Nodular Glomerulosclerosis with Deposition of Monoclonal Immunoglobulin Heavy Chains Lacking C_{H1}

BRUNO MOULIN,* SOPHIE DERET,§ XAVIER MARIETTE,¶ OLIVIER KOURILSKY,‡ HIROKAZU IMAI,** LUC DUPOUET,§ LUC MARCELLIN,† ISABELLE KOLB,* PIERRE AUCOUTURIER,¶ JEAN-CLAUDE BROUET,‖ PIERRE M. RONCO,# and BÉATRICE MOUGENOT#

Departments of *Nephrology and †Pathology, University Hospital, Strasbourg, France; Renal Divisions, ‡Evry and §Chartres Hospitals; †Department of Clinical Immunology, Saint-Louis Hospital, and Institut National de la Santé et de la Recherche Médicale ‡U25 and #U489, Necker and Tenon Hospitals, Assistance Publique-Hôpitaux de Paris, Paris, France; and **Third Department of Internal Medicine, Akita University School of Medicine, Akita, Japan.

Abstract. The objective of this study was to further characterize the clinical and immunopathologic features of heavy chain deposition disease (HCDD), a recently described entity. Four patients were diagnosed as having HCDD on a kidney biopsy. All presented with nodular glomerulosclerosis with deposition of γ1 heavy chains lacking C_{H1} epitopes, but without light chains. Two different patterns were observed in the serum. First, patients 1 and 2 had a circulating monoclonal IgG containing a short γ1 heavy chain lacking C_{H1} epitopes, but with an apparent molecular weight of 40 kD consistent with a complete C_{H1} deletion. Biosynthetic experiments also showed that the deleted heavy chain was produced in excess compared with light chains, and was secreted in vitro together with half Ig molecules, although these abnormal components were not detected by Western blot analysis of whole serum. Second, patients 3 and 4 had a circulating monoclonal IgG1 with an apparently normal, nondeleted heavy chain subunit, but serum fractionation followed by immunoblotting revealed an isolated monoclonal γ1 chain lacking C_{H1} epitopes. These data strongly suggest that renal deposition of a C_{H1}-deleted heavy chain circulating in low amounts in the serum as a free unassembled subunit is a major feature of HCDD. The C_{H1} deletion is most likely responsible for the premature secretion in blood of the heavy chain by a clone of plasma cells.

Since the first description of amyloid light chain (AL)-amyloidosis by Glenner and colleagues (1), the spectrum of glomerular diseases associated with deposition of monoclonal Ig components has expanded dramatically. These diseases can be divided into two categories depending on ultrastructural appearance of the deposits by electron microscopy (reviewed in reference (2)).

Those with organized deposits include diseases with fibril formation, mainly AL-amyloidosis, and diseases with microtubule formation covering mixed cryoglobulinemia and immunotactoid glomerulonephritis.

The second category of diseases is characterized by nonorganized electron-dense granular deposits. These deposits are noncongophilic and are not stained by the other amyloid dyes. They are found in tubular and glomerular basement membranes, where they are they are mostly associated with monoclonal light chains, usually of the κ type, thus defining an entity now termed non-amyloid monoclonal Ig deposition disease. The first cases of myeloma-associated glomerulosclerosis were reported in the late 1950s by Kobernick and Whiteside (3) and by Sanchez and Domz (4), who described glomerular nodules “resembling the lesion of diabetic glomerulosclerosis,” and lacking the staining features and fibrillar organization of amyloid. The presence of light chains in these lesions was recognized later in 1973 by Antonovych et al. (5) and was confirmed by Randall and associates (6), who published in 1976 the first extensive description of light-chain deposition disease (LCDD). In approximately 10% of patients, monoclonal heavy chains are associated with the monoclonal light chains in the renal deposits (7), hence the designation of light- and heavy-chain deposition disease. Biosynthesis experiments performed on bone marrow cells have shown that monoclonal heavy chains with a normal size were produced by four of 27 LCDD patients, although they were not deposited in kidneys (reviewed in reference (8)). In contrast, truncated heavy chains were synthesized in four of the five studied cases of light- and heavy-chain deposition disease, which suggests a close relationship between heavy-chain structural abnormalities and their tissue deposition. The finding of patients with heavy-chain deposition disease (HCDD) was expected since the de-
scription of systemic heavy-chain amyloidosis (AH-amyloidosis) by Eulitz and colleagues (9), who identified the amyloid component as a short γ chain without light chain.

