Further studies on an eleventh case of heavy (Hgamma1) chain disease–clinical studies

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Abstract

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FURTHER STUDIES ON AN ELEVENTH CASE OF HEAVY (Hγ1) CHAIN DISEASE
— CLINICAL STUDIES —

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Received for publication, January 17, 1975

Abstract: An eleventh case of heavy (Hγ1) chain disease (Yok), surviving for more than 10 years and still living showed clinical and pathological findings similar to cases described in the past. The patient was given only glucocorticosteroids, ACTH, antibiotics and gamma globulin, as specific drugs. Precipitation arcs besides the major ones formed by albumin and Fc fragment were disclosed by immunoelectrophoresis. The existence of these minor components were confirmed with antigen-antibody crossed electrophoresis and Sephadex G-200 gel filtration. They did not form precipitation arcs with the other antigens available and they appeared in the same fractions of IgG on gel filtration suggesting their having higher molecular weight than the major ones. In addition to these findings, the clinical course of the patient is described.

IgG-heavy chain disease or “Franklin’s disease” is characterized by the presence in serum and urine of a protein associated structurally with the Fc fragment of IgG. To date, more than 20 cases have been reported (1-14). Studies of these proteins found to be characteristic of the disease have suggested that all patients with this disease seem to produce an abnormal protein which consists of the Fc fragment and piece of the Fd fragment (15-24). It was shown in the first case report that extracellular catabolism is not responsible to the existence of anomalous protein in patient’s serum (1). Structural studies on these proteins suggested that the aberrant protein is a product of internal deletion (19-24).

Alloalbuminemias (25) and transient para-albuminemia (26) were reported in the past. Minor precipitation arcs identified as albumin and Fc-fragment
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appeared in both serum and urine of the eleventh case of heavy (H-1) chain disease. The studies were started to elucidate the presence of proteins.

**METHODS**

The patients serum and urine were pooled and stocked in a frozen state for special studies. Urinary protein was concentrated by lyophilization.

Paper electrophoresis of serum and urinary proteins was performed with Toyo Model SE-2 paper electrophoresis system (27). Immunoelectrophoretic analysis was performed by the method of Scheidegger (28). Moving boundary electrophoresis was carried out in a Hitachi Model HTD-1 apparatus (29). Analytical polyacrylamide electrophoresis was performed according to Maizel (30). Antigen-antibody crossed electrophoresis was performed according to Laurell (31). The immunoglobulins and their molecular subunits were identified by specific monovalent anti-sera obtained commercially. The quantitation of serum protein was performed by using the (Behring Werke AG, Marburg, Lahn, Germany and Handai Biken. Suita, Osaka, Japan) antibody-in agar radial diffusion method (32).

For ultracentrifugal analysis, a Spinco Model E and a Hitachi UCA-1 instruments were employed at a speed of 51,200 or 60,000 r.p.m. and at the temperature of 19.5-20.5°C. Both moving boundary electrophoretic and ultracentrifugal analyses were performed in buffers of sodium barbiturate, pH 8.6 and sodium phosphate, pH 7.2 respectively, and of 0.1 ionic strength. Sodium chloride made up 80% of ionic strength of these buffers. The protein samples were dissolved in the buffers and dialysed against the same buffer overnight at 4°C.

Ten ml of the serum was applied to a Sephadex G-200 column (2.0 × 90cm) which had been equilibrated with 0.14 M NaCl containing the buffer. A flow rate of 10ml per hour was maintained and fractions of 5ml were collected. Aliquots were analysed for protein by phenol reaction (33) by using human albumin as a standard. They were pooled into five fractions, lyophilized, dissolved in and dialyzed against the barbiturate buffer. The protein concentration of each fraction was made up to 2gm per cent with the buffer.

**CASE HISTORY**

A thirty-two year-old Japanese house-wife was seen on March 4, 1963 at Mitoyo General Hospital when she had dizziness with nausea and continuous tinnitus which led to the diagnosis of Ménière's syndrome. Physical examination disclosed hepatomegaly 8 cm below the costal margin. Laboratory examination revealed red cell sedimentation rate of 17mm in the first one hour; albumin/globulin ratio, 1.26; urinary protein, 1-plus without Bence Jones protein. Four months later, on July 29, she was admitted to the hospital for pulmonary tuberculosis. The patient also had episodes of severe attack of bronchial asthma from January to May in 1964. In the course of her
hospitalization, laboratory examination performed on June 12, 1964 showed
white blood cell count of 4,900 per cu mm with neutrophils of 11 per cent,
eosinophils of 8 per cent, monocytes of 4 per cent, and with lymphocytes of
77 per cent. The cause of relative lymphocytosis and eosinophilia was not
studied further but should be noted. She became completely well after 55
weeks hospitalization and was discharged on August 17, 1964.

