Plasma Cell Dyscrasia Associated With the Production of Incomplete (? Deleted) IgGλ Molecules, Gamma Heavy Chains, and Free Lambda Chains Containing Carbohydrate: Description of the First Case

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The clinical, hematologic, and immunoglobulin features of a new form of plasma cell dyscrasia (deleted H and L chain disease) are described. The clinical manifestations are periodic fever and weakness, lymphadenopathy, and hepatosplenomegaly. The hematologic abnormalities are anemia, leukopenia, lymphocytosis, thrombocytopenia, and increased plasma cells in lymph nodes and bone marrow. The protein abnormalities have been identified as (1) monoclonal IgGλ serum globulin (5-6 g/100 ml) with deletions in both H and L chains and an estimated mol wt of 110,000; (2) free γ Fc fragment in serum and urine; (3) urinary excretion (10-20 g/day) of deleted λ-chains (Uλ) with an estimated mol wt of 15,000. Uλ and the λλ chains of the IgG are apparently identical. Uλ was shown to contain approximately 26 moles of carbohydrate, with an average of 2.2 moles of sialic acid per 15,000 mol wt. Uλ displayed marked electrophoretic heterogeneity which was related to a variable number of sialic acid residues. The N terminus of Uλ is blocked (PCA). The deletions of both the λ- and the H chains were localized to their respective V regions and are of similar magnitudes (approximately 10,000 daltons). Possible genetic mechanisms to explain apparently comparable H- and L-chain deletions in a single IgG molecule are considered.

In recent years, increased knowledge of the detailed structure of immunoglobulins has enabled the identification and characterization of several specific types of plasma cell dyscrasia (monoclonal gammapathies) and their associated protein abnormalities. As reviewed in reference 1, these syndromes include multiple myeloma and its many clinical and protein variants, macroglobulinemia, "primary" amyloidosis, and the γ-, α-, and μ-heavy chain diseases (HCDs).

In this paper we describe a previously unrecognized form of plasma cell dyscrasia associated with the production of IgGλ molecules, γ heavy chains and λ light chains, all of which are incomplete and presumed to be deleted. An additional unusual feature of the λ-chains is the presence of significant amounts of conjugated carbohydrate.
CASE DESCRIPTION

E. Sm. (FDH No. 26651) (see case outline, Fig. 1). This white male professor was apparently in good health prior to 1969 (age 51), when he noted the insidious onset of weakness, fatigue, paroxysmal nonproductive cough, night sweats, and chills, but no fever. In August 1969, after approximately 6 mo of these symptoms, examination disclosed enlarged cervical and axillary lymph nodes (1-2 cm), a palpable spleen tip, but no hepatomegaly. Limited laboratory studies showed a normal hemoglobin, but the white blood cell count was 3000 with 75% lymphocytes. Chest x-ray was within normal limits, and the tuberculin (PPD) was negative. Over the next several months, the symptoms subsided spontaneously, but the neutropenia and relative lymphocytosis persisted. In June 1970 (age 52), he was essentially asymptomatic but examination in another clinic disclosed bilateral axillary adenopathy (1-2 cm), splenomegaly (6 cm), and hepatomegaly (2 cm). Hemoglobin was 13.4 g/100 ml, hematocrit 40.1%, white blood count 3850 with 59% lymphocytes, platelets 92,000. The peripheral blood smear showed marked rouleaux formation. Erythrocyte sedimentation rate was 90 mm/hr; blood type B, Rh positive; Coomb's test (direct and indirect) negative; blood urea nitrogen 17 mg/100 ml; uric acid 6.4 mg/100 ml; fasting blood sugar 115 mg/100 ml. Total serum protein was 8.8 g/100 ml, and serum electrophoresis disclosed a markedly abnormal pattern with a broad-based constituent in the fast gamma to beta mobility zone. By immunoelectrophoresis, this was identified as an IgG with \(\lambda\) light chains. Although the urine gave a negative reaction for protein by the Labstix (Ames Corp.) method, the patient was found to be excreting daily over 900 mg of protein which was electrophoretically polydispersed, spanning the gamma to the alpha mobility range. Immunelectrophoresis demonstrated that the urinary protein was related to \(\lambda\)-chains. The Bence Jones heat test was negative. Bone marrow was reported as within normal limits. Skeletal x-rays, GI series, and barium enema were negative.

A right axillary lymph node biopsy was interpreted as showing hyperplasia with increased histiocytes and plasma cells.

Since the latter part of 1970, he has been followed in our clinic, and the major features of his clinical and hematologic course and changes in serum and urine proteins are outlined in Fig. 1. During 1971 and the first half of 1972, he was relatively asymptomatic and was able to pursue his full schedule of activities. However, in this period there was a gradual increase in splenomegaly and hepatomegaly, moderate pancytopenia attributed to hypersplenism, and progressive increase in the abnormal proteinuria. The concentration of the abnormal serum protein (M-p) remained relatively constant. Bone marrow aspiration in April 1972 (Figure 2A) showed an increased number of plasma cells (approximately 25%) with many abnormal forms. This was further documented by electron microscopy (Figure 2B).