Deposits containing monoclonal heavy chains only, in the absence of detectable light chains, were indeed reported in 1993 by Aucouturier et al. (10) in two patients affected with otherwise typical monoclonal Ig deposition disease. Since then, we have observed four additional cases and four more patients have been reported in the literature as isolated cases (11–15).a

We describe herein the clinical, pathologic, and immunochemical features of our four patients who all produced and deposited in kidney an isolated IgG heavy chain lacking epitopes of the first constant domain (C\text{H}1). Two patients had a circulating IgG\text{\kappa} with a truncated heavy chain component. But in the other two, the circulating monoclonal IgG\text{\kappa} was composed of an apparently normal heavy chain, i.e., reactive with all anti-constant domain antibodies, and demonstration of the truncated heavy chain (circulating as a free unassembled subunit) required serum fractionation.

### Materials and Methods

#### Patients

The four patients included in this study were diagnosed as having HCDD on a kidney biopsy. All of them showed typical nodular glomerulosclerosis and γ heavy chain deposits in the absence of light chains by immunofluorescence. Electron-dense granular deposits along renal basement membranes and in mesangial nodules were found in the three biopsies studied by electron microscopy (patients 1, 3, and 4). A careful search for glucose intolerance remained negative in all patients, excluding diabetes-induced glomerulosclerosis. Thus, the four patients fulfilled the diagnostic criteria for HCDD. Informed consent was obtained for all further investigations, including bone marrow biosynthesis experiments.

#### Pathologic Studies

Specimens were fixed in Dubosq-Brazil fixative and processed by standard histologic techniques for light microscopy, as described previously (16). Furthermore, sections were systematically stained with Congo red and visualized under polarized light.

For immunofluorescence studies, cryostat sections were incubated with fluorescein-conjugated specific antisera for human IgG, IgA, IgM, C1q, C3, fibrinogen, κ- and λ-light chains (Hoechst-Behring, Rueil-Malmaison, France; Dako, Glostrup, Denmark; BioWhittaker, Walkersville, MD; Atlantic Antibodies, Bio-Rad, Ivy sur Seine, France). Biopsies were further examined with monoclonal antibodies specific for IgG subclasses and for γ chain constant domains (see Table 1 and below for origin of the antibodies). For this purpose, a polyclonal rhodamine-conjugated goat anti-mouse was used as a second antibody (Immunotech, Marseille, France).

Finally, biopsies were studied by electron microscopy after fixing in 2.5% glutaraldehyde in 0.2 M sodium cacodylate or 0.1 M sodium phosphate, pH 7.4, and post-fixing in 1% osmium tetroxide. After staining with uranyl acetate and lead citrate, sections were examined using an EM 109 Zeiss or a JEM 1010 Jeol electron microscope.

#### Immunochemical Studies

Serum and concentrated urine samples from the four patients were studied by immunoelectrophoresis, and by single pressure immunoblotting after thin-layer agarose electrophoresis as described previously (17). Blots were revealed with monoclonal antibodies specific for κ- and λ-light chain constant domains, and for γ heavy chain constant domains (C\text{H}1, C\text{H}2, C\text{H}3) and hinge region (h) (Table 1). Sources of these antibodies were as follows: clones HP 6053, HP 6054, HP 6044, HP 6017, and HP 6017 were from the Center for Disease Control, Atlanta, GA; clones NL16, GOM2, ZG4, and RJ4 were purchased from Unipath, Bedford, United Kingdom; clone GG7 was obtained from Sigma, St. Louis, MO; clones TM15, ZB8, G7C, and A1 were kindly provided by Dr. Margaret Goodall (Birmingham, United Kingdom). Antigen-antibody reactions were detected

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*Table 1. Epitope analysis of circulating and deposited monoclonal Ig components*

