classical pathway in
plasma, although the clinical symptoms of such patients differ con-
siderably, i.e., most often angioedema for patients with anti-C1
inhibitor autoantibodies, and urticarial vasculitis/glomerulonephrit-
is or SLE1 for those with anti-C1q autoantibodies (9, 10). Cryo-
globulins have been reported to deplete complement in vivo, but
again vasculitis is the main feature of the disease, and not pannic-
ulitis. The two patients described by Pascual (11) had very similar
features of histologically proven recurrent panniculitis with fever
associated with IgGκ paraproteins and low levels of the early com-
ponents of the classical complement pathway (C1q, r, s, C4, and
C2). In the present study, we analyzed the paraproteins of these
two patients and of an additional patient with the same syndrome.

We found some common features that may 1) explain specific
interactions with complement, and 2) be related to the clinical
syndrome.

Patients and Methods

Patients

All three patients investigated had recurrent panniculitis, an IgGκ para-
protein (Table I), and hypocomplementemia in vivo, but no vasculitis. We all
classified as having Weber-Christian dis-
ease. The detailed clinical history of two of the patients has been described
by Pascual (patients B and C) (11). Patient B had one more panniculitis
flare, which improved with steroids. He developed multiple myeloma 2 yr
later and died shortly afterward. Patient C had one major flare of pannic-
ulitis, and was lost to follow-up 3 yr later. Patient F had three flares of
panniculitis with mediastinal involvement between 1994 and 1999. The
symptoms improved rapidly with steroids on each occasion. In 1999, he
had an acute myocardial infarction and died 48 h later. None had
plasmapheresis.

Sera

The sera of the three patients were kept at −74°C until use. As controls we
used the serum of a normal individual as well as six sera from patients with
IgG myeloma and one further serum with an IgG paraprotein and plane
xanthomatosis. These control patients had no history of panniculitis. In two
of the myeloma patients, the IgG paraprotein was a cryoglobulin. Control
sera containing anti-C1 inhibitor autoantibodies (kindly donated by Dr.
Wüthrich, Zürich, Switzerland) and anti-C1q autoantibodies were stored at
−74°C as well.

Complement protein concentrations were measured by radial immuno-
diffusion or nephelometry using standard techniques (12, 13). CH50 and
AP50 using guinea pig cells were conducted as described by Mayer (14)
and Platts-Mills (15, 16).

Cryoglobulins were measured as reported by Trendelenburg (17). Im-
muno fixation electrophoresis was conducted as described by Alper (18).

Anti-C1 inhibitor autoantibodies

The anti-C1 inhibitor autoantibody ELISA was conducted as described by
Alsenz (9), with a slight modification. In short, the wells (Maxisorp Nunc
Immu plates, Roskilde, Denmark) were coated with saturating amounts
of C1 inhibitor (Berinert HS from Centeon Pharma) in sodium hydrogen
carbonate buffer, pH 9.6, overnight at room temperature. After washing,
were regarded as a negative test result. HRP (Jackson ImmunoResearch, West Grove, PA). The serum of a patient (Southern Biotechnology Associates, Birmingham, AL) and streptavidin-
second Ab were confirmed using biotinylated, polyclonal goat anti-human IgG was detected with biotinylated monoclonal mouse IgG anti-human Tween 0.05% containing 1% FCS (PBSTwFCS) and 1 M NaCl. Bound washing, the sera were incubated for 1 ha t37°C 1/25 diluted in PBS-
in sodium hydrogen carbonate buffer, pH 9.6, at room temperature. After
was detected with biotinylated monoclonal mouse IgG anti-human IgG purification
MO) (1:30 v/v) for 20 h at 37°C. After centrifugation (30,000
acetate, pH 4.45, at 4°C, and then incubated with pepsin (Sigma, St. Louis, (21). C1q was dialyzed overnight against freshly prepared 0.1 M sodium
were measured using pepsin-digested C1q prepared as reported by Reid
anti-CLR/C1q. and IgG samples were diluted in PBSTwFCS and tested for the presence of
the column using 0.1 M glycine HCl, pH 2.8. The pH of the fractions
CH50 19 10 0 75–125
C2 54 31 25 65–135
C4 12 5