In October 1972, he had a 2-wk "flu-like" illness characterized by low-grade fever, chills, non-productive cough, and weakness. Following this, he noted scattered petechiae on the trunk and extremities, gum bleeding, and persistent fatigue and malaise. Examination in November 1972 disclosed a further increase in splenomegaly (12 cm) and hepatomegaly (5 cm), but no significant change in the lymphadenopathy. Laboratory studies revealed a fall in hemoglobin to 9.5 g/100 ml and decrease in platelets to 50,000. The white blood count remained in the range of 3000-4000 per cu mm with 50%-60%, lymphocytes and many abnormal "immature" forms. Coincident with these clinical and hematological changes, the concentration of the abnormal serum globulin increased to over 6 g/100 ml, and the abnormal proteinuria increased to 23 g/24 hr. Because of these indications of increased disease activity, a trial of chemotherapy was deemed warranted, and treatment with cyclophosphamide (Cytoxan), 100 mg daily, was begun on November 21, 1972. After 2 wk of this therapy, there was some decrease in the hepatosplenomegaly and in the abnormal serum and urinary proteins, but the anemia and thrombocytopenia worsened. The cyclophosphamide dosage was reduced to 75 mg daily, and prednisone 30 mg daily was added in January 1973. Associated with this therapy, there was an improvement in hematologic and clinical status and a slight decrease in the abnormal serum and urinary proteins for approximately 4 mo. Subsequent to this, however, there again was evidence of disease progression with increasing anemia, splenomegaly, and abnormal protein production. Accordingly, in mid-June, cyclophosphamide was discontinued, and melphalan (Alkeran, 1-phenylalanine mustard) was instituted, initially at 4 mg daily, and subsequently reduced to 2 mg daily. Prednisone, 20 mg daily, was continued. Despite 8 wk of this regimen, there was evident further progression of all disease parameters. In late August, he developed an overwhelming Pseudomonas pneumonia and sepsis which failed to respond to cephalosporin and gentamycin.
Fig. 1. Case E. Smi. (FDH No. 26651). Clinical and hematological course with associated changes in the serum and urinary proteins. Tp, total serum protein; M-p, abnormal monoclonal protein. Hg, hemoglobin; Wbc, white blood cell count; Plts., platelets.
Fig. 2. Bone marrow aspiration in April 1972. (A) Wright Giemsa stain, showing increased numbers of plasma cells with many abnormal forms (x 1500). (B) Electron microscopy showing four plasma cells (PCs) with extensive endoplasmic reticulum (ER), a myelocyte (MC), and an erythroblast (EB). Original magnification, x 5700.

Postmortem examination confirmed the total consolidation of the right lung, but there were no other lung lesions. There was extensive infiltration of the liver and spleen by abnormal lymphocytes and plasma cells. The lymph nodes were only moderately enlarged. The kidneys were grossly and microscopically normal. The apparent immediate cause of death was bleeding into the brain and intestines, most likely due to thrombocytopenia.

In summary, the major clinical and hematological features of this case were lymphadenopathy, hepatosplenomegaly, and hypersplenism. These manifestations were initially low-grade and apparently spontaneously remitting, but subsequently they became more severe and progressive. The overall clinical pattern suggests a "mononucleosis" or "lymphoma-like" condition and, in many respects, resembles the clinical and hematological features displayed by several of the reported cases of γHCD.2-4 Furthermore, the histologic abnormalities seen in the lymph node biopsy, i.e., a pleomorphic hyperplasia with an increase in plasma cells, as well as the superficial appearance of the serum electrophoretic pattern, also resemble those seen in γHCD.2-4 For these reasons, γHCD was the initial diagnostic impression, but subsequent detailed studies of the serum and urinary proteins, described below, clearly established these to be unique and distinctly different from those of γHCD.

MATERIALS AND METHODS

All analyses were performed on freshly obtained serum and urine samples except where noted. Cellulose acetate electrophoresis was performed by the Microzone method (Beckman Instrument Co.). Urine samples were concentrated 10- to 20-fold by dialysis against 50%, polyvinylpyrrolidone (PVP) prior to electrophoretic and immunoelectrophoretic analyses. Since the major abnormal urinary protein (UA) was found to be soluble in 5%, 10%, trichloracetic acid, all determinations of urinary protein concentrations were made on whole urine by the biuret method, subtracting the 540-ma blank of the native urine.

Gel filtrations were done on Sephadex G-100 columns (Pharmacia Co.) with 0.05 M phosphate buffer, pH 7.4. To prepare constituent H and L chains, the serum IgG, isolated by Sephadex G-100, was reduced with 0.1 M 2-mercaptoethanol in 0.55 M Tris buffer, pH 8.0, for 2 hr at 37°C and alkylated with 50% molar excess of iodoacetamide.5 The sample was then run through Sephadex G-100 using 1.0 N acetic acid.

Ion-exchange chromatography was done on DEAE-cellulose (Whatman DE-52) with a continuous gradient (0.01-0.1 M) of phosphate buffer, pH 7.0.

SDS-polyacrylamide gel electrophoresis was done using the Microzone, Model 113 (Beckman Instrument Co.), system with 7%, polyacrylamide gel in Tris-glycine buffer, pH 8.4, containing 0.2%, SDS (sodium dodecyl sulfate, Eastman Organic Chemicals, Inc.) and 1 mg/ml of ammonium persulfate. Protein samples were prepared in 1%, SDS and 8 M urea in the same buffer and were
incubated for 1 hr at 37°C prior to electrophoresis. Electrophoresis was carried out for 1 hr at 400 V, 30-36 mA, following which the gels were stained with Buffalo blue-black dye.

