Polyclonal Gammopathy with Marked Increase in Serum Viscosity

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A 45-year-old man with polyclonal hypergammaglobulinemia (gamma globulins, 102 g/L) had a serum relative viscosity of 13 ν but did not manifest clinical signs of hyperviscosity syndrome (e.g., retinopathy, bleeding diathesis, and neurological alterations), except for fatigue and anorexia. In contrast with other patients with polyclonal hyperviscosity reported so far, this patient did not have detectable rheumatoid factor in serum. Analytical ultracentrifugation of his serum showed aggregates of polyclonal IgG3 of various sizes (between 10 and 36 S). The serum also contained immune complex-like material, as demonstrated by the Raji cell immunoradiometric assay and the C1q solid-phase enzyme immunoassay.

Serum hyperviscosity is found primarily in patients with monoclonal gammopathies, e.g., macroglobulinemia and, less frequently, multiple myeloma. In these patients the increase in homogeneous immunoglobulin (especially of the IgM class) is responsible for the increased serum viscosity. Polyclonal gammopathies, which occur more frequently than monoclonal gammopathies, are not usually associated with clinically important increases in serum viscosity. However, when the amount of polyclonal immunoglobulins in the serum is very large, hyperviscosity syndrome may occur and is similar to that seen in patients with monoclonal gammopathies. We report here a patient with a pronounced polyclonal hypergammaglobulinemia whose serum viscosity was markedly increased but who, surprisingly, showed no clinical signs of hyperviscosity syndrome. The patient had neither circulating antibodies to various antigens for which he was tested nor demonstrable rheumatoid factors and cryoglobulins in serum, but he did have circulating immune complex-like material. Complexes of different sizes, apparently composed of IgG2, were detected in his serum by ultracentrifugation and gel-filtration analyses. This documented case of IgG hyperglobulinemia presents one of the highest serum viscosities for this group of patients and, to our knowledge, the highest asymptomatic serum viscosity.

Case Report

A 45-year-old mentally retarded black man presented to the hospital with fatigue, anorexia, nausea, and weight loss (allegedly 16 kg during the last three months). Past history included a urinary-tract infection six months earlier and pneumonia. Also six months earlier, he had been admitted to another hospital, with the diagnosis of renal failure. At that time, the patient had hypertension of undetermined duration and apparently had bilateral hydronephrosis. One month before the first admission the patient's serum protein concentration was 77 g/L (albumin, 42 g/L). At the first admission the relative viscosity (relative to water) of the serum was 3.2 ν (reference interval, 1.4–1.8 ν), the complement C3 was <0.1 g/L (reference interval, 0.7–1.76 g/L), and C4 was 0.172 g/L (reference interval, 0.18–0.45 g/L). A test for cryoglobulins in serum stored seven days at 4 °C was negative.

On admission to this hospital, the physical examination revealed enlarged submandibular and inguinal lymph nodes. Results from routine laboratory tests included serum protein, 142 g/L; cholesterol, 2.34 mmol/L; phosphorus, 2.90 mmol/L; uric acid, 0.49 mmol/L; aspartate aminotransferase, 24.4 U/L (reference interval, 2.4–19.2 U/L at 37 °C); creatinine, 221 μmol/L; and urea nitrogen, 12.8 mmol/L. A blood cell count showed 6600 × 10⁹ leukocytes per liter; hemoglobin, 128 g/L; hematocrit, 0.28; and platelets, 155 000 × 10⁹/L. We observed rouleaux formation of erythrocytes and clumped platelets in a blood smear, and the percentage of eosinophils was increased (6% to 12%). The erythrocyte sedimentation rate (ESR) was 1 mm/h (Westergren method).6 The ratio of CD4+/CD8+ lymphocytes was 0.4, and a test for human immunodeficiency virus antibodies was negative on two occasions, four months apart.

A renal ultrasound examination showed bilateral hydronephrosis, and a roentgenogram of the upper gastrointestinal tract showed diffuse hypertrophy of the mucosal folds of the stomach as well as of the proximal duodenum. Renal biopsy was not performed.

The diagnosis of normocytic normochromic anemia, hypergammaglobulinemia of undetermined cause, and renal failure was considered, and additional tests were performed.

