Glomerulonephritis With Isolated C3 Deposits and Monoclonal Gammopathy: A Fortuitous Association?

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Summary

Background and objectives Glomerular deposition of monoclonal Ig has been exceptionally described as the cause of membranoproliferative glomerulonephritis, through activation of the complement alternative pathway (CAP).

Design, setting, participants, & measurements We retrospectively studied six adults with monoclonal gammopathy and glomerulonephritis (GN) characterized by isolated C3 deposits.

Results All patients presented with hematuria, associated with chronic renal failure and proteinuria in five patients, three of whom had nephrotic syndrome. Five patients had monoclonal gammopathy of undetermined significance and one had smoldering myeloma. The serum monoclonal IgG (κ four of six, λ two of six) was associated with light chain (LC) proteinuria in five patients. Four patients had low serum C3 and/or factor B levels. C4, factor H (CFH), and I protein levels were normal in five of five patients; none had detectable C3NeF. IgG anti-CFH activity was positive in one case. No mutations in CFH, CFI, and MCP genes were identified in four of four patients. Deposits were intramembranous, subepithelial, and mesangial by electron microscopy, and stained positive for C3 (six of six), properdin, and CFH (two of two) but negative for Ig LC and heavy chains, C4, and C1q (6/6) by immunofluorescence. Five patients progressed to end-stage renal disease over a median period of 47 months, despite chemotherapy in four patients. In one patient, monoclonal λLC deposits were observed on a follow-up kidney biopsy after 4 years.

Conclusions GN with isolated glomerular C3 deposits might represent an unusual complication of plasma cell dyscrasia, related to complement activation through an autoantibody activity of the monoclonal Ig against a CAP regulator protein.


Introduction

A wide variety of glomerular diseases may occur during the course of plasma cell disorders, resulting from deposition of a monoclonal Ig. Based on light microscopy (LM), immunofluorescence (IF), and electron microscopic (EM) studies of kidney biopsies, these disorders may be classified according to the nature, localization, and ultrastructural appearance of monoclonal Ig deposits (1,2). Organized glomerular monoclonal Ig deposits include immunoglobulinic amyloidosis (2), microtubular/immunotactoid glomerulopathy (3,4), and type I cryoglobulinemic glomerulonephritis (GN) (5,6), whereas granular amorphous deposits are mostly represented by Randall-type monoclonal Ig deposition disease (MIDD). MIDD is characterized by peritubular, glomerular, and vascular deposition of either a single monoclonal light chain (LC) or a truncated heavy chain (HC) lacking the first constant domain, or of both monoclonal Ig LC and HC (1,7).

Recently, a novel type of proliferative GN or membranoproliferative GN (MPGN) with monoclonal Ig deposits was described (8–12). This entity mimics immune-complex GN and differs from classic MIDD by the absence of Ig deposition on tubular and vascular basement membranes, and by the nonlinear, granular appearance of glomerular deposits (8,9). Like other monoclonal Ig-related glomerulopathies, it is prone to recurrence after kidney transplantation (11).

Although hypocomplementemia and glomerular deposition of C3 or other complement components are common findings in most types of glomerular disorders related to monoclonal Ig deposition (3,4,8–10,13,14), little attention has been paid to the role of the complement system in the pathogenesis of renal lesions. Evidence of the nephrotoxic property of a monoclonal Ig, through activation of the complement alternative pathway (CAP) involving anticompement factor H (CFH) antibody activity, was first demonstrated in a patient with MPGN, dense deposits of C3, and monoclonal lambda LC (15,16). Rare cases of dense deposit disease (DDD), also referred to as

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MPGN type II, have been reported in association with plasma cell disorders (17–19). We studied six patients with monoclonal gammopathy and glomerular disease distinct from DDD, characterized by glomerular C3 deposits without monoclonal Ig deposits at presentation. Our data suggest that this entity might represent an unusual renal complication of plasma cell dyscrasia not related to monoclonal Ig deposition but to local glomerular CAP activation by the monoclonal Ig, with or without hypocomplementemia.