Heat-aggregated IgG (HAGG) was produced by heating normal human IgG to 63°C for 30 min. After being cooled in an ice bath, the proteins were precipitated with sodium sulfate, resuspended in PBS, and additionally
dialyzed overnight at 4°C against PBS (final concentration of 0.4 mg/ml).
the sera were 1/25 diluted in PBS and incubated for 1 h at 37°C. Bound IgG was detected with biotinylated monoclonal mouse IgG anti-human γ-chain (Southern Biotechnology Associates, Birmingham, AL) and streptavidin-
were tested, from which two (4.3%) were positive (20).
Low affinity binding of serum and of purified monomeric IgG fractions to C1q was investigated by varying the amount of NaCl that was added to the
PBSTwFCS in the first incubation step. Furthermore, the results obtained
with the biotinylated monoclonal mouse IgG anti-human γ-chain as second Ab were confirmed using biotinylated, polyclonal goat anti-human γ-chain Abs (Calbiochem).
Abs binding to the globular region of C1q (GR) were determined using collagenase-digested C1q, as reported by Wisnieski (22). The digest was applied to FPLC-Superdex 200 HR 10/30 (Amersham Pharmacia Biotech), and the third peak containing the GR of C1q was pooled and used for the
coating of the ELISA plates. The sera and IgG samples were diluted in PBSTwFCS and tested for the presence of anti-CLR/C1q.
Abs binding directed to the collagen-like-region (CLR) of C1q (anti-CLR/C1q) were measured using pepsin-digested C1q prepared as reported by Reid
(21). C1q was dialyzed overnight against freshly prepared 0.1 M sodium acetate, pH 4.45, at 4°C, and then incubated with pepsin (Sigma, St. Louis, MO) (1:30 v/v) for 20 h at 37°C. After centrifugation (30,000 × g at 4°C), the supernatant was applied to FPLC-Superdex 200 HR 10/30 (Amersham Pharmacia Biotech, Piscataway, NJ), and the first peak containing the CLR of C1q was pooled and used for the coating of the ELISA plates. The sera and IgG samples were diluted in PBSTwFCS and tested for the presence of anti-CLR/C1q.

IgG purification
The sera were applied on protein G-Sepharose 4B (Sigma) and IgG eluted from the column using 0.1 M glycine HCl, pH 2.8. The pH of the fractions was immediately neutralized with 1 M Tris buffer, pH 9. For the separation of the paraproteins of the patients F, B, and C, the IgG-containing fractions were additionally applied to a Superdex 200 HR 10/30 gel filtration column (FPLC, Amersham Pharmacia Biotech) using a high salt buffer (PBS + 1 M NaCl). The fractions with the paraproteins were pooled, dialyzed against PBS, and concentrated with Microsep 30 K ( Pall Filtron).

Purification of γ-heavy chains
The paraprotein of patient F and control normal human IgG were reduced,
and the heavy chains were separated, as described by Fleischman (23). However, for the separation of the heavy chains by gel filtration, we used a Superdex 200 HR 10/30 with FPLC system (both Amersham Pharmacia Biotech) and 6 M guanidine-HCl as a dissociating buffer. Gel filtration was conducted twice, and the pooled heavy chain-containing fractions were dialyzed overnight against PBS at 4°C. The purity of the heavy chain was assessed by the lack of light chain on SDS-PAGE (24). The heavy chain-containing solutions were applied on the next day (no freezing) to the anti-C1q autoantibody ELISA without further addition of NaCl in the first incubation step.

Bidimensional gel electrophoresis of the paraproteins was conducted, as described by Tissot (25). The paraproteins were reduced, denatured, and separated in the first dimension by isoelectric focusing, and in the second dimension by SDS-PAGE. The polypeptides were silver stained (high m.w. proteins at the top, and acid proteins to the left).