Papain digestions were done with twice-crystallized papain (Worthington Biochemical Co.) at an enzyme : protein ratio of 1:100 in the presence of 0.01 M cysteine for 36 hr at 37°C. Neuraminidase (Worthington Biochemical Co.) treatments were also done at a 1:100 enzyme:protein ratio in 0.15 M NaCl, incubating for 30 min at 37°C.

Initial estimates of the sialic acid content of chromatographically isolated protein fractions were done by the direct Ehrlich reaction as described by Werner and Odin. More precise sialic acid analyses of UA were kindly performed by Dr. Phyllis Sampson of Yeshiva University using the resorcinol method of Svennerholm. Later analyses of the sialic acid contents of UA and
its subfractions were carried out by the thiobarbituric acid method of Warren. There was generally good agreement in the results obtained with these different methods. Neutral sugars were measured by the anthrone reaction with mannose as the standard. Fucose was determined by the method of Dische and Shettles, and total hexosamine by the method of Dische and Borenfreund.

Immuno-electrophoretic and Ouchterlony analyses were performed by standard procedures using 1 agarose (Fisher Scientific Co.) in veronal buffer, pH 8.6, ionic strength 0.025. Antisera to the purified UA and serum Fe fractions were prepared in rabbits using complete Freund adjuvants. Details of the other antisera used are included in the text.

Ultracentrifugal analyses were kindly performed by Dr. Y. K. Yip and Professor Sherman Beychok of the Department of Biological Sciences of Columbia University, New York, N.Y.

RESULTS

Electrophoresis

Figure 3 shows representative electrophoretic patterns of the serum and urinary proteins. In the serum, the major abnormality is seen to be an abnormal, broad-based constituent (M-p), traversing the fast gamma (γ₁) and beta mobility ranges. There is a dominant peak of "fast" gamma mobility with a
"shoulder" on its anodal (beta) boundary. As described below, the $\gamma_1$ component was identified as comprised of IgG$\lambda$ molecules, and the beta constituent as free heavy (\gamma) chains (\gamma Fc fragment). The serum also shows a marked decrease in the cathodal "slow" gamma (\gamma_2) globulins and moderate hypalbuminemia. The concentration of the serum M-p was in the range of 5-5.7 g/100 ml for the first 2 yr. In November 1972, there was an abrupt increase to 7.1 g/100 ml and thereafter, associated with the institution of chemotherapy, the M-p concentration decreased to the range of 5-6 g/100 ml.

The urine protein (UP) electrophoretic pattern (Fig. 3) is unique in our experience and shows an extremely polydispersed (gamma to alpha) component with a suggestive bimodal distribution. The very broad distribution of this constituent is clearly different from the characteristically homogeneous "spike" appearance of most Bence Jones proteins and the relatively homogeneous proteins in yHCD. The minimal amount of albumin in the urine indicates relatively intact renal function and further suggests that the abnormal UP is of small molecular size. The total 24-hr excretion of UP increased to a maximum of 23.4 g prior to chemotherapy and subsequently decreased to the range of 15-16 g/24 hr. Qualitatively, i.e., by electrophoretic analysis, the UP remained unchanged throughout the 2$\frac{1}{2}$ yr of observation.

**Solubility Properties of the Urinary Protein**

The urine consistently gave a negative Bence Jones reaction. It also showed only a negative-to-trace reaction for protein with the standard Labstix test (Ames Corp.), despite the known presence of several grams of protein. It was further noted that the abnormal UP was soluble in 5º-20º perchloric, trichloracetic, sulfosalicylic, and phosphotungstic acids. Because of these unusual solubility properties, determinations of urine protein concentration were done by the biuret method on whole urine after dialysis against water, without prior precipitation.

**Immunoelectrophoresis of Whole Serum and Urine**

Figure 4 shows immunoelectrophoretic analyses of the patient's serum and urine developed with anti-whole serum, anti-gamma, anti-kappa, anti-lambda, and anti-Sm urinary lambda (UA) antisera. With respect to the serum, as seen in Fig. 4C, upper, anti-gamma demonstrates a major precipitin arc with three maxima. The component designated "a" in the slow (cathodal) gamma range is also precipitated by anti-kappa (Fig. 4C, lower) and anti-lambda (Fig. 4E, lower) but not by anti-Sm. UA. This component is interpreted as representing a small amount of residual normal IgG. Component "b" in the mid-to-fast-gamma range corresponds to the distribution of the arcs developed with anti-lambda (Fig. 4E, lower) and anti-Sm. UA (Fig. 4E, upper). This major serum component is identified as a monoclonal IgG$\lambda$. The most anodal of the three serum gamma "bows," designated "c," is not precipitated by either anti-kappa or the two anti-lambda antisera. It is, therefore, interpreted as representing free gamma chain or \gamma Fc fragment.