Materials and Methods

Serum electrophoresis was performed with commercial reagents (Corning, Medfield, MA 02052). In brief,
we used plates coated with agarose gel in sodium barbital buffer (pH 8.6), and the plates were stained with Amido Black and scanned with a Model 720 densitometer (Corning). We measured serum protein concentration with a refractometer (Buffalo Optical, Buffalo, NY 14202).

Serum and urine (50-fold concentrated) immunoelectrophoreses were performed on the same plates used for serum electrophoresis (Corning). Urine electrophoresis was also performed on agarose gel plates (Panagel; Princeton Separations, Inc., Freehold, NJ 07728) after a 50-fold concentration of urine with a Minicon concentrator (Amicon Div., W. R. Grace and Co., Danvers, MA 01923). Monospecific antibodies to IgG, IgA, IgM, IgD, and IgE, as well as antibodies to kappa and lambda light chains, were obtained from Dakopatts (Accurate Chemical and Scientific Corp., Westbury, NY 11590), Hyland (Cooper Biomedical, Malvern, PA 19355), and Cappel (Cappel Lab., Cochranville, PA 19330). Immunofixation electrophoresis was performed on agarose gel plates (Paragon; Beckman Instruments, Inc., Fullerton, CA 92634) with use of the antibodies just mentioned. The plates were stained with Amido Black.

Serum relative viscosity was measured at room temperature with an Oswald-type viscometer (Cannon Instrument Co., State College, PA 16804), as described by Fahey et al. (1), and with a Wells-Brookfield cone/plate viscometer (Brookfield Engineering Lab., Stoughton, MA 02072), both at room temperature and at 37 °C. The latter instrument was calibrated with oils of certified viscosity.

Circulating immune complexes (CIC) that bind complement were measured in the serum with a Raji cell immunoradiometric assay, as previously reported (2). In brief, Raji cells were incubated with the serum and 125I-labeled Protein A (ICN Biomedicals, Inc., Irvine, CA 92713). We plotted a standard curve by using the binding values obtained with increasing amounts of alkali-aggregated human IgG (Sigma Chemical Co., St. Louis, MO 63178). The results were reported as equivalent aggregated human IgG. In addition, CIC were measured with a C1q solid-phase enzyme immunoassay (Cytotech Inc., San Diego, CA 92121).

Concentrations of serum IgG, IgA, IgM, and kappa and lambda light chains, as well as concentrations of complement components C3 and C4, were determined with a rate nephelometer (Array Protein Systems; Beckman). Rheumatoid factor was measured with a latex agglutination test (Wampole, Raritan, NJ 08876), and antibodies to double-stranded DNA (dsDNA) were measured with a *Crithidia luciliae* immunofluorescence test (Wampole). Antibodies to thyroglobulin and to thyroid microsomal antigen were measured with a passive hemagglutination assay (Burroughs Wellcome Diagnostics, Kent, U.K.). Antibodies to U1 snRNP, Sm, Ro (SS-A), and La (SS-B) antigens were measured by precipitation in agar-gel (assay from Behring Diagnostics Inc., Branchburg, NJ 08876). Antibodies to hepatitis B virus (HBV) antigens, cytomegalovirus, and Epstein–Barr virus were measured by enzyme-linked immunoassay with commercial reagents from Abbott Labs. (Diagnostics Div., North Chicago, IL 60064) and Whittaker Bioproducts (Walkersville, MD 21793). Subclasses of serum IgG were measured with an enzyme immunoassay and by radial immunodiffusion (Binding Site, San Diego, CA 92121).

Ultracentrifugation analysis of the serum (diluted eightfold with isotonic saline) was performed at 20 °C with a Model E analytical ultracentrifuge (Beckman Spinco, Palo Alto, CA 94303) at 105 000 × g and a Schlieren angle of 60°. Gel-filtration analysis was performed with columns (C16/100 and C26/1300; Pharmacia, Uppsala, Sweden; and Ace Glass, Inc., Vineland, NJ 08360) of Sephadex G 200 and Sephadryl S-300 Superfine (Pharmacia LKB, Biotechnology AB) equilibrated with phosphate buffer, pH 7.4, and citrate buffer, pH 3.4, at a flow rate of 0.2 mL/min. The effluent was measured with a Model 8300 Uvicord II (LKB Instruments, Rockville, MD 20852) equipped with a 280-nm filter. Fractions of 3.75 mL were collected and tested (after concentration with Minicon filters) with anti-IgG, anti-IgA, anti-IgM, and anti-albumin antibodies by double diffusion in agarose gel. The fractions containing IgG were pooled and tested for IgG subclasses (Binding Site).

