Human IgA$_1$ Half-Molecules: Clinical and Immunologic Features in a Patient With Multiple Myeloma

By Ikunosuke Sakurabayashi, Kohjin Kin, and Tadashi Kawai

Abnormal IgA$_1$, half-molecules consisting of one heavy and one light chain were found in a patient (N.N.) with typical multiple myeloma. The serum and the urine of this patient contained both 7.0S and 3.9S IgA myeloma proteins. The IgA half-molecules (3.9S) were found to have a molecular weight of 59,000 daltons and were composed of one $\alpha$, chain of about 40,000 daltons and one light chain of 22,000 daltons. Furthermore, enzymatic degradation suggested that the $\alpha$ chain of the N.N. half-molecules had a large deletion in its Fc portion. We suggest that its heavy and light chains were probably bound noncovalently, since the interchains connecting the heavy and light chains of these IgA half-molecules were easily dissociated with 1% SDS and 8 M urea. Cytologic studies identified at least two types of myeloma cells, and it is possible that half-molecule IgA production might result from mutation among the myeloma cells producing whole-molecule IgA.

Most patients with typical multiple myeloma carry various types of monoclonal immunoglobulins, and recently abnormalities in the molecular structure of these monoclonal immunoglobulins have been reported. These abnormalities include (1) heavy-chain disease proteins, (2) incomplete myeloma proteins,$^1$ and (3) half-molecule immunoglobulins.

Half-molecule immunoglobulin was first described by Potter and Kuff,$^2$ who reported of a half-molecule IgA consisting of one $\alpha$ heavy chain and one light chain in experimentally induced myeloma in mice. Abnormal half-molecule immunoglobulin of IgG-$\kappa$ type was reported first in man by Hobbs and Jacobs$^3$ in a patient with extramedullary soft-tissue plasmacytoma. Four more such patients have been detected subsequently.$^4$ $^7$

IgA half-molecules in humans were first described by Spiegelberg and Fishkin,$^8$ while four additional cases were reported later.$^9$ $^{12}$ Because the authors$^{12}$ have had the opportunity to study half-molecule IgA in a patient with IgA myeloma, this report will treat the structural abnormality of half-molecule IgA.

CASE REPORT

History. Patient N.N., a 78-yr-old Japanese female, was hospitalized in 1974 because of lumbago, hypertension, and shoulder pain. She had been exposed to radiation from the atomic bomb in Nagasaki at the age of 47 yr, and she had suffered a second-degree burn on her forehead. She complained of increasing pain in the lumbar and shoulder regions again at the end of 1975, at which time she was found to have proteinuria and hypergammaglobulinemia. She was readmitted to the hospital in mid-January of the following year.

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Physical examination. Patient N.N. was a normally developed although emaciated housewife. Significant findings included a 3 × 5 cm mass at the sternal end of the right clavicle and a 7 × 5 cm mass at the right tibia. There was also a 3 × 3 × 3 cm palpable nonpainful mass around the ileocecal region. Scattered rhonchi were audible over both lung fields, although the area of cardiac dullness was normal. The liver was palpable three fingerbreadths beneath the right costal margin, smooth and of firm consistency, and the spleen was also palpable (one fingerbreadth). Fundoscopy turned up stage-II hypertensive retinopathy (Keith-Wegener classification). Heart rate was 76 beats/min and regular, and blood pressure was 170/86 mm Hg. The remainder of the physical examination was within normal limits.

Laboratory data. Hemoglobin was 9.0 g/dl, hematocrit 27%, red cell count 286 × 10⁶/cu mm, reticulocyte count 1.1%, and white cell count 3200/cu mm. The differential white cell count revealed 1% metamyelocytes, 4% band neutrophils, 34% segmented neutrophils, 7% monocytes, 45% lymphocytes, 6% eosinophils, and 2% basophils. Serum total protein was 9.4 g/dl, and the serum electrophoresis showed a monoclonal γ fraction amounting to 43.1%. Blood urea nitrogen was 28.2 mg/dl, and serum creatinine was 5.3 mg/dl. Bone marrow aspiration revealed the presence of 36% pleomorphic plasma cells. Conversely, the megakaryocytes, as well as the erythropoietic and granulopoietic series, were below normal limits. Bence Jones proteinuria was also present. X-ray survey of the skeletal system revealed osteolytic punched-out lesions in the skull, bilateral humeri, radius, ulna, femur, tibia, and fibula.

Hospital course. The patient was treated for multiple myeloma with 10 mg prednisone and 100 mg cyclophosphamide (Endoxan) every other day, and was administered occasional γ globulin infusions and 200-ml blood transfusions. Trichloromethiazide (Fluitran) was also administered for hypertension. Although bone pain at the right clavicle and right tibia continued, the patient’s hospital course was that of gradual improvement.