Her medical history also included severe pneumococcal pneumonia in
1958, keratitis in 1962, anal hemorrhoidectomy in 1964, duodenal ulcer in
May, 1965 and an appendectomy in May, 1967. An exploratory laparotomy
performed on March 21, 1968 under diagnosis of a perforated duodenal ulcer
revealed hepatic enlargement with a soft round edge and with normal color.
The right lobe reached to the large pelvic cavity whereas the left lobe was
walnut-sized. Splenic enlargement was also found to be 10 cm below the
costal margin. Several numbers of small walnut sized lymph nodes of a pink
color were observed in the abdominal cavity and one of them was extirpated
for pathological examination. The cause from which this patient was suffering
seriously could not be found by the operation.

Serum paper electrophoresis performed on April 4, revealed an abnormal,
homogeneous band in the fast gamma globulin mobility range which was
quantitated to be 4.2 gm per cent. A myelogram showed 36 per cent of im­
matured plasma cells. No bony lesion was found on roentgenograms and
serum calcium was 9.2 mg per cent. Serum uric acid was 8.7 mg per cent.
These results led to a diagnosis of an early stage of multiple myeloma. On
July 5, 1968 she was referred to First Department of Internal Medicine, Oka­
yama University Medical School to perform further examinations and found
to be a Fc disease by clinical, hematological and immunological studies. A
report of these results was published (7).

A liver biopsy performed on July 26 revealed only a mild cellular in­
filtration in Glisson's disc. A roentgenological examination of gastroin­
estinal tract performed on November 26, 1969 revealed several numbers of isolated,
round shadows in the ileocaecal region which could be lymph follicles. An
artificial abortion was performed on May 7, 1971. Histological study of the
embryo and placenta showed normal tissues. She had episodes of severe
paronychias in January and a hordeolum in February, 1971. Ovarial dysfunc­
tion began to annoy her from this period. Oral candidiasis and a large fur­
unculosis in the lower abdominal wall attacked her in July, 1973. A toxic
hepatitis induced by chloramphenicol appeared in the summer which subsided
within two weeks. Many warty eruptions diagnosed as verruca plana by histo­
logical examination appeared in September 1973. There was no edema on
the soft palate and uvula. Lymphadenopahy was noted for the first time in
May, 1969.

The patient continued to remain under medical supervision. She had been admitted 9 times for 52 weeks in the six years mostly because of several attacks of pneumonia, bronchial asthma and anemia. The patient had been treated with ampicillin, cephalosporins, ACTH and corticosteroids during that period. The latest laboratory examination conducted on December 10, 1974 showed no essential change in the results.

RESULTS

An abnormal precipitation arc identified as albumin appeared in both the serum and the urine since November 1970 (Fig. 1). This component was still present in the serum collected on December 15, 1974. The abnormal arc did not appear when anti-sera against β-lipo-protein, α2-macrogloblin, haptoglobulin, ceruloplasmin, β1-A, β1-C, prealbumin, hemopexin, transferin, α1-lipoprotein, β2-glycoprotein, α1-antiglycoprotein, α1-anti-trypsin, and α2-HS were used separately. Another arc precipitated with anti-Fc serum (Fig. 1) was observed which was not made by anti-sera against Fab, Fd and light chains of human immunoglobulins. These arcs were also confirmed in a freshly collected serum.

The patient’s serum was subjected to a gel filtration with Sephadex G-200 and divided to five fractions as indicated in the elution diagram (Fig. 2).
They were lyophilized, dissolved and made up to 2g/dl of protein concentration. Each protein fraction on acetate membrane electrophoresis was quantitated (Table 1). Immunoelectrophoretic patterns of the fractions were shown in Fig. 3. Both anti-Fc and anti-albumin made precipitation arcs separately in the same region which were clear in fractions 2 and 3. Two peaks formed by anti Fc anti-serum appeared when the patient’s serum was subjected to antigen-antibody crossed electrophoresis (Fig. 4a). The minor peak could not be found by acrylamide electrophoresis or free electrophoresis when compared

