BLOOD

The Journal of The American Society of Hematology

VOL. 49, NO. 4 APRIL 1977

Gamma Heavy Chain Disease: Clinical Aspects and Characterization of a Deleted, Noncovalently Linked γ1 Heavy Chain Dimer (BAZ)

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This report describes the clinical and immunoglobulin features of a patient with gamma heavy chain disease (HCD), who presented with a clinical picture suggestive of an underlying malignancy rather than the usual picture of lymphoma or granulomatous disease. A unique clinical feature was the nearly total replacement of the submaxillary glands by plasma cells. The patient's serum and urine contained a paraprotein, γHCD protein BAZ, which belongs to the γ1 subclass and forms noncovalently linked dimers with a molecular weight of approximately 60,000 daltons. This mutant protein exhibited a deletion which encompassed most of the variable (V) region, the first constant domain (C_H1), and the hinge region. In addition, preliminary structural analyses demonstrated the replacement of alanine by glycine in position 431 of the carboxyterminal octadecapeptide. This substitution may possibly represent another allotypic marker on IgG1 proteins.

THE DIAGNOSIS of heavy chain disease (HCD) requires the identification in serum and urine of immunoglobulin molecules which consist of complete or incomplete heavy chains and are devoid of light chains. Since the initial description by Franklin et al.[1] of a large amount of the Fc fragment of immunoglobulin G (IgG) in the serum and urine of a patient with a clinical picture of malignant lymphoma, at least 35 cases of γHCD,[2] 59 of αHCD,[3] and 10 of μHCD[2] have been identified. Although expected to occur,[2] δHCD and εHCD have not yet been described. The clinical syndromes of the three currently recognized forms of HCD and the physicochemical, immunologic, and structural properties of the associated HCD proteins have been reviewed in detail recently.[2-6] Extensive studies of these heavy chain variants have contributed to our knowledge of the structure and genetic control of the biosynthesis and assembly of normal immunoglobulins.[2,7] The purpose of the present report is to describe an elderly female patient with γHCD who presented with some un-

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Submitted June 21, 1976; accepted November 21, 1976.

Supported by USPHS Grants CA-15215 and HL-12958.

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usual clinical features and whose serum and urine contained a deleted, non-covalently linked γ1 heavy chain dimer with a previously unrecognized amino acid substitution in the constant region.[8,9]

CASE REPORT

History. Patient BAZ (ETMH No. 133-127), a 73-yr-old white female, was referred to the Talmadge Memorial Hospital in December 1971, with a 2-yr history of frequent occipital headaches and photophobia, with occasional nausea and vomiting. Since the fall of 1969, she had experienced easy bruisability, night sweats, frequent "fever" blisters of the lips, and a 30-lb weight loss. She had been hospitalized on four occasions since 1966 with fever and questionable chills. While a clinical diagnosis of pneumonia was made each time, only on one occasion was the chest x-ray compatible with such a diagnosis. Bacteriological studies remained negative. Blood counts on those occasions are given in Table 1. A facial basal cell carcinoma was excised in 1969. One daughter had died of breast carcinoma.

Physical examination. Positive physical findings on admission included evidence of marked weight loss; an enlarged, soft spleen palpable 4 cm below the costal margin; and bilateral varicose veins. The patient had no lymphadenopathy. The remainder of the physical examination was within normal limits.

Laboratory data. The hemoglobin was 11.0 g/dl, the PCV 0.33, the RBC 3.2 × 10^{12}/liter, and the reticulocyte count 3.6%. The WBC was 2.1 × 10^{9}/liter with 16% PMN, 75% mature lymphocytes, 2% eosinophils, and 7% monocytes. The platelet count was 72.5 × 10^{9}/liter. Blood chemistry findings were normal, except for a uric acid of 8.2 mg/dl and an SGOT of 81 units/dl. The total serum protein was 6.1 g/dl. Microzone electrophoresis of serum and urine revealed a β-migrating paraprotein, which was further characterized by immunoelectrophoresis and analytical ultracentrifugation as described below. LE cell preparation (× 3), anti-nuclear factor, latex flocculation, β1C complement, and ESR (× 3) were negative or normal. A coagulation profile, including PT, PTT, PC, fibrinogen, and protamine tests, was normal. Routine urinalysis was normal. Chest x-ray revealed cardiomegaly, but no hilar adenopathy. Left ventricular hypertrophy was noted on EKG. Intravenous pyelography showed multiple radio-opaque gall stones, splenomegaly, and a double collecting system on the right. A skeletal survey demonstrated degenerative joint changes, but neither osteoporosis nor lytic lesions were identified. A liver-spleen scan showed hepatosplenomegaly. A bone marrow aspirate and biopsy were obtained; the histopathology of these specimens is described below.