Glycosylation of the paraproteins
In a first step, the unreduced Igs were separated by SDS-PAGE and transferred to a nitrocellulose membrane (Hybond ECL; Amersham, Arlington Heights, IL); glycosylated proteins were revealed using the DIG glycan/protein double labeling kit (Boehringer Mannheim, Mannheim, Germany), according to the manufacturer’s indications.

Gel filtration chromatography
Gel filtration analysis of sera or purified Ig was performed using a FPLC
system with a Superdex 200 HR 10/30 (cross-linked agarose and dextran) or a HiPrep16/60 Sephacryl S-300 (cross-linked copolymer of allyldextran,
N,N-methylenebisacrylamide) column (Amersham Pharmacia Biotech) using PBS as a standard buffer. For further investigations, the running buffer was changed to PBS + 1 M NaCl, 50% PBS diluted in water, 20% ethanol in PBS, or 6 M guanidine-HCl.

The presence and relative amount of IgG in the gel filtration fractions were analyzed by the comparison of reduced and unreduced samples on SDS-PAGE

Ultracentrifugation.
To investigate whether the paraprotein of patient F is aggregated, we ultracentrifuged the serum for 2 h at 200,000 × g. Then it was applied to FPLC gel filtration, and the peak heights of the IgM, IgG, and paraprotein peaks were compared with their height before ultracentrifugation. To avoid variations due to small differences in the applied amounts of serum, the peak heights were expressed as a ratio to the height of peak 47, which contains small proteins (<1.35 kDa) or peptides. In the same way, we analyzed the loss of IgG and IgM in normal human serum (NHS) and in the serum of patients B and C.

Electron microscopy.
The paraproteins of patients B, C, and F were visualized by transmission electron microscopy and compared with normal human IgG and heat-aggregated normal human IgG. For the negative stain, purified IgG was adsorbed on 2 min to glow-discharged paraloid B-12 grids. After washing, all samples were stained with 0.75% uranylformate (pH 4.2). All samples were observed in a Zeiss EM 910 electron microscope operated at 80 kV acceleration voltage.

C4 consumption assay.
Complement-activating capacity of the different paraproteins was studied using a C4 hemolytic assay, as described previously (26). In short, washed sheep erythrocytes were coated with rabbit IgM anti-sheep erythrocytes (Ambacoprotector 6000) in barbitone-buffered saline for 20 min at 37°C. In a preincubation step, NHS 1/10 diluted in barbitone-buffered saline was incubated with different amounts of IgG preparations for 30 min at 37°C. The preincubation was stopped on ice and the mixture further diluted with the

Table I. Characterization of the γ-globulins of the patients F, B, and C

<table>
<thead>
<tr>
<th>Type of paraprotein</th>
<th>Patient F</th>
<th>Patient B</th>
<th>Patient C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total IgG (6.5–15 g/L)</td>
<td>17 g/L</td>
<td>13.6 g/L</td>
<td>35 g/L</td>
</tr>
<tr>
<td>Cryoglobulins</td>
<td>IgG1κ</td>
<td>IgG1κ</td>
<td>IgG1κ</td>
</tr>
<tr>
<td>No</td>
<td>No</td>
<td>No</td>
<td></td>
</tr>
</tbody>
</table>

Table II. Complement profiles of the patients F, B, and C with paraproteinemia and recurrent panniculitis