<table>
<thead>
<tr>
<th>Monoclonal Antibody</th>
<th>Serum Specificity</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clone</td>
<td></td>
<td>Serum IgG</td>
<td>Kidney</td>
<td>Serum IgG</td>
<td>Kidney</td>
</tr>
<tr>
<td>HP 6053</td>
<td>κ</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>HP 6054</td>
<td>λ</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>NL 16b</td>
<td>γ1C\text{H}1</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>HP 6014</td>
<td>γ2C\text{H}1</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>GOM2</td>
<td>γ2C\text{H}2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>ZG4</td>
<td>γ3 hinge</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>RJ4</td>
<td>γ4C\text{H}3</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>HP 6044-TM15-ZB8</td>
<td>γC\text{H}1</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>HP 6018-G7C-GG7</td>
<td>γC\text{H}2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>HP 6017-1A1</td>
<td>γC\text{H}3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

*a* All antibodies were shown to react with renal deposits of polyclonal IgG. HC, heavy chain.

*b* Reactivity with NL 16 indicates the γ1 subclass of the abnormal monoclonal protein.

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a One of the four cases was published by Yasuda et al. (13) as having a C\text{H}2 deletion. In fact, other studies cited in this article and performed by Sophie Deret indicated that the C\text{H}1, not the C\text{H}2, was deleted. The patient was therefore included in the present series.
with alkaline phosphatase-conjugated anti-mouse IgG antibodies and revealed with tetrazolium nitroblue and bromochloro-indolyl phosphate, as described previously (10).

In two patients (patients 3 and 4) who had monotypic CH1-deleted γ heavy chain renal deposits, the deleted heavy chain could not be detected by Western blot analysis of crude serum samples. Therefore, fractions were prepared from the patients’ serum using Sephadex G200 (Pharmacia, Uppsala, Sweden) gel filtration followed by diethyaminoethyl-Trisacryl (Sepracor, Villeneuve-La Garenne, France) chromatography in 10 mM Tris, pH 8.0, with a linear 0 to 0.3 M NaCl gradient. Fractions were then analyzed by immunoblotting after thin-layer agarose electrophoresis as described above.

In patients 1 and 2, the serum heavy chain molecular size was determined after reduction by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of a serum fraction prepared by Sephadex G200 gel filtration followed by diethyaminoethyl-Tri sacryl chromatography in 10 mM Tris, pH 8.0, with a linear 0 to 0.3 M NaCl gradient. Fractions were then analyzed by immunoblotting after thin-layer agarose electrophoresis as described above.

Bone Marrow Biosynthesis Experiments
Bone marrow mononuclear cells were isolated from patient 1 on a Ficoll-Hypaque gradient. The cell preparation contained 20% plasma cells, most of them stained by a direct fluorescence assay with antiserum to human γ and λ Ig chains. Cells at a concentration of 5.10⁶/ml were continuously labeled for 3 h with 100 μCi of a mixture of radioactive amino acids (¹⁴C valine, ¹³C leucine, ¹⁴C threonine) in RPMI 1640 depleted of the relevant cold amino acids and supplemented with 5% fetal calf serum and antibiotics.

Supernatant and cells were separated by centrifugation. Cells were lysed in 1% NP40 buffer (30 min at +4°C). Lysate and supernatant were precleared 6 times with Sepharose-4B beads. Protein A-Sepharose beads coated with rabbit IgG directed to human Ig chains (10 μl) were thereafter added for 30 min and extensively washed in 0.5% NP40 buffer. The beads were boiled for 1 min, and the dissolved material was fractionated by 7.5% SDS-PAGE, either unreduced or after reduction with 2-mercaptoethanol (0.15 M).

Statistical Analysis
Results from calculated frequencies of renal manifestations in LCDD and HCDD were compared using the χ² test for statistical significance.

