C3 deposition glomerulopathy due to a functional Factor H defect

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CASE PRESENTATION

Two female siblings (Patient 1: 12 6/12; Patient 2: 7 3/12 years old) of consanguineous parents, presented with hematuria and proteinuria at five years (Patient 1) and six months (Patient 2) of age, respectively. Clinical examination, renal ultrasonography and laboratory analyses resulted normal, and both patients were normotensive.

Detailed complement analyses, however, demonstrated activation of the alternative complement pathway reflected by decreased C3 and Factor B (FB), and increased C3d, while C4 was normal. The Factor H (CFH) gene (\textit{CFH}) had a deletion of a single amino acid (Lysine) in position 224 (\Delta K_{224}) within its complement regulatory region in short consensus repeat 4 (SCR 4). In addition, both patients and also the healthy mother were C3 nephritic factor (C3NeF) positive. Renal biopsy in Patient 1 at five years of age prior to therapy (biopsy 1.1) was initially interpreted as membranoproliferative glomerulonephritis type II/dense deposit disease (MPGN II/DDD). Chronic treatment with fresh frozen plasma (FFP) was initiated in both patients (10–15 mL FFP/kg body weight/14 d). A follow-up biopsy of Patient 1 after two years of periodic FFP-infusion (biopsy 1.2) showed no disease progression as compared to the pre-treatment biopsy. This is to our knowledge the first report of successful long-term treatment with periodic FFP-infusion because of dysfunctional CFH and C3NeF.

Furthermore, in light of the recent description of patients with a phenotypical spectrum of glomerular pathology termed glomerulonephritis C3 (we suggest the term 'C3 deposition glomerulopathy (C3DG)' which more precisely describes the pathological changes in the glomerulus than 'glomerulonephritis C3 (GN C3)' does.) which is also caused by dysregulation of the alternative complement pathway, including complement deposition within the glomerular basement membrane (GBM), the subendothelial and mesangial space, the diagnosis of the two patients could be specified as fitting into this disease group.

In summary, chronic treatment with periodical FFP-infusion was successful in preventing disease progression in two patients with C3 deposition glomerulopathy (C3DG) caused by alternative complement pathway dysregulation because of dysfunctional CFH and C3NeF.

PATHOLOGY

Renal biopsies were performed in patient 1 at 5 years of age before therapy (biopsy 1.1) and in both patients, patients 1 and 2, following 2 years of therapy (patient 1: biopsy 1.2; patient 2: biopsy 2). Results of these biopsies are summarized in the following.

Light microscopy

The renal biopsies had 15 (biopsy 1.1), 25 (biopsy 1.2), and 27 (biopsy 2) glomeruli, respectively. The glomeruli showed mild mesangial widening, without hypercellularity, and very rare and small mesangial protein deposits. In the peripheral loops, no significant abnormalities were found. Tubulointerstitial and vascular lesions were absent (Figure 1).

Immunohistochemistry

All glomeruli in the follow-up biopsy of patient 1 (biopsy 1.2) and the biopsy of patient 2 (biopsy 2) showed prominent mesangial deposits of C3 and even more extensive deposits of C5b-9. Peripheral deposits were far less prominent. Minor
deposits of both complement components were present in the basement membrane of Bowman’s capsule. No other deposits were found in glomeruli and/or vessels. The first biopsy of patient 1 (biopsy 1.1) was positive for C3c and C3d in the mesangium and the peripheral loops only (Figure 1).

**Electron microscopy**

Electron microscopy investigations were performed immediately on the follow-up biopsy of patient 1 (biopsy 1.2) and the biopsy of patient 2 (biopsy 2). In both cases, the glomerular structures were well preserved. By low power magnification, slight mesangial enlargement was seen in a few areas, but no deposits were present in the mesangium or in the peripheral loops. Higher magnifications showed a few small nodular or short linear deposits in the mesangial matrix and along the mesangial basement membrane, respectively. In the peripheral loops, some basement membranes exhibited small roundish deposits, mostly within the basement membrane and rarely in a subendothelial position. Short linear deposits were present only in one area. Typical hyperosmiophilia of the deposits, as seen in membranoproliferative glomerulonephritis type II/dense deposit disease (MPGN II/DDD), was not present. Structured deposits were not found. Complete podocyte foot process fusion and very mild activation of endothelial cells accompanied the lesions described. No abnormalities were noted in the tubulointerstitial space or in the vessels. The primarily snap-frozen biopsy of patient 1 (biopsy 1.1) was first studied by immunofluorescence, then embedded in paraffin and finally in epon, and investigated by electron microscopy. Despite massive artefacts, the character and distribution of deposits was the same as described above (Figure 2).

