Gamma Heavy Chain Disease: Rapid, Sustained Response to Cyclophosphamide and Prednisone

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A patient, CAL, with gamma heavy chain disease is presented who has had a complete remission lasting over 2 yr with combination chemotherapy consisting of pulsatile cyclophosphamide and prednisone. The patient exhibited many features of an autoimmune process including a vasculitis, low serum complement levels, a positive antiglobulin (Coombs) test, Raynaud's phenomenon, and keratoconjunctivitis sicca. The CAL paraprotein was found to have several previously undescribed characteristics. It reacted with antisera to Fd, Fab, and Fab', suggesting that most of the Fd portion of the molecule was intact. CAL protein consists of two polypeptide chains of molecular weight 49,000 covalently linked to form a dimer of 95,000 molecular weight. The covalent linkage suggests that the hinge region of this gamma heavy chain is intact.

Gamma heavy chain disease, first described by Franklin in 1963, may be defined as the presence in serum of a homogeneous population of incomplete heavy chains of the IgG immunoglobulin class. Since the first report, over 30 cases have been described in the literature. Three recent reviews have outlined the clinical manifestations of this disease and have detailed the structural features of several of the proteins. The response to treatment has been highly variable, and no consistent therapeutic approach has been used.

In this paper we describe a patient with gamma heavy chain disease who presents several previously undescribed features in his clinical course, in his response to treatment, and in the characteristics of his gamma heavy polypeptide chain.

CASE REPORT

CAL, a 62-yr-old white male, was referred to Barnes Hospital on October 17, 1972 with a 3-mo history of enlarging lymph nodes beginning in the right supraclavicular region, a 1-mo history of...
drenching night sweats, generalized pruritis, anorexia with a weight loss of 17 lb (from 135 lb to 118 lb), parasthesias of the toes and tongue, red spots on his legs, and increased fatigue. Two years previous to admission, a neurilemoma had been removed from the left supraclavicular region with resultant wasting of the left shoulder muscles. Physical examination revealed a thin, pale, afebrile male. Lymphadenopathy was present in the cervical, supraclavicular, axillary, and inguinal regions. The largest nodes measured 5 cm in diameter in the right submandibular region. There was no palatal edema. The liver had a span of 12 cm and was palpable 2 cm below the right costal margin. The spleen was not palpable. There were multiple 1–3-mm diameter flat hemorrhagic lesions on both feet.

Blood counts on admission were: Hb, 10.2 g/dl; Hct, 30%; reticulocytes, 0.3%; platelets, 260,000 × 10⁹/liter; WBC, 6800 × 10⁹/liter; neutrophil segs, 24%; bands, 7%; lymphocytes, 12%; monocytes, 10%; eosinophils, 46%; basophils, 1%; Serum protein electrophoresis revealed a low total protein of 5.0 g/dl, decreased albumin (1.63 g/dl) and beta globulin (0.61 g/dl), and normal alpha globulin (0.24 g/dl), alpha 2 globulin (0.77 g/dl), and total gamma globulin (1.7 g/dl). There was an abnormal slow gamma paraprotein at a concentration of 0.45 g/dl. Further studies of this paraprotein (designated CAL) are described below. Other abnormal findings were an alkaline phosphatase of 129 K.A. units (46° heat stable); ESR, 17; total hemolytic complement, 67 CH U/ml (normal, 150–250); C3, 78 mg/dl (normal, 101–189); 2+ positive antiglobulin reaction for C3d (negative for IgG, IgA, IgM, C3c, C4b). Results within normal limits included urinalysis, creatinine and uric acid concentrations, creatinine clearance, 24-hr urine for protein (63 mg), urinary electrophoresis for Bence Jones protein, rheumatoid arthritis latex agglutination, antinuclear antibody, C4 concentration, prothrombin time, partial thromboplastin time, and plasma haptoglobin concentration.

A chest x-ray revealed bilateral hilar adenopathy most marked on the left. A skeletal survey was unremarkable. A liver-spleen scan showed a normal liver and a borderline enlarged spleen.

Biopsy of a skin lesion on the left foot revealed upper dermal perivascular inflammatory infiltrates of lymphocytes, with some eosinophils, neutrophils, and histiocytes. Some vessel walls were infiltrated by neutrophils, while others had a fibrinoid appearance. Nuclear dust was present in the infiltrate. This histology was diagnostic of an allergic vasculitis. Pathologic examinations of the bone marrow and lymph nodes are described below.