**Study Population and Methods**

**Patients**

Six patients referred to five nephrology departments between 1990 and 2009 were retrospectively studied. Inclusion criteria were as follows: (1) isolated diffuse granular glomerular C3 deposits, without evidence of Ig LC or HC deposits on IF study of kidney biopsy; (2) presence of serum and/or urine monoclonal Ig; and (3) absence of detectable serum cryoglobulin.

Demographics and clinical and biologic data were recorded at the time of the first kidney biopsy and at the last follow-up visit. Estimated GFR (eGFR) was calculated using the modified MDRD (Modification of Diet in Renal Disease) equation (20).

**Pathologic Studies**

All kidney biopsy samples were processed for light and IF microscopy, as described previously (3). Sections were systematically stained with Congo red and examined under polarized light. The extent of tubular atrophy, interstitial fibrosis, arteriosclerosis, and the abundance of glomerular deposits were graded on a scale from 0 to 3+. For direct IF, 3-µm cryostat sections were stained using polyclonal FITC conjugates specific for α, γ, and μ Ig HC; κ and λ LC (Dakopatts, Glostrup, Denmark); and C3, C4, C1q, and albumin (Morphosys AbD, Duesseldorf, Germany). In two patients, indirect IF was performed using monoclonal antibodies to C5b-C9 membrane-attack complex, CFH and properdin (Morphosys AbD), and a fluorochrome-conjugated rabbit anti-mouse as secondary antibody (Dakopatts). In five patients, ultrathin sections were processed for EM studies and examined under a JEOL JEM-1010 electron microscope (JEOL, Tokyo, Japan) (3).

**Hematologic and Immunologic Studies**

Bone marrow smears or biopsy (with IF studies in one case) were performed in all patients. CH50 and plasma concentrations of C3, C4, factor B, CFH, and I were measured, as described previously (21). CD46 surface expression was determined on granulocytes using flow cytometry (22). C3 nephritic factor (C3NeF) was assessed by the ability of tested plasma IgG to stabilize a preformed cell-bound C3bBb convertase, and anti-CFH Ab by ELISA (23,24). Complement genetic screening was performed in four patients, using direct sequencing of all CFH, IF, and MCP exons. The CFH H402 haplotype was tagged by genotyping the single nucleotide polymorphism rs1061170 (c.1204T>C; p. Tyr402His), as reported elsewhere (25).

Serum samples were collected, processed at 37°C, and tested for cryoglobulins. Serum and urine monoclonal Ig were detected by conventional electrophoretic and immunoelectrophoretic (IEL) analysis. In two patients, Western blots were performed to determine serum monoclonal Ig subclasses, using the same specific monoclonal antibodies as for IF studies (26).

**Results**

**Clinical Data at Diagnosis**

Six Caucasian patients—three women and three men (median age 67.5 years, range 40 to 74)—were included in the study. At the time of kidney biopsy, five patients had hypertension with significant proteinuria (median 3.3 g/d, range 2 to 5.1), and nephrotic syndrome in three patients. Hematuria was found in all patients, two of whom had experienced episodes of gross hematuria. Median serum creatinine level was 150 μmol/L (range 70 to 298 μmol/L). Three patients had stage 3 chronic kidney disease (CKD), two had stage 4 CKD, and one had stage 5 CKD. Fundoscopic examination, performed in two patients, did not show Drusen or macular degeneration. None had lipodystrophy or other extrarenal manifestations, and none had experienced any infectious episode before admission (Table 1).

**Hematologic and Immunologic Findings**

At the time of diagnosis, all patients had a serum monoclonal IgG (kappa four patients, lambda two patients), with LC proteinuria in five patients. Western blot showed that the IgG subclass was κ1 in patients 1 and 2. Three patients had abnormal serum free kappa (two patients) or lambda (one patient) LC levels. In patients 1, 5, and 5, monoclonal gammopathy had been diagnosed 2 to 10 years before admission.

