A Case of Rheumatoid Hyperviscosity Syndrome with Characterization of the Serum Immune Complexes*

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ABSTRACT

The clinical and immunologic data in a case of rheumatoid arthritis complicated by serum hyperviscosity are reported. The elevation in the serum viscosity was accompanied by the presence of intermediate immune complexes which were partially purified from the patient's serum by gel filtration chromatography. These complexes had a molecular weight of approximately 510,000 daltons and contained polyclonal IgG and IgA immunoglobulins. The hyperviscosity syndrome in this patient was likely due to the presence of intermediate complexes and unrelated to the rheumatoid factor activity which was found predominantly within the IgM fraction of the serum. This case demonstrates that the hyperviscosity syndrome may, in rare cases, develop as a complication of rheumatoid arthritis. It is important to consider this possibility before the clinical symptoms of increased serum viscosity become manifest.

Introduction

The vast majority of cases of hyperviscosity syndrome (HVS) have been associated with elevated levels of monoclonal immunoglobulins, particularly IgM in Waldenstrom's macroglobulinemia (responsible for 80 to 90 percent of all HVS). The increased concentration of IgM is directly responsible for the clinical manifestations observed with elevated serum viscosity. These include circulatory overload, bleeding diathesis, somnolence, and decreased vision with dilated retinal veins. Although monoclonal immunoglobulins are by far the most common cause of this syndrome, high molecular weight complexes of polyclonal immunoglobulins have been described more recently. Patients with rheumatoid arthritis having high titers of rheumatoid factor (RF) activity in their serum may demonstrate increased serum viscosity. Marked increase in serum viscosity accompanied by symptomatic manifestations are infre-
quent, however. This report describes a patient with rheumatoid arthritis complicated by severe hyperviscosity syndrome. Immunologic characterization of the patient’s serum proteins demonstrated the presence of intermediate complexes of polyclonal IgG and IgA immunoglobulins as well as high titers of IgM RF.

Procedures and Methods

Protein electrophoresis was performed on cellulose acetate using the Beckman Microzone System.* Serum immunoglobulin levels were quantitated by nephelometry using the Beckman Immunochemistry System. Immunoelectrophoresis was performed in agarose gel by standard techniques. Antisera to all human heavy and light chains were obtained from Calbiochem.† Whole blood and serum viscosity measurements were performed using a Wells-Brookfield cone-plated microviscometer at 12 sec⁻¹ shear rate.‡ Rheumatoid factor (RF) activity was determined by using a latex agglutination technique.§

Agarose Gel Filtration

Serum (1.5 ml aliquots) from a normal individual and the patient were centrifuged at 39,000 g for 30 min and fractionated by gel filtration at 23°C on a 1.5 × 98 cm column of BioGel A-5m.|| The eluent was 0.05 KPO₄, 0.15 M NaCl, 0.001M EDTA, 0.02 percent NaN₃, pH 7.5 (flow rate, 9 ml per hr). Fractions (1.6 ml) were collected, and aliquots of each were assayed for total protein at 280 nm, albumin by BCG dye binding, and immunoglobulins G, A, and M. The gel column was calibrated for use in estimating apparent molecular weights based on the elution volumes of albumin and the immunoglobulins of normal serum. The apparent molecular weights of the proteins in the patient’s serum were calculated, based on their elution volumes, as previously described.³ Pools of individual fractions were analyzed by immunoelectrophoresis and assayed for RF as indicated.

The unfractionated serum, and various protein peaks isolated by gel chromatography, were treated with 2-mercaptoethanol (ME) 0.1M final concentration, at pH 7.3 in order to effect the depolymerization of IgM. The unfractionated serum and the major protein peaks were also treated with 0.17M glycine-HCl buffer, pH 3.1,⁹ in order to dissociate the gamma globulin components of the intermediate complexes.

Case Report

A 61-year-old woman was first admitted in 1979 with a two month history of shortness of breath, blurring vision, epistaxis, and vertigo. Initial examination revealed marked vision loss bilaterally with marked segmental dilations of retinal veins and flame-shaped hemorrhages. There was a jugular venous distension, a loud third heart sound, and evidence of pulmonary edema. The liver and spleen were mildly enlarged.