Results
Clinical Data
Clinical and laboratory features of the four patients are summarized in Table 2. The youngest patient was 35 yr old. Three of them had increased BP, and modest to severe renal failure. Despite severe glomerular lesions, proteinuria was in the nephrotic range in two patients only. Although proteinuria was only 1.0 g/d, patient 1 showed unexplained marked hypoalbuminemia (23 g/L): Liver function tests were normal, tests for protein-losing enteropathy and malabsorption were negative as was the search for amyloid deposits in gastrointestinal biopsies. In patient 4 also, the serum albumin level was decreased (27 g/L) despite a low proteinuria output (0.5 g/d), but neither a liver disease nor a protein-losing enteropathy could be identified. All patients presented with microscopic hematuria. Two patients (1 and 3) fulfilled the criteria of low-mass multiple myeloma, including an excess of plasma cells (>10%) in the bone marrow and the presence of nucleocytoplasmic abnormalities of bone marrow plasma cells. They were classified as stage I according to Durie and Salmon (18).

Pathologic Studies
The renal biopsy specimens from the four patients showed quite similar lesions by light microscopy (Table 3). All were

<table>
<thead>
<tr>
<th>Case</th>
<th>Gender/Age</th>
<th>BP</th>
<th>Renal Presentation</th>
<th>Bone Marrow</th>
<th>Treatment</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M/58</td>
<td>HT</td>
<td>100, 1</td>
<td>+</td>
<td>14</td>
<td>Yes</td>
</tr>
<tr>
<td>2</td>
<td>M/71</td>
<td>HT</td>
<td>280, NS</td>
<td>+</td>
<td>2</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>M/51</td>
<td>HT</td>
<td>133, (→450)</td>
<td>NS</td>
<td>20</td>
<td>Yes</td>
</tr>
<tr>
<td>4</td>
<td>F/35</td>
<td>NT</td>
<td>90, 0.5</td>
<td>+</td>
<td>5.8</td>
<td>No</td>
</tr>
</tbody>
</table>

BP, blood pressure; SCR, serum creatinine; HT, hypertensive; NT, normotensive; NS, nephrotic syndrome; VAD, vincristine + adriamycin + dexamethasone; VMCP, vincristine + melphalan + cyclophosphamide + prednisone; ABSCG, autologous blood stem-cell grafting.
characterized by nodular glomerulosclerosis with an important increase in mesangial matrix and irregular thickening of glomerular basement membranes (Figure 1). The mesangial nodules were periodic acid-Schiff positive. Mild hypercellularity was present, but no crescent formation was seen. Glomerular lesions were associated with variable thickening of tubular basement membranes, prominent in patients 2 and 3, mild and focal in the other cases. Similarly, the artery and arteriole deposits were more important in patients 2 and 3. No myeloma cast was observed. The renal lesions did not show any staining characteristic of amyloid. In particular, there was no green birefringence under polarized light after staining with Congo red.

Electron microscopy was performed in patients 1, 3, and 4. It demonstrated an expansion of the mesangial matrix with deposition of finely granular electron-dense material within the mesangial nodules. Electron-dense deposits were associated with 12-nm-wide fibrils in patient 4. Glomerular basement membranes were irregularly thickened and often wrinkled. They contained a continuous band of granular electron-dense material, located in the lamina rara interna and/or in the lamina densa, often resulting in dense transformation of the whole membrane (Figure 2). Electron-dense deposits were less prominent along tubular basement membranes, Bowman’s capsule basement membranes, and around the arteriolar myocytes.

In spite of the irregular involvement of the tubules, both light- and electron microscopy data were highly suggestive of LCDD. However, immunofluorescence examination of the biopsies with routinely used polyclonal antisera showed a strong staining of mesangial nodules and of glomerular basement membranes with the anti-γ heavy chain conjugate (Figure 3), but no staining with either the anti-κ or the anti-λ antibody. In patients 2, 3, and 4, substantial deposits of γ heavy chain were also seen along tubular basement membranes (Figure 3) and in vascular walls, whereas in patient 1, only a faint staining of