Therapy was instituted with cyclophosphamide, 500 mg intravenously at 4-8 wk intervals (Fig. 1). Later in the patient’s course, cyclophosphamide was given orally. Prednisone was given as a

*WHO complement nomenclature used in this report: C3, intact third component; C3b, activated C3; C3c, 1A; C3d, 2D; C4, intact fourth component; C4b, activated C4.
4-day pulse at 100 mg/day along with each course of cyclophosphamide. Initially, prednisone (50 mg) was also given every other day. However, after 3 wk, prednisone was reduced to twice weekly, and after 6½ mo was completely stopped except at the time of cyclophosphamide administration.

A dramatic response to therapy was seen within 4 wk after the first course of chemotherapy, with disappearance of most symptoms, increase in weight, decrease in the size of the lymph nodes, and disappearance of the cutaneous lesions. Two months after diagnosis, serum complement levels were normal. Three and one-half months after diagnosis, the patient’s weight had returned to normal, and there was no significant lymphadenopathy. However, at this time (mid-winter), Raynaud’s phenomenon appeared along with livido reticularis of the lower extremities. These symptoms disappeared in the spring but returned with cooler weather in the following year.

Complete reevaluation in November 1973 was normal with the exception of persistent Raynaud’s phenomenon, livido reticularis, a positive Schirmer test for keratoconjunctivitis sicca, a persistent weakly positive C3d antiglobulin test, and slight hypocellularity of the bone marrow. The patient has continued to do well and remains in complete remission as of March 1975.

MATERIALS AND METHODS

Tissue was fixed in buffered formalin for routine histologic sections. Tissue for electron microscopy was fixed in formalin overnight and then postfixed with 3% glutaraldehyde and osmium tetroxide. The sections were then examined in a Philips 300 electron microscope.

Immunoelectrophoresis was carried out in 1.5% agar gel in 0.02 M barbital buffer, pH 8.6, according to Scheidegger. Anti-lgG, anti-IgA, anti-IgM, anti-κ, anti-λ, anti-Fab, and anti-Fd sera were obtained from Meloy Laboratories, Springfield, Va. Anti-Fc serum was obtained from Behring Diagnostics, Somerville, N.J. Antisera to pepsin monovalent and divalent fragments of IgG Fab’ and F(ab’)2 were prepared in rabbits as previously described. After absorption with normal κ- and λ-chains, the anti-F(ab’)2 sera reacted with normal intact IgG, gamma heavy chains, and F(ab’)2, but not with the Fc fragment of IgG prepared by papain digestion.

Alkaline starch gel electrophoresis was performed by the method of Smithies. Acid 8M urea starch-gel electrophoresis employed the method of Edelman and Poulik.

The antiglobulin (Coombs) test was carried out employing ten serial dilutions of potent antiglobulin reagents monospecific for IgG, IgA, IgM, C4b, C3c, and C3d, in the manner previously described.

The heavy chain paraprotein was purified initially by gel filtration of whole serum on Sephadex G-200 in 0.05 M borate-buffered saline, pH 7.4. Three main protein peaks were seen. The second peak contained most of the intact IgG and aggregates of the paraprotein, while the nonaggregated paraprotein was confined almost entirely to the low-molecular-weight, albumin-containing third peak. Fractions of the third protein peak were pooled and rechromatographed on Sephadex G-200. A single peak was eluted from the column. The leading edge of the peak was discarded, and the remaining protein solution was equilibrated with 0.0175 M phosphate buffer, pH 6.5, and passed over DEAE-cellulose equilibrated in the same buffer. The paraprotein (accompanied by trace amounts of intact IgG) was not retarded on the column under these conditions.

Sodium dodecylsulfate polyacrylamide gel electrophoresis was carried out by a modification of the method of Weber and Osborne, employing gels containing 5% acrylamide, 3.25% bisacrylamide, and 0.1% sodium dodecylsulfate. Gels were electrophoresed at 8 mA/gel, with samples either unreduced or reduced by boiling for 5 min in 0.1 M 2-mercaptoethanol.

RESULTS

Histology

A bone marrow aspiration was unsuccessful, but a marrow biopsy was 100% cellular with marked increase in mature and immature plasma cells (some binucleate) and an increase in eosinophils and lymphocytes. Erythroid and myeloid elements were normal. Reticulin fibers were moderately increased. The marrow biopsy was interpreted as showing a malignant immunoproliferative disorder, including either multiple myeloma or gamma heavy chain disease.
Fig. 2. Electron micrograph of lymph node from patient with heavy chain disease. Most plasma cells had markedly dilated rough endoplasmic reticulum cisternae containing densely staining material (A). Whorled configurations of the endoplasmic reticulum were present in many plasma cells (arrow). Other plasma cells (B) had a more normal appearance with flattened endoplasmic reticulum. x 7800.