Clinical course. During the year following her discharge from the hospital, she apparently experienced several upper respiratory infections which were treated with antibiotics by her local physician. She was seen again in April 1973. At that time she was noted to have enlargement of the submaxillary glands, prompting a complete reevaluation which included salivary gland biopsy, chromosomal analysis, and in vitro lymphocyte studies. Results of these and other studies are described below. Although cytotoxic chemotherapy was considered, this therapeutic approach was not undertaken by virtue of the slow progression of her disease and her poor compliance. Instead, she was begun on a human gamma globulin regimen (20 ml every 2 wk). Upon discharge from the hospital, however, she was lost to follow-up and received no further treatment. She died in a nursing home 22 mo after the diagnosis of γHCD had been established. An autopsy could not be performed.

Table 1. Blood Counts

	4/22/66	2/6/70	3/11/71	4/8/71
Hemoglobin (g/dl)	11.1	12.2	13.5	13.5
PCV	0.33	0.36	0.39	0.39
WBC × 10^{9}/liter (lymphocytes)	5.0 (NA)*	5.2 (NA)*	4.2 (40%)	3.8 (48%)

NA, not available.

MATERIALS AND METHODS

Cellular Studies

All tissues for histopathology were fixed in formalin for routine histologic sections. For electron microscopic studies, tissues were fixed in formalin overnight and then postfixed in 3% glutaraldehyde and osmium tetroxide. The sections were examined with a Siemens model 1A transmission electron microscope. Chromosomal analysis was performed on direct preparations of aspirated bone marrow specimens according to the method of Tjio and Whang.[10] Subpopulations of circulating B lymphocytes were ascertained by direct immunofluorescence after incubation of the purified cells[11] with monospecific anti-μ, anti-α, and anti-γ antisera (Meloy). The capabilities of circulating lymphocytes to be stimulated by phytohemagglutinin-P (PHA-P (Difco)) were studied, as previously reported.[12]

Protein Studies

Microzone electrophoresis of the patient's serum, concentrated urine, and isolated, purified urinary protein was done on cellulose acetate strips in a pH 8.6 Veronal buffer. Quantitative serum immunoglobulin analyses were done on Hyland Laboratories' Immuno-plates containing specific antisera to IgG, IgA, and IgM. Immunoelectrophoresis and immunodiffusion were performed according to Scheidegger[13] and Ouchterlony[14] using polyvalent anti-whole serum and specific antisera to heavy and light chains.

The γHCD protein BAZ was recovered from the urine by salt precipitation with 50% saturated ammonium sulfate. After extensive dialysis against distilled water, the urinary protein was lyophilized and further purified by gel filtration on a Sephadex G-200 column.[15] The homogeneity of the fractions was evaluated by cellulose acetate electrophoresis, by immunoelectrophoresis using anti-normal human serum, and by Ouchterlony immunodiffusion analyses using specific antisera for IgG, IgA, IgM, and kappa and lambda light chains.

The reduced and alkylated form of protein BAZ was prepared for ultracentrifugal, viscosity, and gel filtration studies by complete reduction with dithiothreitol and carboxymethylation with iodoacetic acid in 7 *M* guanidine hydrochloride–Tris buffer, pH 8.0. The reduced-alkylated protein was then dialyzed against distilled water and lyophilized.

Sedimentation velocity studies of serum and purified urinary protein were done at 25°C in a Spinco Model E analytical ultracentrifuge equipped with an electronic speed control system and schlieren and interference optical systems. Sedimentation coefficients were calculated in the usual manner from the peak positions and corrected to 20°C in water.

The diffusion coefficient of the purified protein was also determined in the analytical ultracentrifuge, as described by Smith et al.[16,17] The observed diffusion coefficient D was calculated from the areas and maximum peak heights of the schlieren patterns, and corrected to $D^0_{20,w}$. The molecular weight of protein BAZ was estimated from the sedimentation and diffusion coefficients using the classical Svedberg equation.