Bone marrow examination showed a 3% infiltration by dystrophic plasma cells, consistent with stage I multiple myeloma, in patient 3. IgG1 κ-positive plasma cells (1.5%) were found by IF in patient 1. None of the patients had lytic bone lesions, lymphadenopathy, or spleen or liver enlargement (Table 1).

Tests for serum cryoglobulins, rheumatoid factor, hepatitis B and C, and HIV infection were negative in all patients. Antinuclear, anti-dsDNA, and M2 antimitochondrial antibodies were detected in patient 4, without any symptom of systemic autoimmune disease.

**Complement Studies**

Patients 2 and 3 had low serum C3 and factor B levels, which suggested CAP activation. Patient 5 showed decreased levels of factor B, with normal C3 levels. In patient 1, low levels of C3 with normal C4 were observed, but further tests were not available. In the five other patients, serum levels of C4, CFH, and I proteins were normal. CD 46 surface expression on granulocytes was normal in the three patients tested. None of the patients presented with a C3NeF. Anti-CFH IgG antibodies were detected before treatment in patient 2. No genetic abnormality was found in CFH, CFI, and MCP genes in patients 2 to 4 and 6. Patients 3 and 6 carried at least one copy of CFH H402 allele (Table 2).

**Pathologic Findings**

In all patients, the light microscopic appearance of glomerular lesions was characterized by mesangial proliferative...
tion, neutrophilic leukocyte afflux in glomerular tufts, and deposits in the mesangium and glomerular capillary walls (CW; Figure 1). Patients 2 and 6 had mild extracapillary proliferation (Figure 1C), and microthrombi within capillary loops were present in patient 3. Few subepithelial deposits (humps) were observed in five patients (Figure 1D). Five patients had degenerative lesions, including global glomerular sclerosis, tubular atrophy, interstitial fibrosis, and arteriosclerosis, of variable severity.

The main feature by direct IF was diffuse and bright granular glomerular deposits of C3 in all patients (Figure 2A, C, D). No significant staining was observed with anti-kappa, lambda, α, γ, μ, C4, and Clq conjugates. Indirect IF studies, available in patients 2 and 3, did not show significant C5b-C9 deposits; however, positive staining was observed with anti-CFH and antiproperdin antibodies (Figure 2B). By EM, performed in five patients, nonextensive amorphous electron-dense deposits, or deposits of intermediate density, with a “sausage-shaped” appearance, were observed within the lamina densa, which appeared to be interrupted on the subendothelial surface (Figure 3). Deposits of lesser density were found in the subepithelial space (humps) or in paramesangial areas (nodular pseudohumps) in most patients (Figure 3A, B).

In patient 1, two follow-up biopsies showed persistence of glomerular C3 deposits with progression of interstitial fibrosis and glomerular sclerosis. In patient 2, a control biopsy at 4 years showed significant amounts of granular monoclonal lambda LC deposits that colocalized with C3 in the mesangium and glomerular CW (Figure 2D, inset; Table 3).

**Treatment and Follow-Up**

Four patients were given chemotherapy with high-dose dexamethasone, either alone (patient 2) or combined with melphalan (patient 3) or cyclophosphamide (patient 4). Patient 5, who progressed to stage III multiple myeloma after 11 years of follow-up, received three courses of bortezomib plus dexamethasone, relayed by melphalan plus...
thalamidomide and dexamethasone. Treatment was introduced less than 1 year after diagnosis in only one patient, and three had at least stage 4 CKD at the onset of therapy. None achieved hematologic response; however, in patient 2, serum titers of IgG anti-CFH autoantibodies decreased and became undetectable after 11 courses of dexamethasone. Post-treatment kidney biopsies were not performed in any patient.

After a median follow-up of 47 months (range 4 to 162 months), five patients had progressed to (ESRD) over a median time of 48 months (range 12 to 90 months). Patient 1 died from sepsis 14 years after diagnosis and 6 years after the onset of hemodialysis.