Biopsy of a supraclavicular lymph node showed loss of normal nodal architecture with a diffuse infiltration of plasma cells and eosinophils. The peripheral nodal sinuses were obliterated, but the process did not extend into the surrounding fat. The plasma cells were occasionally immature and binucleate. Immunoblastic lymphocytes and histiocytes were also present. Amyloid was not present. Electron microscopy (Fig. 2) showed that most plasma cells had markedly dilated cisternae of rough endoplasmic reticulum containing electron-dense proteinaceous material, and many of the cells contained whorled configurations of rough endoplasmic reticulum. The light- and electron-microscopic interpretation of this process was that it was a malignancy of the lymphocytic-plasmacytic series. The nature of the infiltrate in the marrow and lymph node was similar to that described in several other cases of gamma heavy chain disease, although there are no consistent pathologic changes reported in this disease. The electron-microscopic appearance of the plasma cells was not distinctive as compared to other plasma cell proliferative processes; however, the presence of whorled configurations of the endoplasmic reticulum was unusual.

Marrow biopsy 1 yr after the beginning of therapy showed reduction of cellu-
Fig. 3. Immunoelectrophoresis of the patient’s admission serum sample. The slow paraprotein reacts with antiserum to the Fc fragment of IgG but not with anti-κ or anti-λ sera. In the bottom slide, the paraprotein reacts with antisera prepared against the dimeric F(\(ab'\))\(_2\) (upper trough) and monovalent Fab' (lower trough) fragments of pepsin-digested IgG.

Immunoelectrophoresis of Serum and Urine

Figure 3 shows the immunoelectrophoretic analysis of a 1:5 dilution of the patient’s serum. A very slow migrating arc was seen which reacted with anti-whole IgG and anti-Fc sera (top panel) and with anti-Fab’ and anti-F(\(ab'\))\(_2\) sera (bottom panel), but not with anti-κ or anti-λ sera (center panel), and not with antisera specific for α- and μ-chains (not shown). The paraprotein also reacted with anti-Fab, anti-Fd sera, and with anti-F(\(ab'\))\(_2\) sera absorbed to remove reactivity with κ and λ light chains. These latter antisera are directed against antigenic determinants on the Fd and perhaps also the hinge portion of the IgG heavy chain (Fig. 4). The reactivity to antigenic determinants on the Fd fragment of IgG is an unexpected finding, since previously described gamma heavy chain paraproteins have not reacted with antisera directed against Fab.

Fig. 4. Schematic representation of an IgG\(_1\) immunoglobulin molecule. The sites of proteolytic cleavage by papain or pepsin are indicated by the solid arrows. The hinge region is indicated by the wavy line.\(^{16,17}\)
Fig. 5. Alkaline starch-gel electrophoresis of the patient's serum samples obtained over an 18-mo interval. Normal serum (NS) is shown for comparison. Note the slow IgG paraprotein, the paraprotein aggregates, and the reduced albumin concentration in the initial pretreatment sample (October 23, 1972). The paraprotein was barely detectable, and the albumin concentration was approaching normal 25 days later. The sera have exhibited no detectable abnormalities since December 28, 1972.

(Fd plus light chains), or against the carboxyl end of the Fd fragment including the hinge region. The CAL paraprotein belongs to the gamma, subclass (Kindly determined by Dr. E. Franklin). Trace amounts of paraprotein were identified in the urine only after 600-fold concentration. No urinary free light chains were detected even in this concentrated sample. These studies define the paraprotein as free gamma heavy chain on the basis of its reactivity with anti-Fc but not with anti-light chain sera, despite the unusual features of slow mobility and reactivity with anti-Fd serum.

Change in Serum Paraprotein Following the Institution of Therapy

Figure 5 shows alkaline starch-gel electrophoresis of the patient's sera at various times during his clinical course. Three weeks after therapy was begun, the paraprotein was barely detectable, and by 2 mo it could not be seen. Several abnormal bands in addition to CAL protein were seen in the slow gamma region of the starch-gel electrophoresis on the initial and 3-wk serum samples. These were judged to be aggregated paraprotein, since on acid 8 M urea starch-gel electrophoresis of the second Sephadex G-200 peak they had the same mobility as the paraprotein. Furthermore, the bands were not seen on SDS-polyacrylamide gel electrophoresis of the purified paraprotein. Similar unusual bands were described on starch-gel electrophoresis of serum from the first patient reported to have heavy chain disease and on agarose-gel electrophoresis of serum from the seventh reported case. Seligman has attributed such electrophoretic heterogeneity to either a high carbohydrate content or to N-terminal heterogeneity, both of which have been identified in previously studied gamma heavy chain polypeptides.