Viscosity measurements were made at 25°C with Cannon Ubbelohde semimicro dilution viscometers. The specific viscosity η_{sp} was calculated from the flow times and densities of the protein solution and solvent, and the intrinsic viscosity $[\eta]$ was obtained from a plot of η_{sp}/c versus c.[16,17] Molecular weights were estimated from appropriate $s_{20,w}$ and $[\eta]$ values using the equation of Sheraga and Mandelkern.[18]

Molecular weights were also estimated by gel filtration studies on 6% agarose gel columns as described by Fish et al.[19] The distribution coefficients (K_d) of the reduced and reduced-alkylated forms of protein BAZ were obtained from the elution positions of each from calibrated columns, prepared and operated in 6 *M* guanidine hydrochloride. The apparent molecular weights were determined by comparison of the observed K_d values with a calibration curve of log molecular weight versus K_d values for protein polypeptide chains of known molecular weight.

Cyanogen bromide cleavage of 250 mg of protein BAZ was carried out by dissolving the protein in 70% formic acid and adding a twofold (w/w) excess of cyanogen bromide; after 4 hr, the reaction was terminated by lyophilization. Cyanogen bromide (CB) fragments were isolated by gel filtration on a 1.0 × 150 cm Sephadex G-100 column in 30% formic acid; the fragments were detected in the effluent by monitoring the absorbance at 280 nm. The resulting peaks were further

purified by gel filtration and the C-terminal peptide was purified on a SP-Sephadex column according to the procedure of Waxdal et al.,[20] the peptides from the SP-Sephadex column were detected with the ninhydrin reagent after alkaline hydrolysis.[21] Amino acid analysis of the protein and fragments of peptide was accomplished on a Beckman model 120 B amino acid analyzer equipped with a Infotronix Model CRS-11AB integrator, as described by Garver and Hilschman.[22]

RESULTS

Cellular Studies

Bone marrow aspirate and biopsy showed mild hypocellularity. The M:E ratio was 1:1. Myeloid and erythroid cell lines were normal. Three percent of all cellular elements were large nucleolated plasmacytoid cells with pale cytoplasm and eccentric nuclei containing finely granulated chromatin. These cells were found throughout the marrow, either isolated or in small clusters, and were frequently infiltrated with numerous small, mature lymphocytes (Fig. 1). Electron microscopy showed these cells to be plasma cells with markedly dilated cisternae of rough endoplasmic reticulum, which in some cells acquired a whorled configuration (Fig. 1, inset), similar to that shown in one previous patient with γHCD.[23] Mature plasma cells were not increased. The light and electron microscopic appearance of these cells was not distinctive. Megakaryocytes were adequate in numbers, and the iron stores appeared normal. A bone marrow aspirate performed 16 mo later showed a similar picture, but with 10% mature plasma cells.

The salivary glands were massively infiltrated with plasma cells, resulting in total disruption of the normal architecture. About 90% of the cellularity was

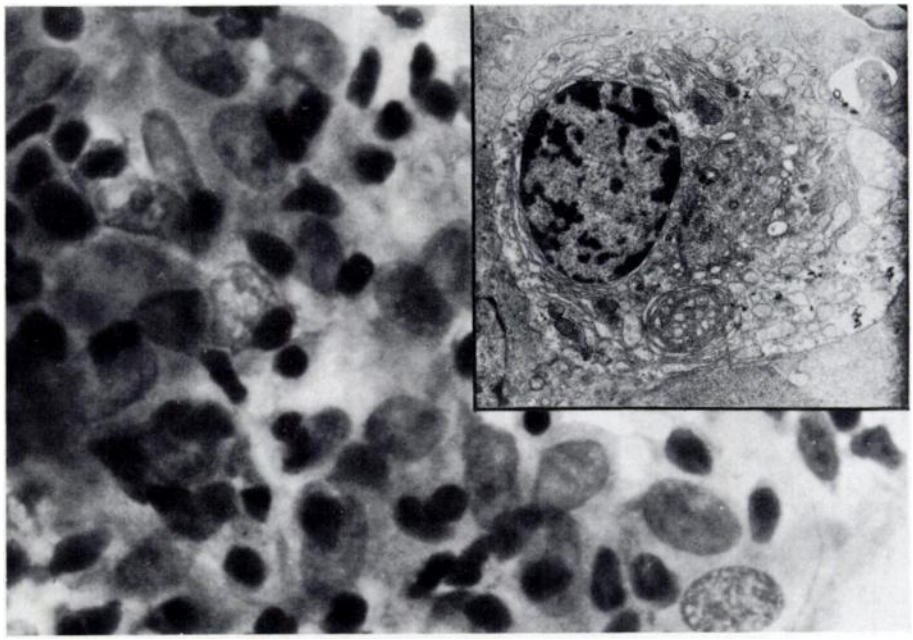

Fig. 1. Light microscopy of bone marrow specimen showing cluster of immature plasma cells and an infiltration of small lymphocytes. × 316. Electron micrograph, inset, illustrates a plasma cell with markedly dilated endoplasmic reticulum of a whorled configuration. × 19,500.