<table>
<thead>
<tr>
<th>Paraprotein</th>
<th>Patient F</th>
<th>Patient B</th>
<th>Patient C</th>
<th>Normal Range (% of normal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1q</td>
<td>17</td>
<td>10</td>
<td>13</td>
<td>57–143</td>
</tr>
<tr>
<td>C1r</td>
<td>10</td>
<td>10</td>
<td>20</td>
<td>75–125</td>
</tr>
<tr>
<td>C1s</td>
<td>22</td>
<td>33</td>
<td>28</td>
<td>75–125</td>
</tr>
<tr>
<td>C4</td>
<td>12</td>
<td>5</td>
<td>5</td>
<td>40–160</td>
</tr>
<tr>
<td>C2</td>
<td>54</td>
<td>51</td>
<td>25</td>
<td>65–135</td>
</tr>
<tr>
<td>C1 inhibitor</td>
<td>25</td>
<td>13</td>
<td>17</td>
<td>60–140</td>
</tr>
<tr>
<td>C3</td>
<td>104</td>
<td>165</td>
<td>93</td>
<td>64–136</td>
</tr>
<tr>
<td>CH50</td>
<td>19</td>
<td>10</td>
<td>0</td>
<td>75–125</td>
</tr>
<tr>
<td>AP50</td>
<td>100</td>
<td>100</td>
<td>120</td>
<td>70–130</td>
</tr>
</tbody>
</table>
barbitone-buffered saline to 1/100. Then, 20 μl of this mixture was added to the IgM-coated sheep erythrocytes together with 15 μl of undiluted guinea pig C4-depleted serum (Calbiochem) and incubated for 30 min at 37°C. Hemolysis was stopped on ice, and unlysed erythrocytes were removed by centrifugation (10 min, 1000 g at 4°C). A total of 300 μl of the supernatants was applied in duplicates to a microtiter plate (Maxisorp Nunc Immuno plates), and the OD was measured at 405 nm. Each hemolytic step was done in duplicate, and the remaining hemolytically active C4 was calculated out of a standard curve with the diluted NHS used in the assays and guinea pig C4-depleted serum. The C4 consumption of the three paraproteins from the patients F, B, and C were compared with normal polyclonal IgG and with the IgG from three of six arbitrarily selected control myeloma patients. Aggregation of the purified Igs as a source of complement activation was minimized by a direct use of the preparations after purification and without further concentration after the gel filtration or by ultracentrifugation of the IgG preparations (1 h, 200,000 g at 4°C).

In a modification of the assay described above, we replaced the NHS with human C1q-deficient serum or C1q-deficient serum purified C1q (5 μg C1q/ml C1q-deficient serum) (both from Calbiochem). In the latter case, reassociation of the C1 complex was allowed for 30 min at 37°C before the IgG preparations were added. C1s-C1 inhibitor complexes were measured by ELISA. Microtiter plates (Maxisorp Nunc Immuno plates) were coated overnight at room temperature with 0.6 μg/well purified, polyclonal goat anti-C1s IgG (Quidel, San Diego, CA) in sodium carbonate buffer and blocked with 2% BSA PBS. After washing, the sera were diluted 1/400 in PBS and incubated for 1 h at 37°C. Bound C1s-C1 inhibitor complexes were detected with biotinylated, polyclonal goat anti-C1 inhibitor IgG (Quidel) and streptavidin-HRP (Jackson ImmunoResearch). The standard curve was established using different dilutions of NHS preincubated for 1 h at 37°C with HAGG (1 μg/ml NHS), which had been shown to lead to a maximal formation of C1s-C1 inhibitor complexes.

For the investigation of the complement-activating capacity of the paraproteins of patients B, C, and F compared with those of three myeloma patients, 5 μl NHS was incubated with varying amounts of IgG for 1 h at 37°C. The reaction was stopped on ice and the sera diluted in PBS to a final dilution of 1/400 before they were applied on the ELISA.

The effects of the paraproteins on the formation of C1s-C1 inhibitor complexes were tested by modifying the Quidel C1-Inhibitor Enzyme Immunoassay (Quidel). The biotinylated activated C1s was preincubated with normal polyclonal IgG; IgG preparations of the patients F, B, and C; or polyclonal goat anti-C1s IgG (Quidel) for 15 min at 37°C before mixing with purified C1 inhibitor (Berinert HS from Centeon Pharma). The final concentrations of C1 inhibitor specimens were 0.0055 mg/ml. The further steps were conducted according to the kit protocol.

Results
Total IgG and the type of the paraproteins are indicated in Table I. All three patients had a depletion of the early components of the classical pathway (Table II).