![Fig. 2. Elution diagram of Sephadex G-200 gel filtration. Tubes with bars were pooled separately.](image)

| Table 1 Analysis of the fractions obtained by gel filtration |
|-----------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| total protein mg             | Alb             | protein fractions | %              |                 |                 |                 |                 |
|                             |                 | $\alpha_1$       | $\alpha_2$     | $\beta$         |                 |                 |                 |
| starting material            | 640             | 38.1             | 2.6            | 6.7             | 3.5             | 48.8            |
| peak 1.                      | 39              | 0                | 16.0           | 81.7            | 2.3             | 0               |
| peak 2.                      | 27              | 5.3              | 41.2           | 19.3            | 4.1             | 25.5            |
| peak 3.                      | 26              | 12.5             | 24.5           | 7.3             | 6.9             | 48.8            |
| peak 4.                      | 234             | 26.5             | 1.5            | 2.9             | 5.2             | 63.7            |
| peak 5.                      | 312             | 50.0             | 1.8            | 0.7             | 3.7             | 43.5            |
Fig. 3. Immunoelectrophoretic patterns of the serum. *s*: original serum. 1-5: fractions from Sephadex G-300 gel filtration. *a-Fc*: anti-Fc fragment. *a-Alb*: anti-albumin. Note arcs precipitated sharply with both anti-sera are seen in the fractions 2 and 3.

to normal one. Two minor peaks were formed by anti-albumin anti-serum by the method (Fig. 4b). One of these migrated in the same region of the one formed by anti-Fc anti-serum. These minor components could not be found out by free electrophoresis, acrylamide electrophoresis or ultracentrifugal analysis.

DISCUSSION

As noted by TERRY and EIN (22), the patient was also affected by active tuberculosis preceding the diagnosis of gamma heavy chain disease. The beginning of disease in this case was insidious and must be earlier than June 12, 1964 when relative lymphocytosis and eosinophilia were found. The symptomatic duration of disease in this case is over ten years and the patient is still surviving. The exact diagnosis of the case was established for the first time on May 14, 1968 as an early stage of myeloma without skeletal
Fever and severe abdominal pain were the initial manifestations. Hepatosplenomegaly and localized lymphadenopathy were found at the time of exploratory laparotomy performed on March 21, 1968. Hepatomegaly was noted for the first time on March 4, 1963 and has remained in the same size throughout the course of disease while spontaneous regression and progression of splenomegaly have been observed. Lymphadenopathy was not so significant and is localized only in the neck and the inguinal regions. Palatal edema was absent. Severe lobar pneumonia attacked her at least three times. She has been suffering by persistent bronchial asthma and low grade fever in the absence of overt infection.

Low grade normocytic, normochromic anemia and thrombocytopenia were noted at the time of initial studies and progressed gradually in the course
of disease. The peripheral blood smears showed leukopenia with relative lymphocytosis and a slight eosinophilia at the beginning of disease. Erythrocyte sedimentation rate remained in normal range regardless of anemia and hypoalbuminemia. Hyperuricemia was also present whereas azotemia and hypercalcemia were absent. Renal function was not impaired despite the proteinuria. The concentration of serum protein was within normal limits.

Extra minor bands adding to the major ones of Fc fragment and albumin were observed by immunoelectrophoresis. These were confirmed by antigen-antibody cross electrophoresis. They made precipitation arcs separately suggesting molecules different from each other and did not react with other antisera against human serum components including Fab, Fd and light chains of the human immunoglobulins. Studies on Sephadex G-200 gel filtration of the serum showed that they might have higher molecular weight than the major ones. They have existed in the serum for the last four years and might be called transient paraproteinemia.

The primary structures of Fc fragment found in the disease have been studied by several investigators concluding that they have internal deletion in their molecules. The physico-chemical properties and biosynthetic studies of the proteins found in the eleventh case will be described in the following papers.

Various therapies have been employed in the treatment of the disease including local radiation to spleen and lymphnodes (1, 2, 13), alkylating agents (1, 2, 14), and glucocorticoides (7). In the eleventh case, a very good response to prednisolone was observed. She is still alive and continues to remain under medical supervision as an outpatient.

Acknowledgements: We wish to express deep appreciation to Drs. K. Kosaka and Y. Shimada for the guidances, and also to Dr. T. Tsuji for his encouragements throughout of this study.

We are also indebted to Drs. H. Shigemoto and H. Mukuno for providing surgical data of an exploratory laparotomy performed on March 21, 1968 and of an artificial abortion performed on May 7, 1971, respectively, and to Drs. T. Izawa, H. Miki, H. Danjo, K. Oka, Y. Mizuno, H. Sato, K. Hata and G. Yamada, for providing the clinical informations during admission in Okayama University Hospital. In pathologic studies Drs. M. Hamazaki, H. Sanada, Y. Ohta, T. Kihara, T. Kobayashi and Y. Kitajima should be acknowledged for their advices. We also wish to express our thanks to Mr. Y. Ishii, Mrs. H. Tsuchida, Mr. S. Katsuura, Mr. H. Miyai, and especially to Mrs. M. Ishii for their excellent technical assistances, to Mr. M. Tsuji for photographic assistance and to Miss. T. Goda for help in preparing the manuscript.
Heavy chain disease, clinical studies.

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Produced by The Berkeley Electronic Press, 1975


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