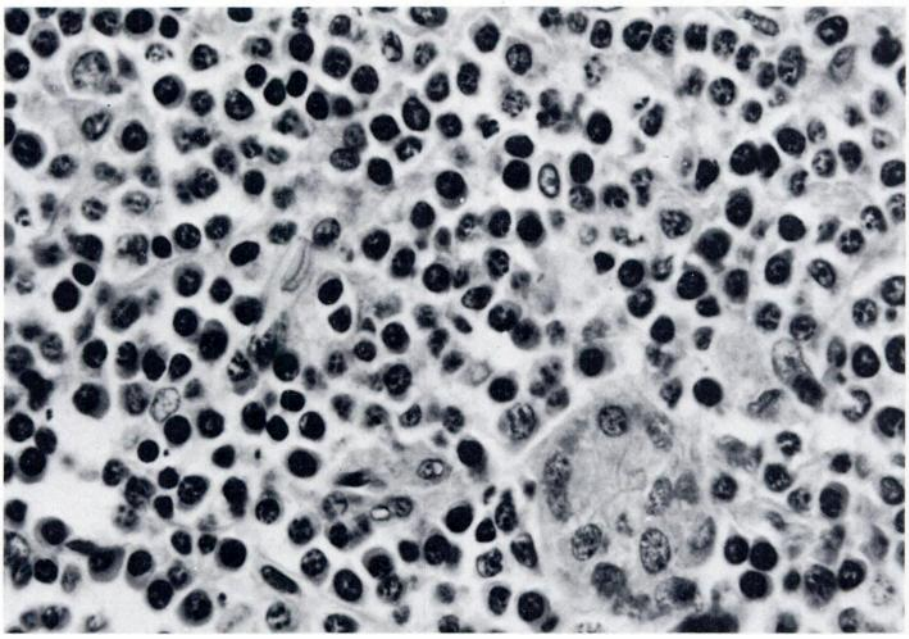

Fig. 2. Light microscopy of salivary gland showing massive replacement of the normal architecture by plasma cells. × 204.

represented by plasma cells, which in most areas formed solid sheets (Fig. 2). Chromosomal analysis performed on the second bone marrow aspirate was normal. Fifty-eight percent of the circulating lymphocytes were B-dependent cells (normal = 22.2 ± 3.4%). The distribution of chain-specific surface immunoglobulins was: α 10% (normal 2.8 ± 1.3%); γ 35% (normal 7.4 ± 1.6%); and μ 13% (normal 13.8 ± 1.0%). The profile of peripheral blood lymphocyte responsiveness to PHA-P stimulation was grossly abnormal and suggested an increased activation threshold and impaired stimulation by this phytomitogen.[24]

Protein Studies

Microzone electrophoresis revealed a broad peak of β-mobility in both the serum (accounting for 37% of the total serum protein) and concentrated urine. Repeated quantitative serum immunoglobulin analyses gave values of 4900–9200 mg/100 ml for IgG, 60 mg/100 ml for IgA, and 20 mg/100 ml for IgM. The values for IgG were inconsistent, but always much greater than the value calculated for the β-γ fraction of the serum protein on the basis of the total protein concentration and the fractional values obtained from the electrophoresis scan. This finding suggested the possibility of an IgG fragment which diffused through the gel at a considerably faster rate than normal 7S IgG.