**Results**

Serum electrophoresis showed an intensely stained band in the gamma globulin region, extending into the beta globulin area (Figure 1). The albumin was 23.7 g/L (reference interval, 37–55 g/L), and the gamma globulins were 105.4 g/L (reference interval, 7–15g/L). The Sia test was positive. Immunoelectrophoresis and immunofixation electrophoreses of the serum showed a polyclonal increase in IgG and a relative decrease in IgA and IgM (Figures 2 and 3). Electrophoresis of urine (after 50-fold concentration) showed three discrete bands (albumin and α1- and α2-globulins) and a diffuse band extending from the fast beta to the gamma region (Figure 4). Immunoelectrophoresis of urine showed mainly albumin, transferrin, and IgG.
g/L (reference interval, 1.17–7.4 g/L), IgG₂ was 85.1 g/L (reference interval, 0.41–1.29 g/L), and IgG₄ was undetectable by radial diffusion. The quantification of light chains showed kappa chains of 38.5 g/L and lambda chains of 35.1 g/L (reference intervals, 5.98–13.2 and 0.28–6.65 g/L, respectively). The ratio kappa:lambda was 1.67 (reference interval, 1.47–2.65).

The relative serum viscosity at room temperature was 13.5 ν (reference interval, 1.4–1.8 ν, relative to water). The absolute serum viscosity was 9.6 cP at room temperature and 7.3 cP at 37 °C. After plasmapheresis of two units of plasma, the relative serum viscosity decreased to 4.9 ν. During the first three months of follow-up, the relative serum viscosity at room temperature fluctuated between 5.5 and 9 ν.

Antibodies to thyroglobulin, thyroid microsomal antigen, streptolysin O, and antibodies to HBV surface and core antigens were not detected in serum. A test for HBV surface antigen was negative. Tests for antinuclear and antimitochondrial antibodies and for rheumatoid factors were negative. Tests for antibodies to Cytomegalovirus, dsDNA, Sm, U1 snRNP, Ro, and La antigens were also negative. A test for Epstein–Barr virus antibodies showed a titer of 64. A Raji cell assay showed 124 mg/L, and a solid-phase Clq assay for immune complexes showed 29.7 mg/L equivalents of aggregated human IgG (reference, <4 mg/L).

Ultracentrifugation analysis of the serum showed an apparent decrease in the 19 S components; the presence of complexes with sedimentation rates of 36, 15, and 10 S; and a small amount of 7 S proteins. Gel-filtration analysis with Sephacryl S-300 showed a wide peak starting before the peak seen with normal serum; this peak consisted of IgG₁ (Figure 5) and some IgA. The low pH buffer did not separate more peaks, as are seen in normal serum, which suggests that the complexes did not dissociate; hence, we conclude they were not composed of antigen and antibody but of aggregates of IgG. Gel-filtration patterns with Sephadex G-200 are depicted in Figure 6.

**Discussion**

Hyperviscosity syndrome is characterized clinically by a triad of symptoms: bleeding diathesis, retinopathy, and neurological alterations. Clinical manifestations of the syndrome are not proportional to the serum viscosity, but vary from patient to patient: each patient has his or her own symptomatic threshold. As a rule, most patients are symptomatic at a relative viscosity of 4–8 ν and almost all patients at a value of ≥10 ν (2, 3). The patient reported here did not present these objective
classical clinical symptoms of hyperviscosity syndrome, even when the relative viscosity of his serum was as great as 13.5 \nu. Fatigue, anorexia, and nausea, mostly nonspecific subjective symptoms, disappeared three days after admission, despite the persistence of serum hyperviscosity.

Hyperviscosity syndrome can occur more commonly in monoclonal immunoglobulin disorders (monoclonal gammopathies, e.g., Waldenström macroglobulinemia and multiple myeloma) and in polyclonal immunoglobulin disorders (polyclonal gammopathies), including rheumatoid arthritis (4–7), Felty syndrome (8), Sjögren syndrome (9–11), systemic lupus erythematosus (12, 13), polymyositis (14), lymphoproliferative diseases (15–16), chronic active hepatitis (17), and acquired immunodeficiency syndrome (18).