Laboratory values on admission were as follows: hemoglobin 11.7 g per dl, leukocyte count 3.2 × 10⁶ per μl with a normal differential count, and platelets of 288 × 10⁶ per μl. Erythrocyte sedimentation rate was 143 mm per hr. Prothrombin time, partial thromboplastin time, fibrinogen, and bleeding times were normal. Whole blood viscosity was 11.3 cPs (N:4.1 to 6.1 cPs); serum viscosity was 6.7 cPs (N:1.21 to 1.8 cPs); total serum protein was 8.7 g per dl; IgG 2600 mg per dl (N:770 to 1500); IgA 296 mg per dl (N:60 to 330); IgM 1395 mg per dl (N:45 to 270 mg per dl). Although the serum protein electrophoresis suggested the presence of a paraprotein, immunoelectrophoresis detected no monoclonal immunoglobulins in either serum or urine. The RF was 1.5120 (N: <40), and antinuclear antibody (ANA) titer as 1.1290 (N: <40). The serum was negative for cryoglobulins. Serum C3 level was normal.

A bone marrow biopsy and aspiration revealed a diffuse increase in plasma cells and small lymphocytes but was otherwise normal.

* Beckman Instruments, Brea, CA.
† Calbiochem Behring Corp., San Diego, CA.
‡ Brookfield Engineering Laboratories, Inc., Stoughton, MA.
§ RH-test, Hyland Laboratories, Los Angeles, CA.
|| Bio-Rad Laboratories, Richmond, CA.
Initially, a diagnosis of Waldenstrom's macroglobulinemia was made and the patient was started on plasmapheresis with prompt relief of her visual loss, epistaxis, and congestive heart failure. The serum viscosity decreased to 3.6 cPs. Therapy with chlorambucil was started but was discontinued because of the development of pancytopenia. Maintenance therapy with plasmapheresis every three to four weeks prevented recurrence of her HVS symptoms, and chemotherapy was never restarted.

In August, 1983, the patient was admitted because of recurrent congestive heart failure and a large presacral hematoma following a fall. A recent biopsy of a left earlobe mass was interpreted as a rheumatoid nodule. A history of mild, non-deforming polyarthritis was elicited. Several firm, non-tender nodules were noted near the elbows and on the right forearm, and the retinal vessels were dilated. The WBC was $2.5 \times 10^9$ per μl, Hgb was 10.8 g per dl, and platelets were $235 \times 10^9$ per μl. Total protein was 10.3 g per dl, and serum albumin was 2.0 g per dl. IgG was 5450 mg per dl, IgA was 1010, and IgM was 1660 mg per dl. Serum immunoelectrophoresis revealed no monoclonal immunoglobulin. Whole blood viscosity was 10.1 cPs, and serum viscosity was 5.0 cPs. The RF was 1:40,960 and ANA was greater than 1:50. The Raji cell assay for immune complexes was positive at greater than 1600 mg/ml antihuman globulin (AHG) Eq per ml (N: <50). A bone marrow aspiration showed mild infiltration with plasma cells and lymphocytes which appeared morphologically normal. Her diagnosis was changed at this time to rheumatoid arthritis with rheumatoid nodules and hyperviscosity syndrome. The frequency of her plasmaphereses was increased, resulting in control of her congestive heart failure, although she was admitted once for an episode of hypotension and tachycardia during one of the procedures.

In early 1984, she was begun on alkylating agent chemotherapy, with little decrease in her immunoglobulin levels. Her final admission was in December 1984 for severe weakness and jaundice. She was found to have severe hepatitis B virus (HBV) and quickly developed hepatic decompensation, coma, and coagulopathy. She expired of terminal gram negative sepsis and shock, five years after initial presentation with HVS.

Results of Special Studies

Serum protein electrophoresis done in August 1983 revealed a large gamma globulin peak with restricted mobility suggesting the presence of a paraprotein (figure 1). Serum immunoelectrophoresis, however, showed no monoclonal immunoglobulin but did demonstrate a double precipitin IgG arc, with the abnormal inflection around the application well producing a "gull winged" pattern. This suggested the presence of an IgG subpopulation that penetrated the agar poorly during electrophoresis, probably because of a higher state of aggregation (figure 2). This pattern was observed with anti-IgG and anti-IgA as well as with both anti-kappa and antilambda sera, but not with antisera to IgM. The abnormal arc persisted following treatment of the patient's serum with ME, implying that IgM was not associated with these aggregates.

Gel filtration chromatography revealed significant differences between
the elution profile of serum proteins of the patient and that of a normal control (figure 3). The profile of normal serum was characterized by major protein peaks containing albumin (fractions 75 to 80) and Ig (fractions 65 to 75). In the normal profile, the peak fractions containing IgA and IgM were fractions 68 and 71.