With respect to the urine, as seen in Fig. 4B, there is a relatively small constituent in the fast gamma-to-beta mobility range precipitated by anti-whole
Fig. 4. Immunoelectrophoretic analyses of the patient’s serum and urine developed with the following antisera: anti-human whole serum, Hyland Laboratories (H No. 204); anti-gamma, anti-IgG absorbed with ϵ and λ BJ proteins; anti-kappa and anti-lambda, antisera to these respective BJ types; anti-Sm. urinary lambda, rabbit antiserum prepared against the chromatographically-purified Uλ of the patient’s urine (see below). The direction of migration is left to right. Alb, albumin. For explanation and interpretation, see text.

serum (Fig. 4B, upper) and anti-gamma (Fig. 4B, lower). This arc, designated “c,” corresponds most nearly to the fastest (“c”) of the three serum gamma constituents and is, therefore, interpreted as representing free gamma chain or γFc fragment. As seen in Fig. 4D (lower) and Fig. 4F (upper), both anti-lambda and anti-Sm. Uλ demonstrate an extremely polydispersed constituent
extending from the fast gamma to the albumin mobility range. This arc shows two maxima of fast gamma ("d") and alpha ("e") mobility, respectively. Neither "d" nor "e" corresponds to the urine arc ("c") developed by anti-gamma (Fig. 4B, lower). This indicates that the lambda-related components in the urine are separate and distinct from the gamma-related components, and this was confirmed by the subsequent studies described below.

**Gel Filtration and Chromatography**

As shown in Fig. 5, when fresh serum was separated on Sephadex G-100, a major peak eluted in the position corresponding to intact IgG with an estimated molecular weight of approximately 170,000. Immunologic analyses of the eluted samples, however, showed extension of the gamma determinants to a mol wt range of 45,000 and of the lambda determinants to a mol wt range of less than 68,000. After the serum had been stored at −20°C for 7–10 mo, there was a reduction in the first peak and an extension of the distribution of the gamma and lambda determinants to lower mol wt elution positions, apparently due to spontaneous degradation of IgG molecules (see below). A similar effect was produced by papain treatment of fresh serum (Fig. 5, lower panel). A small
mol wt constituent eluting in the range of 23,000 was also demonstrated in the stored and papain-treated samples, and this was subsequently identified as Fab fragment.

Figure 6 shows the results of separation of the urinary proteins on Sephadex G-100. The eluates were analyzed for both protein and sialic acid. The pattern of whole urine shows a small initial peak with gamma and lambda constituents (mol wt approx. 68,000) followed by a major polydispersed peak in the mol wt range of 20-40,000 with only lambda determinants (designated UA). Rechromatography of UA on the same column confirmed its elution position (Fig. 6, second panel). As described below, however, UA was ultimately shown to have a mol wt of approximately 15,000, and its elution behavior on Sephadex G-100 is therefore anomalous, possibly due to its carbohydrate and sialic acid content. The whole urine also contained low mol wt constituents (<1000) which absorbed at 280 m\textmu, presumably peptides, amino acids, urates, etc., plus some free sialic acid.

![Gel filtration (Sephadex G-100) of the urinary proteins.](image_url)

Fig. 6. Gel filtration (Sephadex G-100) of the urinary proteins. Identical conditions as in Fig. 5. Samples analyzed for protein (OD 280 \textmu, solid lines) and sialic acid (OD 570 \textmu, after Ehrlich's reagent, broken lines). The major urinary protein peak eluted in the mol wt range of 20,000-40,000 contained only lambda determinants and was designated UA. Rechromatography of UA before and after neuraminidase treatment is shown in the second and third sections. The elution pattern of sialic acid is shown in the bottom section for reference.
Figure 7 shows analyses of whole serum and the isolated UA fraction using DEAE-cellulose and a continuous ionic gradient (phosphate buffer, pH 7.0, 0.01-0.1 M). The serum pattern demonstrates a major initial peak (I) which was immunoelectrophoretically identified as IgG. Peak I also contained a small amount of an unidentified constituent of alpha mobility. The second, more anionic serum peak (II) was shown immunoelectrophoretically to contain gamma but no lambda determinants. This constituent is, therefore, consistent with Fc fragment or heavy chain. The third serum DEAE fraction (III) contained albumin and alpha globulins.

As seen in the lower section of Fig. 7, the UA fraction eluted from DEAE cellulose as a broad peak with asymmetrical boundaries. This was separated into six fractions (a-f). When analyzed by cellulose acetate electrophoresis, these subfractions of UA continued to show a range of electrophoretic mobilities (fast gamma to alpha) which correlated with their relative elution positions on DEAE-cellulose.

**Relationship of the Electrophoretic Heterogeneity of the Abnormal Serum and Urinary Proteins to the Presence of Sialic Acid Residues**

Because of the possibility that the electrophoretic heterogeneity of the abnormal serum and urinary proteins might be due to the presence of sialic acid,

![Diagram of DEAE-cellulose chromatography of whole serum and the isolated UA fraction of urine using a continuous gradient of phosphate buffer, pH 7.0, ranging from 0.01 to 0.1 M. By subsequent analyses, serum peak I was identified as IgG, peak II as Fc, and III as primarily albumin (see below). UA was eluted in a single broad peak and was separated into six fractions (a-f), as indicated.](attachment:diagram.png)
residues, the effect of neuraminidase treatment was investigated. Samples of whole serum and urine and the isolated heavy (H) and light (L) chains of the serum IgG were incubated for 30 min at 37°C with neuraminidase at an enzyme : protein ratio of 1 : 100. As shown in Fig. 8, this treatment resulted in a marked decrease in the electrophoretic heterogeneity and a cathodal shift of the abnormal serum protein, its constituent L chains, and of the abnormal urinary protein, presumably due to the removal of negatively charged sialic acid residues. Neuraminidase treatment of the H chains of the serum IgG resulted in a small but definite cathodal shift in electrophoretic mobility, indicating the presence of some sialic acid residues, but this effect was less than that seen with the L chain or the intact proteins in the whole serum.