Table 3. Immunologic data and renal pathology

<table>
<thead>
<tr>
<th>Case</th>
<th>Serum</th>
<th>Urine M-Component</th>
<th>Features of M-Heavy Chain</th>
<th>Immunohistology</th>
<th>Electron Microscopy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>IgG</td>
<td>C4</td>
<td>λ</td>
<td>γl in MSG and GBM, and arterioles</td>
<td>Granular EDD in MSG and GBM, and arterioles</td>
</tr>
<tr>
<td>2</td>
<td>IgG</td>
<td>C4</td>
<td>γl in MSG and GBM, and arterioles</td>
<td>γl in MSG and GBM, and arterioles</td>
<td>Granular EDD in MSG and GBM, and arterioles</td>
</tr>
<tr>
<td>3</td>
<td>IgG</td>
<td>C4</td>
<td>γl in MSG and GBM, and arterioles</td>
<td>γl in MSG and GBM, and arterioles</td>
<td>Granular EDD in MSG and GBM, and arterioles</td>
</tr>
<tr>
<td>4</td>
<td>IgG</td>
<td>C4</td>
<td>γl in MSG and GBM, and arterioles</td>
<td>γl in MSG and GBM, and arterioles</td>
<td>Granular EDD in MSG and GBM, and arterioles</td>
</tr>
</tbody>
</table>

Figure 1. Light microscopy of renal biopsy specimen from patient 2. Nodular glomerulosclerosis. Note moderate mesangial hypercellularity, some double-contour aspects, and capillary aneurysm formation. Most of the tubular basement membranes are thickened. Masson’s trichrome. Magnification, ×312.
some tubular and vascular structures was observed with the anti-\(\gamma\) heavy chain antibody (Figure 3). The absence of light chains in the deposits was confirmed with the light chain-specific monoclonal antibodies HP 6053 and HP 6054 (Table 1).

In addition, biopsy specimens from patients 1 to 3 were used to perform an epitope analysis of the \(\gamma\) heavy chain deposits with monoclonal antibodies specific for the \(\gamma\) heavy chain constant domains (Table 1). First, this analysis established that the deposited heavy chain belonged to the \(\gamma\)1 subclass since deposits were reactive with NL16, a monoclonal antibody specific for the C\(_{H1}\)2 domain of the \(\gamma\)1 subclass (Figure 4). No reactivity was observed with antibodies specific for the other \(\gamma\) subclasses (Table 1), which indicated that the deposits were monotypic. The IgG subclass in the kidney was the same as that of the serum monoclonal components (see below and Table 3). Second, we failed to detect C\(_{H1}\)1 epitopes with three different antibodies (HP 6044, TM15, ZBB), whereas a strong staining of mesangial nodules and basement membranes was obtained with anti-C\(_{H2}\) and anti-C\(_{H3}\) antibodies (Figure 4). These results suggest that the \(\gamma1\) heavy chain deposited in kidney lacks the C\(_{H1}\)1 domain. No significant complement deposits were shown except in some mesangial areas for patient 4, despite signs of complement activation in patients 2 to 4 (Table 3).

**Immunochemical Studies of Serum and Urine**

The four patients had a circulating monoclonal IgG\(\lambda\) (Table 3). To identify the subclass of the circulating IgG\(\lambda\) and to determine whether its heavy chain component lacked the C\(_{H1}\) as did the deposited \(\gamma\) heavy chain, sera were analyzed by nondenaturating agarose electrophoresis followed by Western blotting with the anti-constant domain-specific monoclonal antibodies (Table 1). The heavy chain isotype was \(\gamma1\) in all four patients as shown by reactivity with antibody NL16 (Table 1). However, a different pattern of reactivity with anti-C\(_{H1}\)1 antibodies was observed.

In patients 1 and 2 (see Figure 5 for patient 2), the circulating IgG\(\lambda\) failed to react with the anti-C\(_{H1}\)1 antibodies, whereas normal reactivity was obtained with the anti-C\(_{H2}\) and anti-C\(_{H3}\) antibodies. To estimate the molecular weight of the abnormal heavy chain, the IgG fraction was purified and submitted to SDS-PAGE under reducing conditions followed by Western blot. Figure 6 shows the presence, together with polyclonal normal-sized (50 kD) \(\gamma\) chains, of a short (40 kD) \(\gamma\) chain of IgG1 subclass that was not revealed by the anti-\(\gamma\) C\(_{H1}\)1 antibody TM15. These results are consistent with a deletion of the whole C\(_{H1}\)1 domain in the monoclonal \(\gamma1\) heavy chain. SDS-PAGE and Western blot analysis of the serum from patient 1 revealed the presence of a short (40 kD) \(\gamma\) chain of IgG1 subclass (not shown).