**Figure 2.** Serum immunoelectrophoresis. Immunoelectrophoresis of control serum (above) and patient's serum (below). Antihuman sera include polyvalent anti-IgG, A and M (upper trough), monospecific anti-IgG (middle trough) and monospecific anti-IgA (bottom trough). Note the "gull winged" appearance of the patient's serum. A similar appearance was seen with monospecific anti-kappa and anti-lambda antisera.

**Figure 3.** Elution profile of serum proteins from the patient and a normal control during gel filtration chromatography. Gel filtration on BioGel A-5m was carried out as described in Methods. Aliquots of each fraction were diluted 10-fold in column buffer and prior to optical density measurements at 280 nm. ———— control serum; ———— patient's serum. The tubes containing the peak fractions of the human protein standards (mol. wt.) were as follows: tube 76, serum albumin (66,200); tube 71, IgG (150,000); tube 68, IgA (160,000); tube 49, IgM (850,000).
48, respectively. The patient’s profile showed a decreased amount of protein in the albumin peak. A large double peak (fractions 65 to 75) eluted in the region of normal IgG and IgA. Most impressive in the patient’s profile was the large protein peak that eluted in fractions 51 to 65. This peak had no correlate in normal serum; its protein constituents were estimated to have an apparent molecular weight of approximately 510,000 Daltons. The patient’s profile also showed an elevated quantity of protein (fractions 45 to 51) that eluted in the same region of the chromatogram as normal IgM.

The results of immunoglobulin assays of the gel filtration fractions from the patient’s serum are shown in figure 4. IgG eluted as two peaks. Peak I eluted with an apparent molecular weight identical to that of normal IgG; however, the amount of IgG in the peak fraction was about three-fold elevated compared to the peak IgG fraction of the normal serum. The second IgG peak, Peak IV corresponded to the 510,000 Dalton protein peak identified by ultraviolet absorption. The material in this peak constituted the majority of the intermediate complexes in the patient’s serum.

The IgA in the patient’s serum eluted as three distinct peaks. The first (figure 4, Peak II) had an elution volume identical to normal IgA; however, the peak fraction in the patient’s profile was approximately 10-fold elevated compared to the peak IgA fraction of normal serum. The second IgA peak (figure 4, Peak III) eluted with an apparent molecular weight of 255,000 Daltons, between that of normal IgA and the intermediate complexes in peak IV. Thus, Peak III appears to represent a second class of intermediate complexes in the patient’s serum. The third IgA peak (figure 4, Peak V) was associated with the tailing edge of peak IV. The IgM in the patient’s serum eluted as a single major peak (figure 4, Peak VI) with an apparent molecular weight identical to normal IgM. The amount of IgM in the peak fraction from the patient was approximately 10-fold elevated relative to the comparable fraction from normal serum.

The intermediate complexes in the patient serum (figure 3, Fractions 51 to 65)
65; figure 4, Peaks III and IV) comprised 35 percent of the total serum protein, 34 percent of the total IgG, and 26 percent of the total IgA. The characteristics of Peaks I to VI, as determined by additional studies of pooled fractions, are summarized in Table I.

Immunoelectrophoresis of each of the six pools identified in Table I showed that the light chains in each peak belonged to both kappa and lambda subclasses, indicating that all immunoglobulins were of polyclonal origin. The majority of RF activity of the patient’s serum was confined to the material in Peak VI (Table I). Following 2-ME treatment of this material, the RF titer decreased from 1:1280 to 1:320. This indicated that the RF activity present in the patient’s serum was due to IgM RF and not associated with the small amount of intermediate complexes that eluted in these fractions.

Discussion

The rare hyperviscosity syndrome (HVS) associated with rheumatoid arthritis has been attributed to unusual IgG aggregates. These “intermediate complexes” (usually having sedimentation rates during analytical ultracentrifugation of 9 to 17 S) were first described by Kunkel et al., in a group of patients including some with rheumatoid arthritis. Although these intermediate complexes are seen occasionally in rheumatoid arthritis and other connective tissue diseases, their presence usually results in only clinically unimportant increases in serum viscosity. Meltzer and colleagues were the first to describe HVS owing to intermediate complexes in a patient with rheumatoid arthritis, and other reports have followed. Four types of intermediate complexes have been described in patients with connective tissue and HVS.