Taken together, these findings indicate the pathomorphological diagnosis of a complement deposition glomerulopathy (glomerulonephritis) with prominent C3 and even more prominent C5b-9 deposits in the mesangium and less in the peripheral capillary loops, minor glomerular abnormalities by light microscopy, as well as few non-hyperosmiophilic deposits in the mesangium and the peripheral capillary loops. Of note, this morphology is similar, if not identical, to the cases described by Servais et al.\(^1\) as glomerulonephritis with isolated C3 deposits.

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**Figure 1** Representative images of biopsies 1.1 and 1.2 of patient 1 (a–c), and of biopsy 2 of patient 2 (d, e). (a) Biopsy 1.1: Minimal mesangial matrix increase, no hypercellularity (PAS stain; × 400). (b) Biopsy 1.2: Minimal mesangial matrix increase, no hypercellularity (PAS stain; × 400). (c) Biopsy 1.2: Immunohistochemistry in paraffin sections for complement C3 showing prominent mesangial and minimal peripheral staining (complement C3 from Dako, Glostrup, Denmark; × 400). (d, e) Minimal mesangial matrix increase without hypercellularity or mesangial deposits, questionable minimal thickening of the peripheral basement membranes (d) (PAS stain, × 400; e: silver stain, × 400). (f) Immunohistochemistry in paraffin sections for complement C5b-9 showing prominent mesangial and peripheral staining (complement C5b-9 from Dako, × 400).

**Figure 2** Typical electron microscopic picture characteristic for both patients. Numerous osmiophilic deposits in the mesangium and in the loop periphery, either intramembranous or subendothelial (arrows). No linear hypereosinophilic deposits. (Primary fixation in buffered formalin (4%), postfixation in glutaraldehyde (3%), × 4400).
**Table 1 | Spectrum of complement-based renal diseases**

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<td>Acute onset with:</td>
<td>Loss of surface complement control:</td>
<td>Segmental thickening of GBM with double contours</td>
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<td></td>
<td>Hemolysis</td>
<td>Mutations resulting in loss (gain*-) of function of CFH, Fi, FB*, MCP</td>
<td>Congestion of capillaries</td>
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<td>Thrombocytopenia</td>
<td>CFHR1/3 deficiency with/without CFH autoantibodies</td>
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<td>Impaired renal function</td>
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<td></td>
<td>Neurological symptoms (rare)</td>
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<tr>
<td>C3DG</td>
<td>Chronic disease:</td>
<td>Loss of systemic complement control:</td>
<td>Mesangial and subendothelial C3 and C5b-9 deposition with/without mesangial proliferation</td>
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<td>MPGN II/DDD</td>
<td>Proteinuria</td>
<td>CFH deficiency</td>
<td>Absence of dense deposits within the basement membranes</td>
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<td>Absence of hematological symptoms</td>
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<td>Linear hyperosmiophilia (electron dense) deposits within the basement membranes and nodular deposits in the mesangium</td>
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**DIFFERENTIAL DIAGNOSIS**

The following stepwise diagnostic approach was applied to the two patients when they first presented with hematuria and proteinuria (Table 1; Figure 3).

Alternative complement pathway activation was examined measuring C3, C3d, C4, CH50, APH50, and in addition, C3 nephritic factor (C3NeF), C3NeF was measured. In both patients, biochemical work-up revealed activation of the alternative complement pathway with increased C3d (patient 1: 156 mU/l; patient 2: 144 mU/l; normal 284–528 mU/l), genetic analyses were performed that identified a compound heterozygous CFH mutation resulting in impaired complement regulatory activity of CFH in both patients. Taking together, these results indicated a glomerulonephritis with activation of the alternative complement pathway.