With respect to the urinary proteins, the presence of sialic acid was further indicated by gel filtration studies (Fig. 6) of whole urine and the Uλ fraction. In these studies, the sialic acid analyses were done by the method of Werner and Odin.6 After neuraminidase treatment, there was a shift in the elution position of Uλ to a lower mol wt range and the appearance of a small mol wt fraction corresponding in elution position to free sialic acid.

Carbohydrate Analyses of Uλ and Uλ Subfractions

Determinations of the carbohydrate content of Uλ, expressed as moles per 15,000 mol wt were as follows: neutral sugars 12.3, fucose 1.7, total hexosamine...
Table 1. Carbohydrate Content of U\(\lambda\) Compared With Reported Values for Carbohydrate-Containing \(\lambda\) Bence Jones Proteins\(^*\)

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Neutral Sugars</th>
<th>Fucose</th>
<th>Total Hexosamine</th>
<th>Glucosamine/Galactosamine</th>
<th>Sialic Acid</th>
<th>AA Residue With CHO Attachment</th>
</tr>
</thead>
<tbody>
<tr>
<td>U(\lambda)-Sm</td>
<td>This study</td>
<td>12.3</td>
<td>1.7</td>
<td>10.4</td>
<td>ndt</td>
<td>2.2</td>
</tr>
<tr>
<td>BJ(\lambda)-Hul</td>
<td>12</td>
<td>7.2</td>
<td>1.99</td>
<td>nd</td>
<td>1.82/2.02</td>
<td>2.0</td>
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<td>BJ(\lambda)-Pet</td>
<td>13</td>
<td>6.3</td>
<td>1.3</td>
<td>nd</td>
<td>6.2/nd</td>
<td>3.0</td>
</tr>
<tr>
<td>BJ(\lambda)-Hug</td>
<td>13</td>
<td>4.8</td>
<td>nd</td>
<td>nd</td>
<td>4.6/nd</td>
<td>3.7</td>
</tr>
<tr>
<td>BJ(\lambda)-Bla</td>
<td>13</td>
<td>5.5</td>
<td>nd</td>
<td>nd</td>
<td>nd/nd</td>
<td>3.0</td>
</tr>
<tr>
<td>BJ(\lambda)-Ful</td>
<td>14</td>
<td>10.0</td>
<td>nd</td>
<td>nd</td>
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<td>1.5</td>
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<tr>
<td>BJ(\lambda)-Nei</td>
<td>15</td>
<td>5.1</td>
<td>nd</td>
<td>nd</td>
<td>5.3/nd</td>
<td>1.2</td>
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\(^*\)Values expressed as moles per 15,000 mol wt for U\(\lambda\) and per 22,500 mol wt for other proteins.

10.5, and sialic acid 2.2 (method of Warren).7 In Table 1, these values are compared with the reported analyses of six other carbohydrate-containing \(\lambda\) Bence Jones proteins.12-15 The relatively high neutral sugar content of U\(\lambda\) is noteworthy. The total sialic acid content of U\(\lambda\), expressed on the basis of molar ratios, is comparable to that of the other proteins.

Sialic acid analyses were also made on the subfractions of U\(\lambda\) separated by DEAE chromatography (see Fig. 7). As shown in Table 2, the sialic acid content of these subfractions, expressed as moles per 15,000 mol wt, ranged from 1.1 (subfraction a, most basic) to 3.4 (subfraction f, most acidic). These results appear to establish that the charge heterogeneity of U\(\lambda\) is related to the presence of a varying number of sialic acid residues. Whether the U\(\lambda\) subfractions differ in their other carbohydrate constituents remains to be determined.

Effects of Storage (\(-20^\circ C\)) and Papain Digestion on the Serum IgG

As shown in Fig. 9, storage of whole serum at \(-20^\circ C\), with at least one cycle of freezing and thawing, was associated with a decrease in concentration and marked reduction in the electrophoretic heterogeneity of the abnormal gamma-beta component and the appearance of two narrow-banded components in the more cathodal (mid-to-slow gamma) mobility range. Papain digestion of serum produced similar effects, except that there were three narrow-banded cathodal components instead of the two seen after storage, and the additional third component was the most cationic.

Figure 5 shows Sephadex G-100 analyses of these same fresh, stored, and papain-treated serum samples. It is evident that after storage or papain diges-
Fig. 9. Electrophoretic patterns of fresh, stored (7-10 mo at -20°C), and papain-treated serum. Following both storage and papain digestion, the heterogeneity of the abnormal component is decreased and additional narrow-banded components appear in the more cathodal (mid-to-slow gamma) mobility range.

tion, there was a decrease in the first peak and an increase in the fraction in the mol wt range of 55,000. This component was shown immunologically to have gamma but no lambda determinants and is, therefore, interpreted as representing the Fc fragment. An additional single peak in the mol wt range of 20,000 appeared in the stored and papain-treated samples. By electrophoresis, this smaller mol wt fraction was found to correspond with the narrow-banded cathodal components shown in Fig. 9, and immunologically it was shown to have lambda but no gamma determinants. It is, therefore, considered to represent the Fab fraction. It is noteworthy that the estimated mol wt (approx. 20,000) of this fraction is considerably lower than that of usual Fab fractions (mol wt ~ 40,000), indicating that either the L-chain or the Fd fragment (or both) are incomplete, i.e., deleted.