(1) Most commonly, the intermediate complexes consist of IgG rheumatoid factor aggregated with IgG. Both IgG components are polyclonal, and the complexes are acid labile, consistent with their being of the antigen-antibody type. (2) A second type of intermediate complex was reported in two patients by Jasen et al. Large amounts of 11 to 16 S IgG intermediate complexes without RF activity were found, but high viscosity was seen only when these complexes interacted with the patient’s IgM RF. M’Seffar et al. and Lovy et al. described cases in which intermediate complexes of both of these types were present. (3) Meltzer et al. reported a third type of intermediate complex in a patient who

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<th>Peak</th>
<th>Fractions Pooled</th>
<th>Apparent Mol. Weight X10^-3</th>
<th>Total IgG mg/dl</th>
<th>Immunoglobulins Percent of Total</th>
<th>RF Titer</th>
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<td>890</td>
<td>187</td>
<td>13 2 85</td>
<td>1:1280</td>
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*Peak fractions representing each of the immunoglobulin peak shown in Figure 4 were pooled and characterized. The difference in the RF titer in the pools as compared to the original titer of the serum was likely due to dilution during fractionation.
presented with acid-labile, 10 to 45 S IgG complexes. The complexes had RF activity and demonstrated cold precipitable characteristics. One of the cases described by M'Seffar and colleagues had, in addition to intermediate complexes, high molecular weight complexes (an IgM RF interacting with IgG aggregates) that were cryoprecipitable. Finally, Abruzzo et al described a single case in which 13 IgG complexes were found to be loosely associated via their Fc regions. The Fc fragments prepared from these complexes had RF-like activity but the Fab fragments did not. In almost all of the cases reported, the intermediate complexes have been found to contain polyclonal immunoglobulins regardless of the mechanism of complex formation. However, in one instance a monoclonal IgA immunoglobulin complexed with polyclonal IgG has been reported in a patient with HVS but with no evidence of connective tissue or immunoproliferative disease. Our patient had severe HVS associated with relatively mild, nodular rheumatoid arthritis. Immunoelectrophoresis of the patient's serum revealed an abnormal IgG precipitin line around the application well as has been noted in all previous cases of HVS owing to intermediate complexes. Our patient's serum contained polyclonal intermediate immunoglobulin complexes composed of IgG and IgA. The complexes did not possess RF activity, although an IgM RF that was not associated with the intermediate complexes was also present. Although it was not directly confirmed that the intermediate complexes were responsible for the high serum viscosity, this can be clearly inferred from the facts that the serum viscosity decreased following each plasmapheresis and that the serum IgM concentration never rose above 1.7 g per dl. In addition, viscosity measurements were performed on the unfractionated serum as well as serum treated with glycine-HCl buffer. A fifty percent decrease was observed following dissociation of the immune complexes (IC) by the buffer, indicating the serum hyperviscosity (SHV) was due, at least in part, to the complexes per se rather than to the increased concentrations of the individual molecular components. The abnormal IgG precipitin line on serum immunoelectrophoresis was not affected by disulfide bond cleavage with 2-ME, confirming the lack of involvement of IgM in the intermediate complexes. It was therefore hypothesized by us that this is a case of intermediate complexes of the type described by Jasin et al in which large quantities of IC devoid of rheumatoid activity are present.

Initially, because of the impressive HVS and abnormally restricted gamma globulin peak on serum protein electrophoresis, our patient was diagnosed as having Waldenstrom's macroglobulinemia. Review of the original laboratory data and reevaluation of the clinical data in 1983 established that this was, in fact, a case of HVS owing to rheumatoid arthritis and intermediate immunoglobulin complexes. Fortunately, the treatment of these two conditions (predominantly for symptoms of HVS) is very similar. Because of the mostly intravascular location of both IgM and the relatively large intermediate complexes, plasmapheresis is effective and is the treatment of choice. This modality is usually successful in controlling the acute effects of hyperviscosity. However, if the serum viscosity is difficult to control over a long course with plasmapheresis alone, corticosteroids and/or alkylating agents may be used in either condition. Towards the end of our patient's course and after four years of adequate control, plasmapheresis alone was not as effective as previously, probably because of her progressively rising IgG and intermediate complex levels. A course of multiple alkylating agent therapy and prednisone
was not effective in lowering her serum viscosity. Her terminal severe HBV hepatitis may well have been related to the replacement therapy with blood products that is necessary during frequent plasmapheresis.

Even in the absence of monoclonal immunoglobulins, individuals with connective tissue diseases (especially rheumatoid arthritis and Sjogren’s syndrome) and high serum concentrations of immunoglobulins and rheumatoid factor activity must be observed closely for increased serum viscosity owing to intermediate complexes. Measurement of serum viscosity may detect this potential problem before clinically important and sometimes devastating serum hyperviscosity develops. Plasmapheresis alone may control this clinical problem for years with relatively little toxicity, although more aggressive immunosuppressive therapy may, on occasion, be indicated.

References