In contrast, the circulating monoclonal IgG1\(\lambda\) detected in patients 3 and 4 exhibited normal reactivity with all tested antibodies, which indicated that the deposited heavy chain was not deleted (not shown). We therefore hypothesized that the deposited heavy chain circulated in low amounts possibly as a free heavy chain. To test this hypothesis, sera from patients 3 and 4 were fractionated as described in Materials and Methods, and a 5S Sephadex fraction was analyzed by nondenaturating agarose electrophoresis followed by Western blotting. A monoclonal \(\gamma1\) heavy chain lacking C\(_{H1}\)1 epitopes was then revealed (Figure 7A, patient 3; Figure 7B, patient 4). In the urine, small amounts of an isolated \(\gamma\) chain lacking C\(_{H1}\)1 epitopes were detected in patient 2.

**Bone Marrow Biosynthesis Experiments**

SDS-PAGE of Ig precipitated from bone marrow cell lysates or supernatants from patient 1 showed two main bands at 70 and 40 kD (Figure 8). After reduction, two bands of 40 and 30 kD were observed (Figure 8). These data are consistent with the synthesis of a short \(\gamma\) heavy chain (40 kD) and normal-sized A chain (30 kD) that partly assembled covalently in half Ig molecules (70 kD).

**Outcome**

Patients 1, 3, and 4 were treated with various regimens in their respective hospitals (Table 2). All three showed a marked improvement of renal abnormalities. In particular, renal function dramatically improved in patient 3 after chemotherapy followed by autologous blood stem-cell grafting. In contrast, patient 2, who was left untreated, reached end-stage renal failure.

**Discussion**

This article reports the clinical, pathologic, and immuno-logic characteristics of four patients with \(\gamma\) HCDD, who shared a deletion of the C\(_{H1}\). To our knowledge, only eight other cases of HCDD have been reported thus far (10–15, 19). Although they were not recognized as HCDD, the first two cases were described by Tubbs and associates (19) in 1992, who proposed the term “pseudo-\(\gamma\) heavy chain deposition disease.” Both patients presented with “nodular glomerulopathy” and massive deposition of \(\gamma4\) heavy chain in vascular, tubular, and glomer-
ular basement membranes. Light chain deposits were absent in one patient, very faint and limited to the glomerular basement membrane in the other. In 1993, Aucouturier et al. (10) first described in two patients gross abnormalities of the circulating or deposited heavy chains that exhibited a deletion of the $C_H^1$ in one case, and of both the $C_H^1$ and the $C_H^2$ in the other case. Since 12 cases including ours have been reported thus far, it is now possible to give a more detailed description of the disease, to ask whether LCDD and HCDD can be considered variants of the same disorder, and to propose a pathophysiologic scenario.

Figure 3. Direct immunofluorescence with polyclonal fluorescein-conjugated anti-$\gamma$ antibody. (Left) Patient 1. Glomerular nodules, all mesangial areas, and peripheral capillary walls are brightly stained (top), whereas only a faint staining was observed in some tubular basement membranes and in arteriolar walls (bottom). (Right) Patient 2. Diffuse strong staining of the tubular basement membranes (bottom) and of the glomerular structures, especially mesangial nodules (top). Staining with the anti-light chain antibodies was negative in both patients. Magnification, $\times 312$.