Analysis of the paraproteins by gel filtration
To characterize the paraproteins and exclude the presence of aggregates, we started to analyze the serum of the patients by gel filtration using FPLC-Superdex 200 HR 10/30. The analysis of the serum of patient F conducted with PBS as running buffer is shown in Fig. 1. In contrast to NHS, serum of patient F had a low IgG, but an additional peak between 17 and 1.35 kDa was shifted to the region of small peptides, indicating an increased retardation in the gel filtration column.

![FIGURE 1. Gel filtration pattern of NHS (A) and serum of patient F (B). The serum of patient F had, in comparison with NHS, a smaller peak at about 158 kDa (representing the IgG), but an additional peak between 17 and 1.35 kDa. Gel filtration pattern of NHS (C) and serum of patient F (D) if PBS + 1 M NaCl instead of PBS is used. Whereas the pattern of NHS remains mainly unchanged, the additional peak in the serum of patient F between 17 and 1.35 kDa was shifted to the region of small peptides, indicating an increased retardation in the gel filtration column.](http://www.jimmunol.org/)

For the investigation of the complement-activating capacity of the paraproteins of patients B, C, and F compared with those of three myeloma patients, 5 μl NHS was incubated with varying amounts of IgG for 1 h at 37°C. The reaction was stopped on ice and the sera diluted in PBS to a final dilution of 1/400 before they were applied on the ELISA.

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size of 150 kDa. After reduction, the IgG split into the expected heavy and light chains (50 and 25 kDa). Thus, the paraprotein behaved unusually by being retarded on Superdex gel filtration. This retardation was also seen on HiPrep16/60 Sephacryl S-300 gel filtration (data not shown). Several different running buffers were used to see whether we could abolish this retardation. PBS + 1 M NaCl led to a further retardation with elution in the range of small peptides (Fig. 1). PBS/water (1:1) also increased the retardation slightly. Reduction of the pH of PBS to 4 had no influence on the elution characteristics. With PBS + 20% ethanol as a running buffer, the paraprotein was eluted together with albumin. Finally, 6 M guanidine-HCl as a running buffer blocked the retardation completely. As controls, we used six sera of myeloma patients containing IgG paraproteins as well as the serum of a patient with plane xanthomatosis and an IgG paraprotein. In none of them could we demonstrate a retardation in PBS, or PBS + 1 M NaCl.

As controls, we used six sera of myeloma patients containing IgG paraproteins as well as the serum of a patient with plane xanthomatosis and an IgG paraprotein. In none of them could we demonstrate a retardation in PBS, or PBS + 1 M NaCl.

Because of this unexpected retardation phenomenon on gel filtration, the initial question, as to whether the paraproteins were forming aggregates, had to be investigated by a different technique.

**Ultracentrifugation**

The loss of the paraprotein in the serum of patient F after ultracentrifugation (20% after 2 h, 200,000 × g) was similar to the loss of IgG in the same serum (15%) or in NHS (16%), but less pronounced than the loss of the IgM in serum of patient F and NHS (38% and 34%, respectively). Similar observations to those made in patient F were made in the sera of patients B and C.

**Electron microscopy**

The purified monoclonal IgGκ of the three patients were analyzed by electron microscopy using normal IgG and normal HAGG as controls. The monoclonal Igs were dissociated, and never formed any aggregates, which was in clear contrast to Igκ, which had been heated at 63°C (Fig. 4). The IgG paraproteins had the expected morphological characteristics of IgG (with distinguishable F(\(ab\))\(^2\))
and Fc portions) and were microscopically not different from normal polyclonal IgG.