Although analytical ultracentrifugation of the patient's diluted serum (1.5 g/100 ml protein concentration) showed virtually no 7S component, in marked contrast to what is normally present, there were increased amounts of a component with a sedimentation coefficient of about 4S (Fig. 3A). The

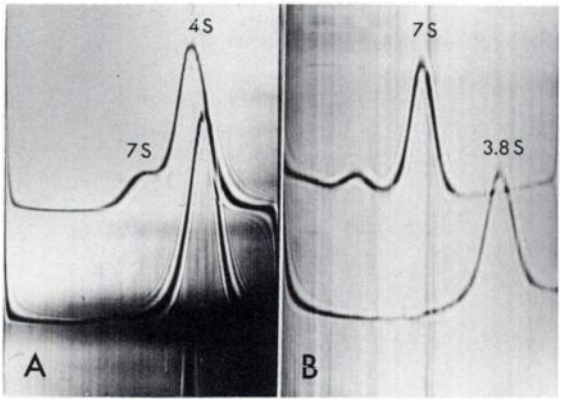

Fig. 3. (A) Sedimentation pattern of diluted serum from normal control (upper tracing) and patient BAZ (lower tracing), 56 min after reaching 60,000 rpm. Direction of sedimentation is from right to left. Total protein concentration of each sample was approximately 1.5 g/100 ml in 0.1 M NaCl. (B) Sedimentation pattern of normal human IgG Cohn fraction II (upper tracing) in 0.1 M phosphate buffer, pH 7.3, as compared to isolated γHCD protein BAZ (lower tracing). Protein concentrations were 10 mg/ml.

$s^0_{20,w}$ value of the isolated, purified protein in 0.1 *M* potassium phosphate buffer was 3.83S (Fig. 3B).

Immunoelectrophoretic patterns of the patient's serum demonstrated the presence of an abnormally fast migrating anodic band in the β-γ region reacting with anti-IgG (Fig. 4). As seen in the figure, there was a marked decrease in the amount of normal IgG. The fast-migrating protein band did not react with either type of L chain antisera (anti-κ or anti-λ). Furthermore, no precipitin reaction was visible with anti-Fab, whereas anti-Fc gave a distinctly positive reaction (Fig. 5). These results were sufficient to establish tentatively a diagnosis of γ-heavy chain disease.

Dr. Arthur Steinberg, Case-Western University, analyzed protein BAZ for the presence of the Gm markers 1, 3, 5, 17, and 21. BAZ contained the Gm (1) factor; therefore this protein belonged to the IgG1 subclass.

The mol wt of protein BAZ in 0.1 *M* phosphate buffer, pH 7.3, was calculated from the sedimentation and diffusion coefficients, and also from the sedimentation coefficient and intrinsic viscosity. Using an $s^0_{20,w}$ value of 3.83S and a $D^0_{20,w}$ value of 6.1×10^{-7} sq cm/sec in the Svedberg equation, yielded a mol wt of 58,700, while a value of 59,800 was calculated from the Sheraga-Mandelkern equation using values of 3.83S for $s^0_{20,w}$ and 5.1 ml/g for $[\eta]$.[17]

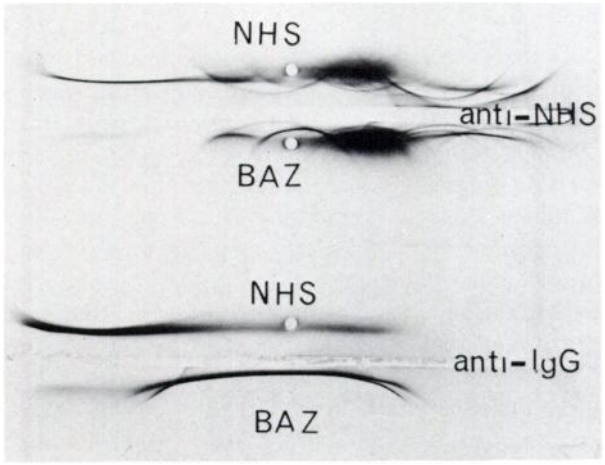

Fig. 4. Immunoelectrophoresis of normal human serum (top wells) and serum of patient BAZ (bottom wells). Top slide developed with anti-normal human serum, lower slide with anti-IgG. Anode, right; cathode, left.

