

Human Myeloma IgA Half-Molecules

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ABSTRACT A λ , IgA1 myeloma protein that formed two chain half-molecules was obtained from a patient who had typical multiple myeloma. His serum contained 1.3 g/100 ml of an IgA paraprotein of γ -1 electrophoretic mobility, his urine predominantly λ Bence Jones protein, and only small amounts of IgA paraprotein. Analytical ultracentrifugation of the isolated serum IgA protein showed 7.0S and 4.5S protein peaks but no IgA polymers. When the 7.0S and 4.5S protein peaks were tested with an anti-serum specific for α chain, both fractions were antigenically deficient compared to control IgA myeloma proteins but showed a line of identity to their F(ab')₂ fragments. The serum and 7.0S protein fraction showed double precipitin lines in IgA radial immunodiffusion plates and in immunoelectrophoretic analysis, one line being formed by the myeloma protein and the other by residual normal IgA. The myeloma protein did not form a precipitin line with antisera specific for the IgA Fc fragment. Sodium dodecylsulfate-urea-polyacrylamide gel electrophoresis demonstrated that both the 7.0S and 4.5S fractions of the myeloma protein consisted of covalently linked heavy and light chains, the 4.5S fraction being apparently the half-molecule of the 7.0S protein. The heavy chain had a mol wt of 46,500 daltons compared to 55,000 daltons for normal α chains. Reduction and alkylation in aqueous solutions resulted in dissociation of the 7.0S myeloma protein fractions into smaller units, probably half-molecules, suggesting that the noncovalent interactions between the α chains were substantially weakened or absent, presumably as a result of a deletion in the Fc portion of the α chain. The catabolic rates of the radio-

labeled 7.0S and 4.5S proteins in rhesus monkeys were similar to those of control IgA myeloma proteins; the excretion of protein-bound radioactivity of the IgA half-molecules into the urine was no greater than that of the 7.0S or of control IgA myeloma proteins. It is suggested that the myeloma IgA half-molecule is probably derived from an IgA1 mutant that is carried in the human genome and that it is unlikely a representative of a rare IgA subclass or an IgA 1 allotypic variant.

INTRODUCTION

IgG half-molecules consisting of one heavy and one light chain were first described as a normal component in sera of colostrum deprived piglets (1, 2). Subsequently, IgA half-molecules were detected in the urine of mice bearing mineral oil-induced plasmacytomas (3). Recently, several patients with myeloma who excreted IgG half-molecules into their urine have been described (4-6). Structural analysis of the murine and human half-molecules indicated that these immunoglobulins (Ig)¹ had a deletion in the heavy chain (7-9). The deletion caused a weakness or absence of the noncovalent interactions normally present between Ig heavy chains and apparently resulted in an incomplete assembly of typical four chain Ig. The "hinge" region containing the half-cystine residues forming the inter-heavy chain disulfide bonds, appeared to be normal in the single case of IgG half-molecules that was studied for this parameter (8). This explained why this patient's serum contained two chain half-molecules and small amounts of four chain Ig molecules that were produced by the same clone of tumor cells.

The human IgG half-molecules were antigenically deficient in the Fc fragment (8) and were detected by their abnormal reaction in IgG specific radial

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¹ Abbreviation used in this paper: Ig, immunoglobulin.

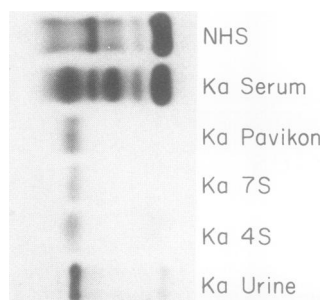


FIGURE 1 Cellulose acetate electrophoresis of normal human serum (NHS), Ka serum, and concentrated urine, and the isolated IgA fractions obtained by Pevikon block electrophoresis and Sephadex G-200 gel filtration (Ka 7S and Ka 4S).

immunodiffusion plates (6). The patient's serum showed a double precipitin ring, one formed by the antigenically deficient myeloma protein and the other by the residual normal IgG. We describe in this report an IgA myeloma protein that formed half-molecules which simulated IgG half-molecules in both its antigenic and structural features.