Bidimensional gel electrophoresis

Having established that aggregation was not the explanation for the retardation on the gel filtration and the hypocomplementemia of the patients, we analyzed the charge of the three paraproteins isolated by affinity chromatography on protein G. The monoclonal heavy chains from patients presenting monoclonal gammopathies were easily differentiated from polyclonal heavy chains, according to their different two-dimensional electrophoretic patterns (25) (Fig. 5). Monoclonal heavy chains from patients presenting monoclonal gammopathies were easily differentiated from polyclonal heavy chains, according to their different two-dimensional electrophoretic patterns (25) (Fig. 5). Monoclonal heavy chains showed charge microheterogeneity, whereas polyclonal heavy chains were highly heterogeneous and were resolved as unspotted diffuse zones extending in a zone corresponding to pI from 5.5 to more than 9. When the purified IgG fractions from the three patients were analyzed, the spots corresponding to the monoclonal γ-chains were not observed onto the gels, as exemplified for patient F (Fig. 5A), indicating that their charges were highly basic, contrasting with the control myeloma patient depicted in Fig. 5B, whose monoclonal γ-chain appeared as a set of well-resolved spots in the area of the gel corresponding to a pI of about 7 (Fig. 5B). No apparent particular electrophoretic properties were evidenced when analyzing the monoclonal Ig light chains from the three patients and from the control myeloma patients. Monoclonal light chains from patients and control myeloma patients disseminate in more than one spot, and were characterized by different pI. These patterns were clearly different from that of polyclonal Ig light chains (Fig. 5C).

Glycosylation

Finally, heavy glycosylation of the three paraproteins as a possible explanation for the retardation described above was tested on immunoblots using a DIG glycan/protein double labeling kit. However, using that assay, the glycosylation of the paraproteins of patients F, B, and C was not found to be different from the glycosylation of normal polyclonal IgG (data not shown).

Paraprotein binding to complement proteins

Because similar complement profiles as found in these three patients were described in patients with either anti-C1 inhibitor or
anti-C1q autoantibodies, we tested for the presence of both types of autoantibodies. The three sera were negative for anti-C1 inhibitor autoantibodies.

The possible binding of the paraproteins to C1q was investigated using C1q-coated plates, as done for the anti-C1q autoantibody ELISA. In high salt buffer, i.e., the classical anti-C1q autoantibody assay (19), no binding of Ig to C1q could be seen in the sera of patient F and C, whereas there was a clear signal in the serum of patient B. Lowering the ionic strength below the physiological value allowed the detection of specific binding of IgG to C1q in sera F (Fig. 6) and C as well. This signal was not influenced by varying the pH between 6 and 9. Replacing the detecting monoclonal mouse anti-human heavy chain by a polyclonal Ab did not modify the results. Testing all fractions eluted from FPLC-Superdex directly, the IgG binding to C1q was shown to be restricted to the IgG paraprotein in all three sera. Interestingly, under these conditions, the specific C1q binding of the two paraproteins F (Fig. 7) and C was evident even at physiological ionic strength, suggesting that this binding was hindered in whole serum and unmasked when the eluted FPLC fractions were tested separately. However, such weak interactions with C1q were not entirely specific for the paraproteins investigated in this study. In low ionic strength buffer, three of the six control sera of myeloma patients were also showing some binding, although two of these three controls that were positive had strong cryoglobulinemia (>1 mg/ml).

The specificity of anti-C1q autoantibodies has been defined by their binding to the CLR of C1q. In a specific ELISA for anti-CLR Abs, all three paraproteins showed no binding, similarly to HAGG and in contrast to the control sera containing anti-C1q autoantibodies (Fig. 8). When GR of C1q were used as the Ag, there was no drop of the signal as seen for anti-C1q autoantibodies (Fig. 9). These results indicated the globular heads of C1q as the binding site for the paraproteins.

The next question was whether the positively charged heavy chain provided an advantage for such binding. After purification of the heavy chain of paraprotein F, we could demonstrate that this heavy chain bound several fold more efficiently to bound C1q than normal polyclonal heavy chains (Fig. 10).