Discussion

The association of proliferative glomerular disease with isolated C3 deposits is an extremely rare condition in adults (27). Isolated intramembranous diffuse C3 deposits is characteristic of DDD (27,28), but disseminated granular glomerular CW and mesangial C3 deposits, without IgG deposits, are sometimes observed in late stages of poststreptococcal GN. These two conditions result from CAP activation. C3NeF, an autoantibody with anti-CAP C3 convertase activity, is found in more than 80% of DDD cases and in some cases of poststreptococcal GN (23,27). Recently, Servais et al. (29) introduced the term glomerulonephritis C3 (GNC3) to describe glomerular disease in a series of 19 patients, mostly adults, with isolated glomerular C3 deposits distinct from classical DDD and poststreptococcal GN. Thirteen patients displayed features of type I MPGN, whereas five patients had mesangial and epimembranous deposits without mesangial proliferation and subendothelial deposits. Circulating C3NeF and low serum C3 levels, indicative of systemic CAP activation, were frequent in patients with MPGN features. By contrast, C3 levels were normal in most patients without MPGN features who displayed mutations in CFH or factor I in two thirds of cases. Mutations in the C3 gene have been described in DDD (30), whereas mutations in CFH or CFI genes have been identified in DDD and GNC3 (21,31,32). These data suggest that different mechanisms of CAP activation might result in various patterns of glomerular damage. In the present series, all six patients had similar glomerular lesions, with isolated glomerular C3 deposits and overlapping features of type III MPGN and DDD. Clinical manifestations were homogeneous, including heavy proteinuria, with nephrotic syndrome and progressive renal failure in most cases. Three patients had low serum C3 levels (with low factor B levels in two), and one showed low factor B levels with normal C3 levels. Serum levels of CFH and I proteins were normal in five of five patients, as was C46 expression in three of three patients, and no mutations in CFH, CFI, and MCP genes were identified in four of four patients. Strikingly, all had evidence of monoclonal gammopathy. None of the patients had circulating C3NeF, indicating that an autoantibody activity against the CAP C3 convertase was not involved in the pathogenesis of glomerular lesions.

The prevalence of monoclonal gammopathy in adults with isolated glomerular C3 deposits appears to largely exceed that of the general population, which is around

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<td>Patient No.</td>
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<td>Before Chemo</td>
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<tr>
<td>CH50 (%)</td>
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<td>C3 antigen (mg/L)</td>
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<td>CD46 (MFI)</td>
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Chemo, chemotherapy; MFI, mean fluorescence index; C3NeF, C3 nephritic factor; NA, not available.

Normal values: CH50, 70% to 130%; C3 antigen, 660 to 1250 mg/L; C4 antigen, 93 to 380 mg/L; Factor B, 90 to 320 mg/L; H protein, 65% to 140%; I protein, 70% to 130%; MCP, 600 to 1400 MFI.
1% to 2% in adults and increases from 3.2% to 7.5% in patients aged over 50 years and over 85 years, respectively (33,34). Since the first description of MPGN and isolated C3 deposits with monoclonal gammopathy (17), few similar cases have been described. Nasr et al. (18) reported that four adults (22%) out of a series of 32 DDD cases had a history of plasma cell disorder. All had typical ultrastructural features of DDD and sole or dominant C3 deposition without LC restriction. The type of the circulating monoclonal component was not detailed.

In a recent series of 81 hepatitis-negative MPGN patients, 28 had evidence of monoclonal gammopathy, three of whom had isolated C3 deposits (12). Recently, Sethi et al. found that among 14 patients with DDD, 10 (71.4%) had a serum monoclonal IgG (19). By IF, only C3 deposits were observed. Two patients with monoclonal gammopathy and either DDD (19) or GNC3 (35) carried one or two copies of the CFH H402 allele variant, which has been found to be associated with increased risk of DDD (36). However, as the frequency of the H402 allele is high in the general population, and as it was not present in two patients from the present series, it is unclear whether a genetic permissive background is required for the development of DDD or GNC3 in patients with monoclonal gammopathy.