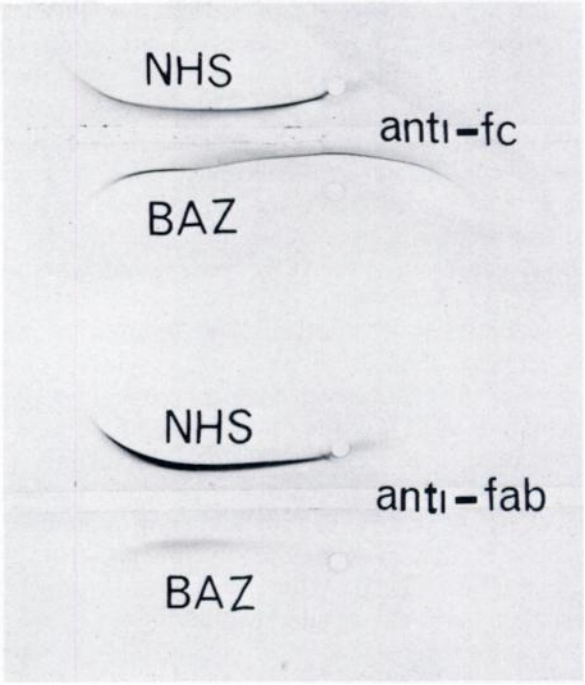

Fig. 5. Immunoelectrophoresis of normal human serum (top wells) and serum of patient BAZ (bottom wells). Top slide developed with the anti-Fc fragment, lower slide with anti-Fab fragment. Anode, right; cathode, left.

Table 2. Amino Acid Composition of γ1 Heavy Chains and BAZ C-Terminal Peptide (CB-III)*

	BAZ	DAW†	EU‡	BAZ γ^1-CB-III	Normal γ^1-CB-III
LYS	18.8	29	31.1	1.2	1.0
HIS	5.9	8.9	8.8	2.9	3.0
ARG	7.4	11	10.5	—	—
ASP	21.7	34	31.7	1.1	1.0
THR	16.4	35	33.3	1.0	1.0
SER	23.1	47	56.0	3.0	3.0
GLU	25.9	36	44.3	2.3	2.0
PRO	22.4	34	38.6	1.0	1.0
GLY	14.1	26	32.8	2.1	1.0
ALA	9.1	20	22.1	—	1.0
VAL	23.9	41	46.5	—	—
MET	2.1	3.6	5.9	—	—
ILE	4.2	8.1	9.5	—	—
LEU	16.6	36	30.2	3.0	3.0
TYR	9.1	17	18.7	0.9	1.0
PHE	7.4	13	16.1	—	—
TRP	4.5	7.5	8.3	—	—
CYS	4.2	11.0§	11.8	—	—

*Amino acid residues are expressed as moles of amino acid/mole protein or peptide based upon a mol wt of 30,000 daltons for protein BAZ.

†Reference 25.

‡Reference 27.

§Reference 26.

The molecular weights of the reduced alkylated and of the unreduced forms of protein BAZ in 6 *M* guanidine hydrochloride were estimated. A mol wt of 33,500 was calculated for the reduced alkylated protein, based on a value of 1.9S for $s^0_{20,w}$ and a value of 20.0 ml/g for $[\eta]$. A value of 30,000 was obtained for the unreduced form, for which $s^0_{20,w}$ and $[\eta]$ were 2.15S and 11.0 ml/g, respectively. The mol wts determined from gel filtration studies on a 6% agarose gel column were 31,000 for reduced-alkylated BAZ and 29,100 for the unreduced protein. These results established that the γ_1 heavy chain disease protein BAZ was a dimer linked by noncovalent type bonds, rather than by covalent type disulfide bridges.

Preliminary structural analyses[15] established that protein BAZ had a blocked N-terminal amino acid, presumably pyrollidone carboxylic acid (PCA). Treatment of the protein with hydrazine liberated glycine as the C-terminal amino acid, indicating that the protein had both amino and carboxy termini homologous to normal γ chains. However, the amino acid composition of the protein showed that it contained only 4 cysteine residues, compared to 11 for normal γ_1 chains (Table 2). The carbohydrate content was high, 13.9%, in contrast to a normal value of about 2.5% for IgG proteins.

After cyanogen bromide cleavage of the protein, the amino acid composition of the C-terminal octadecapeptide indicated an absence of 1 mole of alanine and one extra residue of glycine (Table 2). Sequence analysis of this peptide established an Ala → Gly substitution at position 431.

DISCUSSION

Our patient exhibited features generally associated with γHCD, including an insidious onset with prolonged prodromata of upper respiratory infections, splenomegaly, pancytopenia, hyperuricemia, and hypogammaglobulinemia. Unlike most patients with γHCD, however, our patient's presentation with a 30-lb weight loss was more suggestive of an underlying malignancy than an overt lymphoma or granulomatous disease. However, she subsequently exhibited clear evidence of plasmacytic proliferation, as evidenced by the increasing marrow plasmacytosis and the massive plasma cell infiltration of the salivary glands. This later finding, not previously observed, was somewhat reminiscent of the thyroid involvement by plasma cells in a previous case of γHCD.[28] The neoplastic nature of the plasmacytic proliferation was supported by the extent of the glandular infiltration with total effacement of the normal architecture.