At present, the molecular basis for the unusual susceptibility of the abnormal IgG to degradation after storage and freezing and thawing is not known. Previous studies have shown that IgG molecules of the gamma-3 subclass are particularly susceptible to storage and papain digestion, but the present protein was found to have the specific antigenic determinants of the gamma-1 subclass.

Molecular Weight Estimations by Ultracentrifugation and SDS-Acrylamide Gel Electrophoresis

On ultracentrifugal analysis, the native IgG fraction sedimented as a broad, polydispersed complex probably reflecting both aggregation and dissociation, and it was not possible to calculate a sedimentation constant. As shown in Fig. 10, native IgG also failed to completely penetrate the SDS-acrylamide gel, although a dominant band was demonstrated in the mol wt range of 170,000. This behavior is consistent with aggregation and/or nonspecific interaction with the gel. After reduction with 2-mercaptoethanol, the IgG showed a major band in the mol wt range of 45,000, interpreted as the heavy chain, and a fainter band in the mol wt range of 15,000, interpreted as the light chain. Figure 10 clearly demonstrates that both of these components are relatively smaller than their counterparts in the reduced reference IgG.

After storage and spontaneous cleavage of the IgG, two major bands were seen in the SDS-acrylamide gel pattern in the mol wt range of 52,000 (the Fc fragment) and 29,000 (the Fab fragment), respectively. A third faint band was
Fig. 10. SDS-acrylamide gel electrophoretic analyses of the following samples: isolated Sm. IgG before and after reduction with 2-mercaptoethanol (2 ME) and storage; the Fab fragment of the IgG before and after reduction; Sm. IgG before and after treatment with neuraminidase (NMase).

A reference mixture of IgG (mol wt 170,000) Bence Jones dimer (BJ-d, mol wt 45,000), Bence Jones monomer (BJ-m, mol wt 22,500), and human lysozyme (LZM, mol wt 14,700) is shown at the top. The reference IgG after reduction with mercaptoethanol is also shown for comparison with the Sm. IgG after mercaptoethanol reduction.

also seen in the mol wt range of 15,000. The Fc fragment was isolated by DEAE chromatography and confirmed as the 52,000 mol wt component. After reduction with 2-mercaptoethanol, it yielded a single band in the mol wt range of 26,000, indicating that it is a disulfide-bonded dimer. The fragment identified as Fab was also isolated by DEAE chromatography and confirmed as the component in the mol wt range of 29,000. As seen in Fig. 10, when this was reduced by 2-mercaptoethanol, two major bands were obtained in the mol wt range of 14,000 and 15,000, along with a faint band in the mol wt range of 30,000. These results are interpreted as evidence that the Fab fragment contains a 15,000 mol wt (L-chain) and a 14,000 mol wt (Fd fragment) subunit which are disulfide bonded. The third faint band in the mol wt range of 30,000 is unidentified, but it possibly represents a small amount of L-chain dimer.

The sedimentation constants (S20,w) of both the isolated L-chain of the IgG and the native UA were determined to be 1.5S, i.e., consistent with an estimated mol wt of 15,000, and considerably smaller than the usual Bence Jones proteins and light chains. As seen in Fig. 10, however, native UA showed a
broad polydispersed band in SDS-acrylamide, spanning the mol wt range of 20,000-40,000, but after neuraminidase treatment, it displayed a major 15,000 mol wt band, and a less prominent band in the 14,000 mol wt range. These results appear to establish that the anomalous behavior of the native UA on SDS-acrylamide is related to its sialic acid residues and that the major component of UA has a mol wt of 15,000. It should be noted that reduction of UA either before or after neuraminidase treatment did not alter its SDS-acrylamide behavior, indicating an absence of interchain disulfide bonds. The smaller 14,000 mol wt component in neuraminidase-treated UA has not yet been identified. It may possibly represent some free Fd fragment, a more extensively deleted L chain, or a polypeptide chain identical to the 15,000 mol wt component but lacking conjugated carbohydrate.

Further Antigenic Analyses of Uλ and Comparisons With λ Bence Jones Proteins

As previously shown in the immunoelectrophoretic analyses in Fig. 4, Uλ was precipitated by a reference anti-lambda antiserum as well as by its homologous (anti-UA) antiserum. Further immunodiffusion analyses of Uλ are shown in the Ouchterlony patterns in Fig. 11, in which Uλ is seen to be antigenically deficient as compared with all Bence Jones λs, when analyzed with four different heterologous anti-lambda antisera. Further evidence of the antigenic deficiency of Uλ was provided by analyses with an homologous anti-Uλ antiserum. This antiserum clearly recognized lambda determinant(s), but there was no demonstrable idiotypic specificity indicative of variable-region deficiency. Very faint spurs were developed with two samples which were previously shown to have a deficiency in the constant peptide (L7).16

Additional Studies

Preliminary studies of the tryptic peptides of Uλ have disclosed a pattern identifiable as lambda-related, but several peptides appear to be missing. Uλ
was found to have a blocked (PCA) N-terminus, characteristic of a large proportion of λ-chains. Detailed amino acid sequence studies are in progress.