In keeping with a complement-based pathogenetic concept, the light microscopic picture as well as the immunohistochemical findings with C3 and C5b-9 deposits in the glomeruli were interpreted as MPGN II/DDD (Figure 1). At the ultrastructural level (electron microscopy), however, the findings were not compatible with MPGN II/DDD. The diagnosis MPGN II/DDD requires linear dense (that is, hyperosmiophilia) deposits within the peripheral basement membrane and in the mesangium (Figure 4). As, however, such deposits were not present in the biopsies of the two patients but numerous osmiophilic deposits in the mesangium and in the loop periphery, either intramembranous or subendothelial, were found (Figure 2), this represents a novel subgroup of glomerulonephritis that we defined as C3 deposition glomerulopathy (C3DG).

**Diagnosis**

C3 deposition glomerulopathy.

**DISCUSSION**

During recent years, a crucial role of the alternative complement pathway for the pathogenesis of certain glomerulopathies, for example, MPGN II/DDD and atypical hemolytic uremic syndrome, has been established. Disease-causing are defects in proteins composing or regulating the alternative complement pathway C3 convertase C3bBb, which mediates activation of the central complement factor C3. Numerous soluble (CFH), CFH-related proteins 1 and 3
In patient 1, complement analysis revealed a prompt increase of C3 after FFP infusion that gradually decreased to pretreatment levels after 120 h. In patient 2, post-FFP C3 increase was followed by a more rapid decrease to pretreatment levels than in patient 1.

Glomerular lesions seen at the ultrastructural level in the biopsies of the two reported patients were different from MPGN II/DDD in so far as mesangial deposits were more prominent than peripheral and, even more so, because no linear hypereosinophilic deposits were seen in the peripheral basement membrane. The predominance of C3 (and C5b-9) deposits seen by immunohistochemistry was nonetheless suggestive of a diagnosis in the MPGN II/DDD spectrum. The morphology, however, was similar if not identical to the cases recently described by Servais et al. as glomerulonephritis with isolated C3 deposits.

Interestingly, the CFH mutation of the two presented patients was localized to the regulatory domain SCR4 of CFH. Using in vitro assays we have previously shown that while binding to endothelial cells and C3b was unaffected, cofactor and decay-accelerating activity of the mutant CFH protein was severely reduced. Like CFH deficiency, loss of complement regulatory activity of CFH seems to predispose for a glomerulopathy similar (but not identical) to MPGN II/DDD-type picture or—structurally spoken—from a disease affecting endothelial cells to a disease affecting the GBM. The type of underlying alternative complement pathway dysregulation is relevant for the phenotype as risk factors for atypical hemolytic uremic syndrome (for example, mutations in CFH, immunohistochemical finding, or membrane cofactor protein) direct injury toward the endothelial cells, and risk factors for MPGN II/DDD (for example, C3NeF) toward the GBM, respectively.

As impaired control of the alternative complement pathway caused by a functional CFH defect was identified as underlying cause, chronic therapy with fresh frozen plasma (FFP) to substitute intact CFH was initiated. On the basis of a CFH half-life of 6 days, FFP infusions (10–15 ml/kg body weight/infusion at an infusion rate of 5–7.5 ml/kg body weight) were given every 14 days for a total of 36 months. A representative time course of C3 levels over the period of one treatment cycle indicates treatment efficacy with immediate C3 increase upon FFP infusion in both patients (Figure 5).
During chronic plasma therapy, patient 1 never showed symptoms of disease relapse. Patient 2, however, had several episodes of gross hematuria and proteinuria triggered by upper airway infections. These flares were successfully treated with additional FFP infusions. In both patients, renal function remained normal, blood pressure did not exceed normal range, and persistent proteinuria did not develop over the entire observation treatment period of 36 months. Chronic FFP treatment was well tolerated and no severe side effects occurred. Of note, despite chronic exposure to large amounts of plasma proteins, development of anti-CFH autoantibodies was not observed in either patient.

In summary, we here report—to our knowledge for the first time—successful long-term plasma therapy (36 months) of two patients with C3 deposition glomerulopathy because of defective alternative complement pathway control caused by a functional CFH defect. The patients could efficiently and safely be treated with FFP infusions in the long-term and relapses responded favorably to additional FFP infusions. Anti-CFH autoantibodies, although not detected in the presented patients, may challenge treatment efficacy in the long run and must therefore be monitored.

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REFERENCES