Gamma HCD may progress relentlessly with death in a few months or may wax and wane, allowing a survival of several years. The impairment of humoral and cellular immunity exhibited by our patient most probably contributed to her recurrent febrile episodes, thus providing a rationale for passive, specific immunotherapy in the form of human gamma globulin. Such a therapeutic modality may be beneficial in patients with slowly progressive disease.[29] In spite of uncertainties surrounding the use of chemotherapy and radiotherapy for γHCD, a number of long-lasting remissions, including the disappearance of the paraprotein from serum and urine, have been associated with their use in selected patients with rapidly progressive disease.[5,23,28-31]

Although approximately 35 cases of γHCD have been reported to date, the number of γHCD proteins for which detailed immunologic, physicochemical, and primary structural analyses are available is considerably less. Of 28 γHCD proteins typed according to subclass, 19 are γ1, 2 are γ2, 6 are γ3, and 1 is γ4.[2]

Physicochemical studies have shown these proteins to have electrophoretic mobilities in the fast γ to β region and sedimentation coefficients in the range of 2.8–4.0S. The mol wt of the heavy chain monomer averages about 35,000 daltons, but ranges between 25,000 and 58,000. The carbohydrate content is often quite high (up to 20%), compared to that of normal gamma chains (approximately 3%). γHCD proteins which were originally thought to consist primarily of the Fc fragment, have been demonstrated to range from intact heavy chains to molecular structures containing internal deletions of 100–200 amino acid residues.[2] In addition, several of these proteins with deletions of the entire variable (V) and first part of the constant regions (C_H1 domain) may be enzymatic degradation products of a larger HCD protein, since the hinge region is known to be very susceptible to proteolytic cleavage.

γHCD proteins with internal deletions appear to be of two major types:[2,6] one type is characterized by deletions of parts of the V region and C_H1 domain with resumption of normal sequence at the glutamic acid residue in position 216 at the beginning of the hinge region. Since the hinge region (amino acid residues 216 to 232 of protein Eu[32]) is intact, normal inter-heavy chain disulfide bonds are formed by the cysteine residues in position 226 and 229. The γHCD proteins in this group are covalently bound dimers which dissociate only in the presence of a reducing agent. The mutants CRA (γ1),[33] GIF (γ2),[34] and ZUC (γ3)[35] belong to this category. In the second type of internally deleted HCD proteins, the deletion also encompasses the hinge region. Such proteins lack the inter-heavy-chain disulfide bridges and therefore form noncovalently linked dimers which split into monomers in nonreducing, dissociating solvents such as urea or guanidine hydrochloride. Proteins PAR (γ1),[36] HI (γ1 or γ2),[37] and HAL (γ4),[38] belong to this group.

Protein BAZ must also contain a deletion that extends beyond the hinge region, since the native protein has a mol wt of 60,000 daltons, forms a 30,000-dalton monomer in 6 *M* guanidine hydrochloride, and has only four cysteine residues.[8,17] Although precise localization of the initiation and termination points of the deletion of protein BAZ has not yet been achieved, sequence studies to date have established that the deletion includes the inter-heavy chain half-cysteines at position 226 and 229. The defect includes most of the V region and the entire C_H1 and hinge regions. Normal synthesis resumes immediately beyond the hinge region, forming complete C_H2 and C_H3 domains.[15] Amino acid sequence analysis of the cyanogen bromide (CB) fragments of protein BAZ have demonstrated that the carboxy-terminal octadecapeptide contains a substitution of glycine for alanine at position 431.[9,15] This substitution has not been previously recognized in any other human γ1 chain; recently, however, this replacement has also been demonstrated in the γHCD protein YOK.[39] These findings suggest that the ALA → GLY exchange may possibly represent another genetic marker on IgG1 proteins. Structural and immunochemical studies of this exchange in normal human serum are currently under investigation to examine this possibility.

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Blood (print ISSN 0006-4971, online ISSN 1528-0020), is published weekly by the American Society of Hematology, 2021 L St, NW, Suite 900, Washington DC 20036.