Summary of the Available Protein Data

Figure 12 summarizes the presently available data concerning these proteins. The major urinary protein, Uλ, has been identified as an incomplete and presumably deleted lambda chain with a mol wt of approximately 15,000 (S20 = 1.5 S) containing about 26 moles of conjugated carbohydrate including an average of 2.2 moles of sialic acid. The marked electrophoretic heterogeneity of Uλ has been shown to be due to its sialic acids. The demonstration of lambda antigenic determinants on Uλ and the failure to demonstrate idiotypic antigenic determinants suggests that the major deletion is in the variable (V) region. However, the finding of a blocked (PCA) N-terminus and the fact that, in previously reported studies of carbohydrate-containing Bence Jones proteins,12-15
the carbohydrate has been shown to be attached in the V region would indicate that at least a portion of the V region is present. It is also noteworthy that Uλ does not display the characteristic thermal solubility properties of typical Bence Jones proteins, and these properties have been shown to be primarily determined by the V region. Thus, we presently believe that Uλ is mainly deficient in the V region, but detailed sequence studies are needed to clarify this point.

The predominant abnormal serum protein has been identified as an IgGλ. The L-chains are apparently identical to Uλ, having the same sedimentation constant (1.5 S), SDS-acrylamide gel behavior, and also containing sialic acid. The H-chains obtained by reductive cleavage have an estimated mol wt of 45,000. They are thus distinctly smaller than the H-chains of a reference IgG, and the deletion is apparently in the Fd (VH) region. As shown in Fig. 12, present evidence indicates a comparable extent of deletion of the H and L chains. The estimated mol wt of the IgG is 110,000. It has been necessary to derive this value from the sedimentation and SDS acrylamide analyses of its component fragments and chains, since the intact molecule appears to aggregate and dissociate spontaneously. The tendency to dissociate suggests an anomaly in the region of the junction between the Fc and Fd (hinge region), and this is indicated by a break in continuity of the H chains in the schematics shown in Fig. 12. The Fc fragments of the two H chains have been shown to be disulfide bonded, but the number of interchain disulfides has not been determined.

DISCUSSION

This case exhibits a complex combination of clinical and immunoglobulin abnormalities which have not been previously described. The clinical features are similar in many respects to those observed in cases of γHCD, i.e., a lymphomalike pattern with lymphadenopathy and hepatosplenomegaly, moderate anemia, leukopenia, and thrombocytopenia, consistent with hypersplenism, and the nonspecific symptoms of weakness, malaise, and periodic fevers (100-102°F). There was no palatal erythema and edema, but the increased susceptibility to bacterial infections was comparable to that observed in γHCD. Significantly, there were no clinical or radiographic signs of skeletal disease and no amyloidosis.

The clinical manifestations were relatively low grade and well tolerated for approximately 3 yr and then increased in severity along with an increased production of abnormal immunoglobulin products, indicating an acceleration of the disease process. Treatment with cyclophosphamide (Cytoxan) and prednisone produced temporary objective and subjective improvement, but melphalan was apparently ineffective.

The histological findings in the lymph node and bone marrow were also similar to those described in γHCD. The lymph node biopsy showed hyperplasia with some distortion of the architecture and increased numbers of plasma cells and histiocytes. The proliferation of plasma cells was particularly evident in the bone marrow, and this was further documented by electron microscopy. In contrast with many of the reported cases of γHCD, no increase in eosinophils was seen in the peripheral blood, bone marrow, or lymph node.
The immunoglobulin abnormalities in this case differ in several respects from those described in cases of \( \gamma \)HCD or other plasma cell dyscrasias. In their recent excellent review of \( \lambda \)HCD, Frangione and Franklin have classified the reported \( \gamma \)HCD proteins in the following four categories:

- **Type I.** Partial deletion of the Fd fragment and resumption of normal synthesis at position 216 (proteins Zuc, CRA, and GIF).
- **Type II.** Deletion of the hinge region (protein McG).
- **Type III.** Partial deletion of the Fd fragment and hinge region (proteins PAR and HAL).
- **Type IV.** Enzymatically derived from a larger H chain (proteins RIP and SAC).

If our present formulation is correct, the Sm IgG would appear to most closely resemble the SAC IgG which has been shown to have deletions in both H and L chains and to have an estimated mol wt of 125,000. This protein is an IgG1 with kappa L chains. The deletions have been demonstrated to consist of the N-terminal 102 residues of the H chain and 68 residues within the variable region of the L chain. The L chain deletion begins with residue 18 or 19 and is thus clearly internal. However, since the H-chain deletion includes the N-terminus, the possibility of partial in vivo proteolytic degradation cannot be excluded. Since the N-terminus of the Sm IgG H chain has not as yet been characterized, we must also consider this possibility with our protein. In regard to the SAC IgG, Parr et al. have suggested that the L chain deletion might have been the primary structural lesion and have secondarily increased the susceptibility of a “normal” H chain to proteolytic digestion.

In the case of SAC, the patient was a 53-yr-old female with an 18-yr history of a scleroderma-like condition associated with hyperpigmentation and pruritus. The major abnormalities at the time of development of the plasma cell dyscrasia were moderate anemia, leukopenia and thrombocytopenia, splenomegaly, and an increase in marrow plasma cells (8%). Thus, the clinical features in both the SAC case and our patient were atypical and suggestive of a “lymphomalike” condition. The serum concentration of abnormal protein in SAC was reported to be 5.5 g/100 ml, i.e., in the same range as in Sm. The SAC IgG had a beta electrophoretic mobility with a narrow-banded distribution in contrast to the electrophoretic polydispersity of Sm IgG. Studies of its carbohydrates, if any, have not been reported. The proteinuria was quite variable and inconsistent in quantity and, unfortunately, these proteins were not precisely characterized. It would have been of interest to determine whether the proteinuria represented an excess of free L chain and/or H chain or Fc fragment.