MATERIALS AND METHODS

Serum proteins and concentrated urine were analyzed using the following procedures: cellulose acetate electrophoresis (barbital buffer, pH 8.6, 1/2 0.07), agar gel immunoelectrophoresis (Grabar-Williams), immunogel filtration (Sephadex G-200 superfine), and single radial immunodiffusion.

Half-molecule IgA was isolated from the patient’s serum and urine by means of Sephadex G-200 gel chromatography employing a 0.1 M Tris-HCl buffer at pH 8.6, containing 0.15 M NaCl (column size 2.5 × 80 cm), and cyanogen bromide (CNBr)-activated Sepharose 4B (Pharmacia Fine Chemicals) affinity chromatography coupled with anti-α chain antiserum (DAKO-immunoglobulins, Copenhagen, Denmark) according to the method of Cuatrecasas. That is, CNBr-activated Sepharose 4B was suspended in cold borate buffer (0.2M, pH 8.0) to which anti-α antibodies (rabbit) were coupled by incubating at 4°C for 24 hr. After a glass column (15 × 50 mm) was filled with the pretreated Sepharose 4B, 1 ml of sample was applied to the column. The IgA was eluted with 0.2 M glycine-HCl buffer at pH 3.0. The eluted protein was then neutralized and concentrated. Molecular weight was determined by 7.5% polyacrylamide gel electrophoresis (PAGE) containing 1.0% sodium dodecyl sulfate (SDS), as well as by analytical ultracentrifugation (MSE Centriscan 75, England) using a u.v. (280 nm) filter. Sedimentation was determined using a standard procedure to give an extrapolated Sₐw value, and sedimentation equilibrium was determined by the short-column method.

Reduction and alkylation with 0.75 M mercaptoethanol and 0.75 M monooiodoacetamide, high-temperature trypsin digestion (60°C) as described by Calvanico and Tomasi, papain digestion employing the method of Porter, and pepsin digestion using the method of Nisonoff and Wissler were also performed on the isolated IgA half-molecules.

Cytologic studies of the bone marrow were carried out using Wright-Giemsa stain, immunofluorescence technique (FITC labeled), and electron microscopy.

RESULTS

Cellulose acetate electrophoresis of the N.N. serum showed a relatively broad monoclonal band in the γ region, amounting to about 4 g/dl. The urinary pattern also showed a well-defined band in the γ region. Immunoelectrophoresis revealed IgA-λ type monoclonal immunoglobulin and λ Bence Jones protein (Fig. 1.). Serum and urine showed two distinct precipitin arcs of identical mobility against the anti-α
antiserum. In the immunogel filtration examinations, IgA molecules were found to consist of two different components, larger (7S) and smaller (5S) molecules that formed a spur against the anti-\(\alpha\) antiserum (Fig. 2). Single radial immunodiffusion using anti-\(\alpha\) antiserum produced only a single precipitin ring.

The serum IgA reacted against anti-\(\alpha_1\) antiserum showed a partial identity with two control IgA molecules and failed to react against anti-\(\alpha_2\) antiserum. Thus the N.N. monoclonal IgA was identified as belonging to the subclass IgA1.

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Fig. 1. Immunoelectrophoresis of N.N. serum (A) and 30-fold concentrated urine (B). N.N. serum shows double arcs against anti-\(\alpha\) antiserum having the same mobility (arrow). PS, N.N. serum; PU, N.N. urine; AWH$\$ anti-whole human serum; A.IgA, anti-\(\alpha\) chain antiserum; A.K\', anti-\(\kappa\) antiserum; A.L\', anti-\(\lambda\) antisemur.

Fig. 2. Immunogel filtration pattern of N.N. serum. N.N. serum shows two arcs with a partial identity against anti-\(\alpha\) chain antiserum (arrow), representing the IgA half-molecules and the larger IgA molecules (7S); a small amount of \(\lambda\) Bence Jones protein is also seen.
The low molecular weight IgA was isolated from the N.N. serum and urine using Sephadex G-200 column chromatography (Fig. 3) and purified by affinity chromatography employing CNBr-activated Sepharose 4B to which anti-α antiserum was coupled. This produced a single band on 7.5% PAGE (Fig. 5A). On analytical ultracentrifugation, the abnormal low molecular weight IgA showed a single protein peak with a sedimentation constant of 3.9S and a sedimentation equilibrium of 59,000 daltons, standing in sharp contrast to the 155,000 daltons for the

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**Fig. 3.** Elution pattern on NN serum (---) and urine (-----) on Sephadex G-200 column chromatography. Solvent was Tris-HCl buffer (pH 8.0). Half-molecule IgA was eluted at tube No. 34–42. (----).