Figure 4. Epitope analysis of $\gamma$ chain deposits by indirect immunofluorescence in patient 1. Monoclonal antibodies specific for $\gamma$ chain constant domains and for $\gamma 1$ heavy chain were used. Deposits reacted with NL16, a monoclonal antibody specific for the $\gamma 1$ subclass. Note staining of the deposits with anti-$C_H^2$ and anti-$C_H^3$ antibodies (respectively, G7C and HP 6017), but not with anti-$C_H^1$ antibody (TM15). Magnification, $\times 312$. 
The distinguishing mark of HCDD is the deposition in kidney of heavy chains only. The deposited γ heavy chain belongs to a single subclass: γ1 in five patients including ours, γ4 in four, and γ3 in one (10–13,15,19). Only one patient with an α HCDD was reported (14). The most remarkable characteristic of the monoclonal heavy chain is the deletion of the C\textsubscript{H1}, which was established in our four patients by three different approaches. First, an epitope analysis of the constant domains performed in the serum by immunoblotting and in the biopsy specimen by immunofluorescence showed the absence of C\textsubscript{H1}-specific epitopes defined by monoclonal antibodies HP6044, TM15, and ZB8. Second, SDS-PAGE revealed that the circulating IgG1 in patients 1 and 2 contained a short γ1 heavy chain with an apparent molecular weight of 40 kD consistent with the complete deletion of a single domain. Third, biosynthetic experiments performed in patient 1 were also consistent with the synthesis of one domain-deleted heavy chain (and a normal-sized light chain). In patient 2, the complete deletion of C\textsubscript{H1} was confirmed by sequencing the corresponding cDNA after reverse transcription-PCR amplification of the bone marrow-extracted total RNA (24). In the three other patients in whom it was searched for in previous reports, a deletion of the C\textsubscript{H1} was also found, together with a C\textsubscript{H2} deletion in two patients (10,14).

Western blot analysis of the serum showed that the C\textsubscript{H1}-deleted γ heavy chain was either assembled with a light chain to form a complete IgG (patients 1 and 2), or circulated in low amounts as a free unassembled heavy chain, the detection of which required serum fractionation (patients 3 and 4). It is likely that in patients 1 and 2 also, unassembled or partially assembled truncated γ chains circulated in blood. Indeed bio-

**Figure 5.** Agarose electrophoresis (EP) in non-denaturing buffer and immunoblotting study of the serum of patient 2. Blots were revealed with monoclonal antibodies against lambda (\(\lambda\)) and kappa (\(\kappa\)) chain constant regions, \(\gamma\) chain C\textsubscript{H1} (clone TM15, \(\gamma\text{C}1\)), C\textsubscript{H2} (clone G7C, \(\gamma\text{C}2\)) and C\textsubscript{H3} (clone HP6017, \(\gamma\text{C}3\)), and IgG1 subclass C\textsubscript{H2} domain (clone NL16, \(\gamma\text{C}1\text{H}2\)). Blots incubated with anti-\(\kappa\) and anti-\(\gamma\text{C}1\) antibodies were purposely overexposed to demonstrate lack of reactivity of the monoclonal component revealed by EP. The weak narrow band in the \(\kappa\) blot corresponds to a minor monoclonal IgG2\(\kappa\), revealed with the anti-IgG2 antibody (not shown).

As indicated in Table 4, both diseases show about the same male to female ratio and age distribution. The prevalence of renal failure is also similar. However, that of hypertension and hematuria seems to be significantly higher in HCDD, which may be accounted for by the greater severity of renal lesions. Indeed, nodular glomerulosclerosis was observed in all 12 patients. In addition, crescents were described in three of them (12,14,19). In contrast, in the largest biopsy series of LCDD (7), nodular glomerulosclerosis was found in only 61% of the 49 biopsies, whereas milder lesions, \(i.e.,\) increased mesangial matrix, were identified in 28% of the biopsies, and no glomerular lesion was visible by light microscopy in 10% of them. No crescent was described. The more advanced glomerular lesions observed in HCDD may be related to more prolonged course of the underlying hematologic disease since myeloma accounts for only 25% of the 12 HCDD cases, compared to 45% of those of LCDD (20–23). Furthermore, although nonfibrillar, granular electron-dense deposits are found in mesangial nodules and in basement membranes in both diseases, the prevailing topography of monoclonal Ig components may be slightly different, at least in some patients. Indeed, monoclonal light chain deposits in tubule basement membranes are a constant feature of LCDD, but glomerular deposits may be absent (7). An inverse pattern was observed in our patient 1 who had very faint γ1 deposits in tubular basement membranes contrasting with intense deposits in glomeruli. Despite these slight differences, the overall renal presentations of LCDD and HCDD look very similar.
synthetic experiments in patient 1 revealed that the Ig chains assembled mostly as half molecules, and that the deleted heavy chain was produced in excess compared with light chains and was secreted either as a free subunit or assembled with a single light chain to form half molecules. Because neither half molecules nor unassembled heavy chains were detected by Western blotting of unfractionated serum samples, it is most likely that they circulated in very low amounts, possibly due to their high affinity for basement membranes.