In the present series, the diagnosis of DDD was unlikely, as characteristic glomerular patterns (“tram tracks,” mesangial rings, continuous glomerular intramembranous dense deposits) were not observed, and because circulating C3NeF, a common finding in DDD, was absent. Whereas, in previous reports, typical ultrastructural features of DDD were mostly observed in patients with monoclonal gammopathy (17-19), C3GN has been also described as in the present patients (35).
The frequency of complement activation in patients with plasma cell disorders is likely to be underestimated. Deficient alternative and terminal complement pathways have been reported in 30% of patients with multiple myeloma (37). In the present series, a role of monoclonal Ig in the local or systemic activation of CAP, leading to subsequent glomerular proliferative lesions and C3 deposits, is questionable. Such a hypothesis is strongly suggested in patient 2, in whom MPGN with C3 deposits, but no evidence of monoclonal Ig deposition, was diagnosed simultaneously with IgG1/H9261 monoclonal gammopathy and circulating anti-CFH IgG autoantibody. A control biopsy at 4 years, before treatment, revealed colocalization of glomerular C3 and lambda LC deposits, suggesting that isolated C3 deposits might be an initial step before subsequent monoclonal Ig deposition. This case is reminiscent of the MPGN case LOI, in which a dimeric monoclonal V/H9261 LC was shown to behave as a mini autoantibody to CFH. By binding to the short consensus repeat domain 3 at the N-terminal end of CFH, the LOI dimer blocked the interaction between CFH and C3b, thereby inhibiting the activity of CFH and inducing uncontrolled CAP activation (15,16). We later reported a similar case (L0P) with MPGN and lambda LC, C3, CFH, and C5b-C9 deposits, without evidence of C3NeF. A serum fraction enriched in monoclonal lambda LC induced C3 conversion by the AP, with cleavage of factor B and increased Bb level, while the C4 level remained normal (2). The association of isolated glomerular C3 deposits with MGUS, rather than with high-mass myeloma (12,18,19,35), might suggest that prolonged CAP activation by the monoclonal Ig is required for the development of glomerular lesions.

In the present case, and in previously reported cases, of glomerular C3 deposits with monoclonal gammopathy, renal outcome was poor, with progression to ESRD in most
Figure 3. Electron microscopic findings. (A) Patient 2, second kidney biopsy (original magnification: ×4000): electron-dense voluminous subepithelial deposits (humps) (asterisks). (B) Patient 2, second kidney biopsy (original magnification: ×6000): round, nodular (pseudohumps) electron-dense mesangial deposits (asterisk) and interrupted intramembranous dense deposits (arrow). (C) Patient 1, third kidney biopsy (original magnification: ×3300): glomerular basement membrane thickened by highly electron-dense interrupted intramembranous deposits displaying a sausage-shaped (arrow) or bead-like (arrowhead) pattern. (D) Patient 1, third kidney biopsy, original magnification: ×3000): intramembranous interrupted electron dense deposits (asterisks). Note the disruption of glomerular basement membrane, with protrusion of podocyte epithelium (between short arrows) into the capillary lumen (white arrow) and neoproduction of basement membrane-like material (arrowheads). US, urinary space; CL, capillary lumen.
patients (12,18,19). The demonstration of improvement of renal function with reduction in proteinuria following chemotherapy would reinforce the role of monoclonal gammopathy in the pathogenesis of isolated glomerular C3 deposits. In our series, treatment was introduced too late in the course of the disease to draw any valid conclusion on its potential benefit. However, as the anti-CFH autoantibody disappeared after dexamethasone therapy in patient 2, early chemotherapy aimed at controlling the underlying clonal disorder should be discussed with regard to preventing progression toward ESRD (12). Eculizumab therapy, which may improve renal symptoms in DDD (38), also represents an interesting alternative (39).

Conclusions
Monoclonal gammopathy should be considered in adult patients with MPGN. Isolated C3 deposits might represent an unusual complication of plasma cell dyscrasia related to CAP activation by the monoclonal Ig. Further studies, based on testing the ability of purified monoclonal Igs to activate CAP through an autoantibody activity against CFH or another complement regulator protein, are needed to confirm this hypothesis.

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Disclosures
None.

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