**Fig. 4.** Ultracentrifugal analysis of sedimentation velocity ($S_{20w}$) of the purified half-molecule IgA isolated from N.N. urine by CNBr-activated affinity chromatography (conjugated anti-α chain antiserum). Ultraviolet pattern at 280 nm was taken at 10-min intervals after reaching the maximum speed (50,000 rpm, 200,000 G) by using a single-sector cell (Centriscan 75, MSE, England).
Fig. 5. Polyacrylamide gel electrophoresis of the purified IgA half-molecule in 1% sodium dodecyl sulfate and 8 M urea. (A) Unreduced 3.9S IgA without SDS and 8 M urea, (B) 3.9S IgA with 1% SDS, (C) reduced IgA half-molecule, and (D) reduction by heating (60°C) after incubation of 1% SDS and 8 M urea. IgA half-molecules easily dissociated into heavy and light chains. The top protein band of B and C shows intact half-molecule IgA. The second protein band of C and D shows α chains (40,000 daltons), and third protein band of C and D shows λ chains (20,000 daltons).

whole-molecule IgA of this patient (Fig. 4). The molecular weight of the reduced and alkylated purified low molecular weight IgA was estimated by SDS PAGE to be around 40,000 daltons for the α chain and 22,000 daltons for the λ chain, compared to 56,000 and 23,000 daltons, respectively, for the whole-molecule IgA (Fig. 5C). These were more susceptible to reduction and alkylation than the control IgA myeloma proteins, and they readily dissociated into a λ chain and an α chain upon being incubated at 60°C for 30 min in the presence of 1% SDS and 8 M urea (Fig. 5D). Following papain digestion, the molecular weight of Fab portion of the

Fig. 6. Immunofluorescent micrograph of N.N. myeloma cells after incubation with anti-α chain antiserum, showing many strongly stained cells and smaller faintly stained cells (arrows). × 1000.
Fig. 7. Electron micrograph of N.N. myeloma cells showing two types of cells, a larger cell with well-developed endoplasmic reticulum and a smaller cell with poorly developed endoplasmic reticulum. × 10,000.

low molecular weight IgA was ~37,000 daltons, and its high temperature trypsin-olysis antigenetically failed to show the Fc fragment or its equivalent portion.

Immunofluorescent study of the N.N. myeloma cells in the bone marrow, using FITC-labeled anti-α antiserum, demonstrated two distinct populations of IgA-producing cells: the major cells showed a typical strong green staining and the minor cells a faint orange-green discolored staining (Fig. 6).

Electron microscopy of the myeloma cells also revealed two distinct cell types, the major cells showing well-developed ribosomes, mitochondria, Golgi apparatus and focally dilated rough-surfaced endoplasmic reticula, and the smaller, minor cells showing poorly developed endoplasmic reticula and ribosomes (Fig. 7).

DISCUSSION

IgA half-molecules appear to be a very rare type of monoclonal immunoglobulins (M protein). To our knowledge, such immunoglobulins have been found in only four other patients, two of whom suffered from classical multiple myeloma, one from plasma cell leukemia, and one possibly from pulmonary tuberculosis.

Half-molecule IgA in human myeloma was first described by Spiegelberg and Fishkin, who showed that the heavy chain of the half-molecule IgA had a molecular weight of 46,500 daltons and that it was antigenically deficient of the Fc fragment. A case of plasma cell leukemia was described by Bernier and Berman, who also presented the heavy chain of half-molecule IgA as having a molecular weight of 45,000 daltons. Biewenga and Ban Loghem described a case of half-molecule IgA-producing multiple myeloma in which the molecular weight of
its heavy chain was determined to be 45,000 daltons and suggested that either a molecular deletion in the DNA coding for this portion of the chain had occurred or, alternatively, that a rapid intracellular degradation mechanism existed. The fourth case of half-molecule IgA was investigated by Kang and Shim, who, using SDS-PAGE, determined the molecular weight of its α chain to be 53,000 daltons and suggested the absence of the hinge region in the abnormal molecule. The heavy chain of half-molecule IgA (N.N.) in case described here was significantly smaller than that of the four-chain IgA (N.N.). The physicochemical data of human half-molecule IgA reported so far are summarized in Table I.

These findings agree with the data of Mushinski, who, using analytical ultracentrifugation, determined molecular weights in experimentally induced plasma cell tumor in mice as follows: the two-chain IgA molecule, 68,000 daltons, and the heavy chain from 6C protein, 46,000 daltons, in contrast with the ordinary 6A heavy chain, which was determined to be 54,000 daltons. Seki and Appella published similar data: the two-chain IgA molecules (47A), 63,000 daltons, and the heavy chain from 47A protein, 40,000 daltons, in contrast with 6A heavy chain, 55,000 daltons.