Complement activation by the paraproteins

The capacity of the three paraproteins to activate complement in vitro was investigated by a C4 hemolytic assay. Whereas normal

![FIGURE 6. Binding to C1q. Anti-C1q autoantibody ELISA of NHS and serum of patient F with varying concentrations of NaCl at the first incubation steps. At 1.1 M NaCl, concentration in the incubation buffer (concentration used for anti-C1q autoantibody determination), the binding to C1q was not different. However, decreasing of the ionic strength in the incubation buffer led to an increased signal in the serum of patient F, indicating the presence of IgG-containing material with low affinity to C1q.](http://www.jimmunol.org/)

![FIGURE 7. Localization of the C1q-binding material. Application of the different gel filtration fractions of the serum of patient F to the anti-C1q autoantibody ELISA in a physiological ionic strength (0.15 molar NaCl) allowed a localization of the C1q-binding material to the paraprotein peak. The left vertical axis indicates the relative peak height of the serum proteins eluted after gel filtration. The right vertical axis indicates the OD of the signal of these fractions after being applied to the anti-C1q autoantibody assay (dotted line).](http://www.jimmunol.org/)

![FIGURE 8. Anti-C1q autoantibody and anti-CLR/C1q ELISA. Sera of patients with hypocomplementemic urticarial vasculitis syndrome (HUVS) and SLE with known anti-C1q autoantibodies, NHS, serum of patient B, as well as purified heat-aggregated human γ-globulin (HAGG), IgG from patient F and C were tested on an anti-C1q autoantibody and an anti-CLR of C1q (CLR/C1q) ELISA using physiological ionic strength (0.15 M NaCl) in the incubation buffer. The two anti-C1q autoantibody-positive sera lost only about 20% of the signal if the C1q were digested to CLR/C1q. In contrast, the signal from the serum of patient B and from the IgG preparations of patients F and C dropped similarly to the signal of HAGG (>95%), indicating that the paraproteins did not bind the CLR of C1q (CLR/C1q).](http://www.jimmunol.org/)
human IgG only slightly consumed C4 (<10%) in concentrations up to 50 mg IgG/ml NHS, the three paraproteins showed a dose-dependent consumption of C4 in NHS. In direct comparison, the paraprotein of patient B caused the strongest C4 loss (Fig. 11). Three IgG paraproteins purified from the serum of myeloma patients produced only a minimal loss of C4.

The loss of C4 function might have resulted from activation of complement by the classical pathway or the lectin pathway, and it is conceivable that the paraprotein inhibited C4 by directly binding to it. The C4 loss was therefore reanalyzed using a C1q-deficient human serum, before and after repleting C1q. In C1q-deficient serum, up to 50 mg of the paraproteins/ml serum had no effect on C4 function. When the serum was repleted with C1q, there was, even in the absence of paraprotein, a small loss of C4 probably related to activation of the classical pathway due to immune complexes in this serum. Strikingly, however, was that the C4 was totally consumed when the paraproteins were added, which was similar to the result observed in NHS. These results indicate that C1q is necessary for the loss of C4 function (Fig. 12). In addition, in all three patients, the preincubation of the paraproteins with NHS led to a dose-dependent formation of C1s-C1 inhibitor (Fig. 13).

A further assay was performed to see whether the paraproteins might have interfered with the binding of C1 inhibitor to C1s. In the presence of the paraprotein, the in vitro binding of C1 inhibitor to activated C1s was unchanged as compared with normal IgG (Fig. 14).