In our case there was evidence of free Fc fragment in both the serum and urine, but it could not be ascertained if this represented de novo synthesis of incomplete immunoglobulins or the result of in vivo dissociation or enzymatic cleavage of complete IgG molecules. The latter possibility is unquestionably favored by the demonstrated tendency of the Sm IgG protein to dissociate spontaneously into its constituent Fc and Fab fragments. Since the Sm IgG is of the gamma-1 subclass, this susceptibility to dissociation into Fc and Fab fragments also suggests the possibility of a deletion in the hinge region of the
H chain. It is noteworthy that the Fc fragments present in fresh serum and urine, as well as those derived from spontaneous (storage) and papain cleavage, were disulfide-bonded dimers, indicating that the sequence which provides the H–H interchain disulfide(s) is probably intact.

The L chains in our case are apparently unique in two respects. First, they appear to represent truly deleted polypeptide chains rather than fragments of complete lambda chains. The evidence for this consists of the finding of a blocked N-terminus, indicating the presence of a part of the variable (VL) region, plus the demonstration of antigenic determinants and tryptic peptides of the constant (CL) region. Furthermore, in the majority of the previously reported cases in which VL and/or CL fragments of Bence Jones proteins have been found, some complete Bence Jones molecules were also demonstrable. Thus, in these cases it has been considered most likely that the fragments were derived from the enzymatic cleavage of complete L chains rather than de novo synthesis. This in vivo cleavage has been postulated to be related to the demonstrated in vitro susceptibility of L chains to cleavage at the VL-CL junction by pepsin and other endopeptidases. In our case, however, the finding of apparently identical deleted L chains on the intact IgG molecules and in the urine (UA), the absence of demonstrable complete lambda chains, and evidence for both VL and CL components would all argue against a catabolic explanation and favor de novo synthesis of a deleted L chain. It is also noteworthy that the estimated mol wt of the Sm. L chains (15,000) exceeds that of a half L chain (approximately 11,000 mol wt), and this difference is greater than the possible contribution of the attached carbohydrate.

The second unusual feature of the Sm. L chains and the UA is the associated carbohydrate. Whereas carbohydrate is consistently found on the H chains of IgG and other immunoglobulin molecules, it has only occasionally been demonstrated on L chains (both λ and κ) and Bence Jones proteins. Spiegelberg et al. have studied six IgG proteins with carbohydrate on the Fab fragment and have localized the carbohydrate to the L chains of four (3λ, 1κ), the Fd fragment of one, and to both the L chain and the Fd fragment of one. In the first group of four, there was associated Bence Jones proteinuria, and the carbohydrate moieties of the Bence Jones proteins were the same as those of the corresponding L chain, except for a greater number of sialic acid residues (L chains, 1 mole per mole protein; Bence Jones proteins, 1–6 moles per mole protein). By peptide mapping and sequence studies, the carbohydrate of L chains and Bence Jones proteins have been localized to either of the two hypervariable regions within the V region, i.e., around residue 25 from the N-terminus, or residues 82–93 near the C-terminus (see Table 1). Sox and Hood have further established that the carbohydrate is attached to the asparagine residue in the sequence Asp-Ser-Thr by a glucosamine linkage between the amide N of asparagine and the C-1 carbon of N-acetylglucosamine. They postulate that the occurrence of this characteristic triplet in the V region serves as a receptor for the transglycosolase, whose primary function is to glycosolate H chains and other proteins. Thus, the attachment of carbohydrate to L chains is presently regarded as a chance phenomenon with no specific functional significance.
With respect to the Sm. L chains and UA, the site of carbohydrate attachment remains to be determined by additional peptide mapping and sequence studies, but we presently assume it to be in the V region despite the evidence that the postulated deletion also involves this region. It is of interest to speculate on the possibility that the deletion per se may have resulted in an Asp-Ser-Thr sequence which in turn served as the carbohydrate receptor. It should be noted that carbohydrate may also be present on the Fd portion of the Sm. H chain, and this possibility should also be elucidated by sequence studies.

The evidence obtained in the present studies and in those of the SAC protein of apparently similar deletions involving both the H and L chains of an IgG molecule suggests interesting and potentially important possibilities regarding underlying molecular mechanisms. Until the N-terminus of the Sm. H chain is defined, it is not possible to exclude secondary (postsynthetic) proteolytic cleavage of the H chain which might be facilitated by the (primary) L chain deletion. This possibility has been considered by Parr et al. with respect to the SAC IgG. Alternatively, and perhaps of greater interest, if evidence is obtained that both the H and L chains of the Sm. IgG have "internal" deletions, corresponding deletions of their respective structural genes would be indicated. As noted by Parr et al., this would favor a multiple-stranded or branched DNA model for the immunoglobulin genes, since the independent deletion of two genes of a single DNA strand would seem improbable.

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Plasma Cell Dyscrasia Associated With the Production of Incomplete (?) Deleted) IgGλ Molecules, Gamma Heavy Chains, and Free Lambda Chains Containing Carbohydrate: Description of the First Case

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