In a case of half-molecule IgG described by Spiegelberg and Heath, it was suggested that the carboxyl terminus of its γ chain was probably intact and that the deletion might have involved half-cystine residues in the CH3 domain that normally form an interchain disulfide bond.

Our data indicate that the molecular weight for the heavy chain of half-molecule IgA (N.N.) is about 14,000 daltons smaller than that of the heavy chain from whole-molecule IgA (N.N.) and that, antigenically, the smaller α chain appears to have a large deletion in the Fe fragment. The apparent deletion is assumed to be localized in the CH3 domain, since a deletion of about 14,000 daltons is almost equivalent to the size of the CH3 domain and would seem to be a likely cause of a lack of four-chain molecule formation.

Although Bernier and co-workers established the fact that the light-heavy disulfide bond of half-molecule IgA protein was present, our data indicate that the light-heavy interchain bond of the half-molecule IgA is noncovalent, inasmuch as the protein easily dissociated in 1% SDS and 8 M urea.

Regarding the biologic activity of half-molecule immunoglobulins, the following results were described by Spiegelberg: The unaggregated half-molecule failed to form a complex with Clq and failed to bind the Fe receptors of human lymphocytes, neutrophils, or monocytes or to guinea pig mast cells, while the aggregated half-molecule bound to complements as well as to each of the above-mentioned cell types. It was suggested that these half-molecules did not lack all the structures on the Fe fragment responsible for these biologic activities.

It is suggested that the half-molecule IgA, myeloma protein was formed by an IgA, mutant clone of cells that either evolved during the malignant transformation of the plasma cells or was an aberrant form of an IgA, line that existed as a component of the normal genome. In fact, the high frequency of mutation was demonstrated by experimentally producing mouse myeloma cell lines.

Half-molecule IgA secreted by patient N.N. cells had a large deletion of its heavy chain, and we suggest that one of two abnormal plasma cells lines, probably corresponding to the minor cells of discolored fluorescence with their scanty
Table 1. Clinical and Physicochemical Data of Patients With Half-Molecule IgA Immunoglobulin

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yr)/Sex</th>
<th>Class, Subclass</th>
<th>Type of L Chain</th>
<th>Mol. Wt. of HM Ig</th>
<th>$\delta_{\text{HM}}$</th>
<th>Mol. Wt. of HMâ€¢ chain</th>
<th>Mol. Wt. of HM L Chain</th>
<th>Deletion of H Chain</th>
<th>Genetic Marker</th>
<th>Mol. wt. of WMâ€¢ Chain</th>
<th>Clinical Diagnosis</th>
<th>Ref.</th>
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</thead>
<tbody>
<tr>
<td>1. V.L.A.</td>
<td>65</td>
<td>IgA₁</td>
<td>k</td>
<td>70,000</td>
<td>40,000–45,000</td>
<td>Fc fragment</td>
<td>Multiple myeloma</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Wal.</td>
<td>59/F</td>
<td>IgA₁</td>
<td>$\lambda$</td>
<td>70,000</td>
<td>4.05</td>
<td>45,000</td>
<td>23,000</td>
<td>CH₃ domain, 13,000</td>
<td>Normal A₂m(2)</td>
<td>58,000</td>
<td>Plasma cell leukemia</td>
<td>10, 23</td>
</tr>
<tr>
<td>3. Kø.</td>
<td>57/M</td>
<td>IgA₁</td>
<td>$\lambda$</td>
<td>4.55</td>
<td>46,500</td>
<td>Fc portion, 8,500</td>
<td>Multiple myeloma</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Cha.</td>
<td></td>
<td>IgA₁</td>
<td>k</td>
<td>75,000</td>
<td>53,000</td>
<td>Hinge region</td>
<td>Pulmonary tuberculosis?</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. N.N.</td>
<td>78/F</td>
<td>IgA₁</td>
<td>$\lambda$</td>
<td>59,000</td>
<td>3.95</td>
<td>40,000</td>
<td>22,000</td>
<td>Fc portion, 14,000</td>
<td>Multiple myeloma</td>
<td>55,000</td>
<td>This work</td>
<td></td>
</tr>
</tbody>
</table>

Mol. Wt., molecular weight (daltons); HM Ig, Half-molecule immunoglobulin; WMâ€¢ chain, $\alpha$ chain of whole-molecule IgA in patient serum.
endoplasmic reticula (ER) that were revealed under electron-microscopic examination, might be producing the abnormal heavy chain with a large amino acid deletion. This possibility is supported by the analysis of membrane-bound poly-somes\textsuperscript{26,27} in which rough ER of the smaller-sized cells was composed of 7 and 9 ribosomes, whereas that of larger-sized cells was composed of 7 and 13 ribosomes.\textsuperscript{28}

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