The first documented report of hyperviscosity syndrome associated with a rheumatic disease was by Meltzer et al. (19); by 1987, 13 patients with rheumatoid arthritis and hyperviscosity had been reported (4). In all of these patients rheumatoid factors were present in high titer in the serum, and in most instances the ESR was also markedly increased. In most of the patients the hyperviscosity syndrome was due to the presence of “intermediate complexes,” i.e., immunoglobulin complexes with a sedimentation rate between 7 S (that of monomeric IgG) and 19 S (pentameric IgM).

Kunkel et al. (20) first showed the presence of intermediate complexes in the sera of patients with rheumatoid arthritis and allied conditions. Several intermediate complexes have been reported in patients with connective tissue disease and hyperviscosity syndrome (21). The intermediate complexes most commonly found consist of IgG rheumatoid factor aggregated with IgG. Both IgG components are polyclonal, and the complexes are acid-labile (i.e., of antigen–antibody type). Another type of intermediate complex is represented by large amounts of 11 to 16 S complexes without rheumatoid-factor activity. High viscosity was found only when these complexes interacted with a patient's IgM rheumatoid factor (5). Another type of intermediate complex was acid labile, with sedimentation rates from 10 to 45 S, composed of cold-precipitable IgG with rheumatoid-factor activity (19). Finally, 13 S complexes of IgG have been described in which molecules were loosely associated via their Fc regions. The Fc fragments prepared from these complexes, but not the Fab fragments, had rheumatoid factor-like activity (14).

The patient just described had a polyclonal hypergammaglobulinemia of undetermined cause (no identifiable connective tissue or lymphoproliferative disease). He had a high concentration of IgG, in serum, an immunoglobulin that produces serum hyperviscosity at lower concentrations than IgG, probably because of concentration- and temperature-dependent aggregation (22, 23). Polymerization of 7 S IgG monomers into large complexes (24, 25) and an abnormal shape of the IgG molecule have also been implicated in some cases of monoclonal gammopathy with hyperviscosity syndrome (26).

The patient reported here had a fictitious hypotension and a low ESR because of the serum hyperviscosity. Indeed, hyperviscosity is a known cause of decreased ESR (27), and decreased ESR has been reported
both in monoclonal gammopathies (28, 29) and in polyclonal gammopathies (14, 30, 31) associated with serum hyperviscosity. The cause of other laboratory abnormalities in this patient, such as decreased serum cholesterol concentration, is not clear. This patient had some peculiarities when compared with previously described patients with polyclonal hyperviscosity. He did not have symptoms of hyperviscosity syndrome (even when the relative serum viscosity was 13 ″), presumably because his serum viscosity was lower at 37 °C than at room temperature. He also had neither rheumatoid factors nor cryoglobulins, but he did have increased serum IgG, decreased C3, and evidence of CIC-like material. Patients reported with CIC associated with hyperviscosity syndrome (31–34) commonly have lymphadenopathy, liver involvement, and detectable rheumatoid factor in serum. Recently, Levinson et al. (34) reported that, in patients with CIC and serum hyperviscosity, nephelometry did not accurately estimate the concentration of serum immunoglobulins, and the Raji cell assay or the Clq-binding assays might not accurately represent the CIC. Our Raji cell immunoradiometric assay, which is based on 125I-labeled Protein A, missed the aggregates of IgG, because Protein A does not bind IgG. This suggests that IgG1 and IgG2 were part of CIC-like material measured by this assay, whereas the Clq solid-phase assay also measured aggregates composed of IgG.

Addendum. The patient has been followed for 33 months as an outpatient. At the last visit (March 1991), his total serum proteins were 115 g/L (albumin, 32 g/L), his serum creatinine was 194 µmol/L, and a differential leukocyte count showed an increase in eosinophils (25%). The relative serum viscosity was 3.9 ″, and the patient remained asymptomatic. During the last 30 months the patient was treated only for hypertension. His total serum protein ranged between 105 and 125 g/L, creatinine between 94 and 212 µmol/L, and eosinophils between 18% and 29%. His serum electrophoretic and immunoelectrophoretic patterns remained unchanged. He had waxy and waning lymphadenopathy, but lymph node biopsies showed only nonspecific hyperplasia.

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References