Discussion

In this study, we have analyzed the paraproteins and interactions between these paraproteins and complement in three patients with a unique clinical presentation of recurrent panniculitis and fever, which is different from previous syndromes known to be associated with complement depletion. These paraproteins had unusual physicochemical properties that included retardation on gel filtration chromatography, as well as strongly positively charged γ-chains. Finally, the paraproteins bound the globular heads of C1q, unlike anti-C1q autoantibodies, and activated the classical pathway of complement via C1q despite being monomers in solution.
The retardation by gel filtration was evident using different gel matrices and was not abolished by high salt concentrations. Thus, charge-charge interactions between the paraproteins and the gel matrix could not be responsible for these retardations, although the highly cationic charge of the heavy chains of all three paraproteins might have suggested such interactions. In contrast to that, the paraproteins of patient F and B were even further retarded by a high salt buffer, and in patient C, the retardation of the paraprotein could only be observed under high salt conditions. In addition, the low salt buffer enhanced the retardation of the paraprotein of patient F. These observations suggest a more complex interaction between the paraproteins and the gel filtration matrix, which could be completely prevented only by a strongly dissociating buffer such as 6 M guanidine-HCl. We analyzed six other paraproteins from myeloma patients, and in addition the paraprotein of a patient with plane xanthomatosis and low C1q but otherwise normal complement, which all eluted similarly to normal polyclonal IgG. We have not been able to find a description of a similarly retarded paraprotein in the literature. It will be interesting to see whether this unique retardation profile for a paraprotein, enhanced by high salt, will be found exclusively in patients with the clinical syndrome of recurrent panniculitis and hypocomplementemia. The IgG paraprotein of all three patients bound C1q. Whereas the binding of the paraproteins to C1q could be inhibited by a high salt buffer in patients F and C, this was not possible in patient B. In the most often used anti-C1q autoantibody assay (19), this patient would have been declared as having anti-C1q autoantibodies. However, anti-C1q autoantibodies are described to bind to the CLR of C1q. In contrast to that, we were able to demonstrate that the paraprotein of patient B, as those of patients F and C, bound to the GR of C1q and not to the CLR. It can be speculated that the strong binding of paraprotein B to C1q was responsible for the stronger complement activation compared with the two other paraproteins investigated in this study.

It was striking that the binding to C1q, although almost undetectable in serum for two patients, leads to an efficient activation of C1 and cleavage of C4. According to our findings, this is not due to an impaired formation of C1s-C1 inhibitor complexes. However, considering the high concentration of the paraproteins in vivo compared with the concentration of C1q, it seems to be likely that already the weak affinity of the paraproteins to C1q was sufficient for complement activation.

Although three of six control myeloma paraproteins had a similar low affinity binding to C1q as the paraproteins of patients F and C, they did not activate complement. It is possible that the unique biochemical properties of these paraproteins were responsible for this apparent discrepancy. The strongly positively charged heavy chains in all three patients could explain the measured complement activation. Polycationic molecules have been described as reacting with C1q to induce complement activation via the classical pathway. At the same time, they are known to inhibit the assembly of the alternative pathway C3 convertase by interfering with the binding of C3b to factor B, and they exert an inhibitory effect on C7, C8, and C9 (27). These findings could explain the profile of complement components found in our three patients, in whom the early classical pathway components were depleted, but C3, the AP, and the lytic pathway were normal.

The finding of a monomeric IgG capable of binding C1q and trigger complement activation in the fluid phase would be unique. For this reason, we set out to see whether the paraproteins of the three patients had the property to associate and form polymers in the fluid phase. Such associations could be shown neither by ultracentrifugation nor by electron microscopy. In particular, the general structures of the paraproteins appear completely normal on electron-microscopic pictures.

The link between an ongoing complement activation in these patients and the occurrence of recurrent panniculitis, if there is one, remains speculative. Very probably the hypocomplementemia is due to the presence of the abnormal paraprotein. However, the clinical history of all three patients suggests the need of an additional triggering mechanism such as a trauma or an infection to cause panniculitis. Adipocytes are known to produce all the components of the alternative pathway (C3, factor B, and factor D) (28–30). Furthermore, adipocytes had been shown to fix C1q at their surface (31). Thus, it may be that, in the presence of the paraproteins, at the time of a minimal change in the local synthesis or binding capacity of complement proteins by adipocytes, the cascade is triggered and responsible for local inflammation.

In conclusion, we present three patients with recurrent panniculitis who had highly cationic IgG paraproteins with abnormal retardation on gel filtration. We demonstrated that these soluble monomeric IgG paraproteins directly activated the classical complement pathway. It is likely that they were responsible for the hypocomplementemia of these patients. Our findings provide further evidence that the entity of recurrent s.c. and visceral panniculitis, paraproteinemia, and hypocomplementemia is a unique and well-defined syndrome.

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References