Sodium Dodeyl Sulfate-Polyacrylamide Gel Electrophoresis

Two bands, one of apparent molecular weight 95,000 and a less prominent band of apparent molecular weight 49,000, were seen in gels of the unreduced
purified paraprotein. However, after reduction of disulfide bonds with 2-mercaptoethanol, only a single band of apparent molecular weight 49,000 was seen. Thus, CAL protein appears to exist as a dimer linked by disulfide bonds. The molecular weight of the monomer approaches the molecular weight of normal monomeric gamma heavy chain of 55,000.

DISCUSSION

Our patient presents several features previously described in gamma heavy chain disease, including lymphadenopathy, fever, weight loss, anemia, and marked eosinophilia. However, bone marrow involvement by plasma cells to the degree seen in our patient has only been described once previously and that in a patient who died 5 wk after diagnosis.

Unlike most patients with gamma heavy chain disease, CAL had only trace quantities of paraprotein in his urine. This finding may possibly be attributed to the unusually large molecular weight of the gamma heavy chain polypeptide.

CAL has exhibited multiple autoimmune phenomena. Manifestations of autoimmune diseases in previously reported cases have included rheumatoid arthritis, Sjögren’s syndrome, myasthenia gravis, systemic lupus erythematosus, discoid lupus erythematosus, hemolytic anemia, and thyroid tumor or thyroiditis. Our patient presented with a positive antiglobulin test for C3d coating of his red blood cells in the absence of obvious hemolysis, a cutaneous vasculitis, and low serum complement levels. The latter two phenomena have not been described previously in gamma heavy chain disease. Despite rapid response to therapy in all other parameters, the positive antiglobulin reaction has persisted, and both Raynaud’s phenomenon and keratoconjunctivitis sicca have developed.

Not all patients with gamma heavy chain disease have had an identifiable malignant process, and response to treatment has been extremely variable. Well-documented, long-lasting, complete remissions in patients with malignant disease have been obtained in single isolated cases with local radiation (8 mo +), prednisolone (6 yr +), MOPP for two courses followed by cytoxan maintenance (4 yr), and cyclophosphamide with prednisone (2½ yr +) (present case). The patient treated with MOPP died September 1974 of a myocardial infarction. Our patient has had a sustained complete response to relatively conservative doses of pulsatile cyclophosphamide and prednisone. Despite suggestions in the literature that alkylating agents are generally not beneficial, of the five patients (including our own) who have received an adequate trial of combination chemotherapy which included alkylating agents and prednisone, two have had long-term (>2 yr) complete remissions, and two have had partial remissions of short duration. One seriously ill patient received a course of nitrogen mustard and prednisone but continued to deteriorate rapidly and died with extensive tumor infiltration 5 wk after the initiation of chemotherapy.

Several types of structural abnormality have been described in the IgG molecule of gamma heavy chain disease. All include a deletion of the Fd and/or hinge regions of the gamma heavy chain. The usual pattern of immunoreactivity of these proteins includes reactions with anti-IgG and anti-Fc sera.
but not with sera directed against light chains, Fab, Fd, or the heavy chains of IgA or IgM. Unexpectedly, the CAL protein reacted with anti-Fab, anti-Fd, and anti-F(ab\(^2\))\(_2\) sera. These results have not been found in previously described proteins from patients with gamma heavy chain disease and suggest that a significant part of the Fd portion of CAL protein is intact. Further support for this hypothesis is derived from the molecular weight of CAL, which is 95,000 for the dimeric structure and 49,000 after reduction. These molecular weights are only slightly lower than those of the intact heavy chain dimer and monomer. Most previously described gamma heavy chain paraproteins have had molecular weights of 25,000-40,000, with the exception of the hinge deletion paraprotein Mcg, which has a molecular weight of 45,000. However, CAL does not appear to have a hinge deletion since its two polypeptide chains are covalently linked by disulfide bonds, bonds which are clustered in the hinge region in IgG heavy chains.\(^{16}\)

Further definition of the defect in the CAL protein awaits sequence analysis which is currently in progress in the laboratory of Dr. E. C. Franklin.

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