It is noteworthy that in patients 3 and 4, a monoclonal IgG1λ with a nondeleted heavy chain component was also found in serum. This is similar to a previously described patient (10) who had two circulating monoclonal IgG1A exhibiting normal reactivity with anti-γ constant domain antibodies (including the two anti-γCH1 tested) and an isolated γ chain of IgG1 subclass (reactivity with NL16) lacking CH1 domain epitopes (arrow). The fraction from the serum of patient 3 also included a free λ light chain (A).

Deletions of the CH1 are also found in heavy chain disease, a lymphoproliferative disorder in which renal involvement is extremely rare, and in AH-amyloidosis, in which deposits have a fibrillar organization. In heavy chain disease, however, the variable domain is also almost always partially or completely deleted (reviewed in reference (24)), which suggests that the VH is required for kidney involvement. Sequence analysis of two HCDD proteins including that of patient 2 (24) did show unusual residues in complementarity determining regions and framework regions of the VH that had never been reported before. Even more intriguing is the apparent identical molecular structure of protein THR, responsible for HCDD (10,24), and of protein ART, responsible for AH-amyloidosis (9), both of which exhibit a complete deletion of CH1 and CH2 domains. Alignment of the amino acid sequences of the two proteins identified unusual substitutions in the VH of the HCDD protein (24). The most remarkable was a Cys residue that replaced the invariant Trp47 in the framework region 2. It was associated with other substitutions responsible for charge inversion or increased surface in hydrophobicity.

The following pathophysiologic scenario can thus be constructed, in which abnormalities of both the constant and the variable domains may play a role. First, since the retention of free heavy chains in the endoplasmic reticulum requires binding of chaperone proteins, including BIP (heavy chain-binding protein), to the CH1 domain (25,26), the CH1 dele-
tion may facilitate the secretion of free unassembled heavy chains that are rapidly cleared from the circulation by organ deposition. Second, conformational singularities of the V\(_h\) probably contribute to deposition since heavy chains from patients with heavy chain disease do not yield tissue deposits. Third, these singularities are also most likely responsible for the granular aspect or the fibrillar organization of the deposits. Additional studies are needed to test the second and third steps of this scenario. They are hampered by the low serum concentration of the nephritogenic heavy chain, which prompts us to establish transgenic murine models.

Finally, it is not easy to define the prognosis of HCDD patients, because the first cases were recently reported and because our series is small. It should be noted, however, that a favorable outcome was observed in our three treated patients. In our opinion, HCDD patients over 60 yr of age with a myeloma should be treated with conventional chemotherapy, but intensive therapy with blood stem-cell autografting should be discussed in younger patients. Treatment of nonmyeloma patients should be modulated according to clinical presentation.

In conclusion, this first significant series of HCDD patients provides important information on the renal presentation and pathophysiology of the disease. It shows that deposited heavy chains lack C\(_{H1}\) and circulate in low amounts as free unassembled molecules, as a consequence of the C\(_{H1}\) deletion. Our data thus indicate that all kidney biopsies showing only heavy chain deposits without light chain should be analyzed with antibodies specific for heavy chain constant domains and that antibodies specific for heavy chain constant domains and that a free circulating truncated heavy chain should be searched for by serum fractionation in those patients with HCDD and an apparently normal circulating IgG (or IgA).

**Note Added in Proof:** An additional case of \(\gamma\)-HCDD was reported by Rott et al., *Nephrol Dial Transplant* 13: 1825–1828, 1998. Studies of the serum by Dr. Deret revealed a complete IgG3 with a C\(_{H1}\)-deleted \(\gamma3\) heavy chain.

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