Clinical history. Patient Ka, a 57 yr-old man was admitted to a hospital on 7/1/74 because of a traumatic fracture of the right foot. He had back pain for 4 mo before admission which had intensified 10 days before admission and was not relieved by Butazolidin and "pain pills". 3 days before admission, the patient became apathetic and confused. On admission the patient was dehydrated, confused, and disoriented and had a swollen and discolored right foot. The pertinent laboratory data were: hemoglobin, 9.2 g/100 ml; calcium, 17.4; phosphorus, 4.5; blood, urea, nitrogen, 107; and uric acid, 15.5 mg/100 ml. A skeletal survey demonstrated radiolucencies in the skull, iliac wings, inferior pubic rami, right greater trochanter, and anterior body of the fifth cervical vertebra. A compression fracture of the first lumbar vertebra and traumatic fractures of the second and third metatarsal bones were present. A bone marrow aspiration failed to yield cells, but a bone marrow biopsy showed an anaplastic multiple myeloma. Prednisone (200 mg/day) and saline therapy reversed the hypercalcemic state and the patient became alert and his renal status improved. The serum electrophoresis values were: total protein, 6.5; albumin, 3.1; α_1 , 0.2; α_2 , 0.7; β , 2.2; and γ , 0.2 g/100 ml. Quantitative immunoglobulins were: IgG, 280; IgA, 2,900; and IgM, 44 mg/100 ml. Alkeran therapy was instituted on 7/13. Serum and urine specimens were submitted to our laboratory on 7/16; the serum electrophoresis values were: total protein, 7.1; albumin, 2.7; α_1 , 0.4; α_2 , 1.1; β , 1.0; γ_1 , 1.3; and γ_2 , 0.6 g/100 ml. The urine contained 88 mg protein/100 ml and

electrophoresis demonstrated a β mobility paraprotein of 62 mg/100 ml. Immunochemically, the serum and urine showed λ , IgA1, and λ light chains, respectively. The patient was discharged from the hospital on 7/22 but readmitted on 7/28 because of recurrence of the hypercalcemic state. The serum electrophoresis values on 7/29 were: total protein, 8.6; albumin, 2.8; α_1 , 0.3; α_2 , 0.7; β , 4.6; and γ , 0.2 g/100 ml. The patient expired on 8/8/74. An autopsy was not performed.

METHODS

A single specimen of serum and urine obtained 23 days before the patient expired was available for study. The urine was dialyzed against 0.01 M phosphate buffer, pH 8.0, and lyophilized and dissolved in phosphate buffered 0.15 M NaCl, pH 7.0 (saline). The myeloma protein was isolated from the serum by Pevikon block electrophoresis followed by Sephadex G-200 gel filtration. (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) Protein concentrations of the isolated IgA fractions were determined by optical density employing an extinction coefficient of $E_{280 \text{ nm}, 1 \text{ cm}^1\%} = 15$.

IgA myeloma proteins isolated from serum of other patients by Pevikon block electrophoresis and Sephadex G-200 gel filtration served as controls. They were digested with pepsin (10) for 6 h at 37°C to prepare $F(ab')_2$ fragments. A longer digestion period of 18 h resulted in almost complete digestion of the IgA proteins. The $F(ab')_2$ fragments were isolated from undigested IgA by Sephadex G-200 gel filtration. The undigested IgA separated poorly from the $F(ab')_2$ fragments and the $F(ab')_2$ preparations contained traces of undigested IgA. Reduction and alkyla-

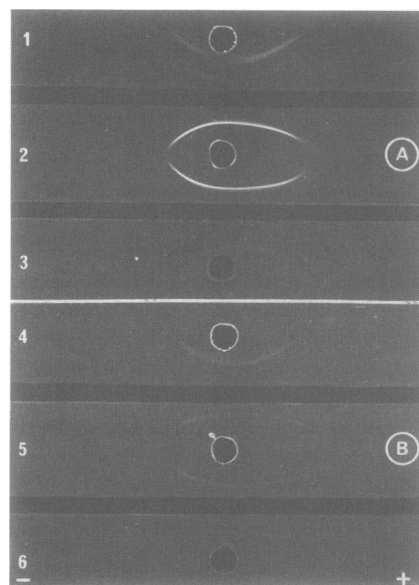


FIGURE 2 Immunoelectrophoresis of normal serum (2, 5), and Ka serum (1, 4), and 50-fold concentrated urine (3, 6) employing specific antisera to α chain (A) and IgA Fc fragment (B).

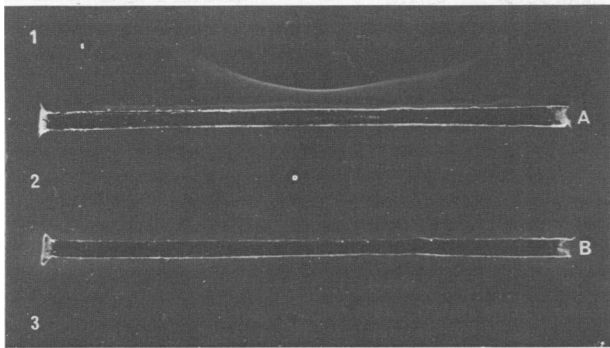


FIGURE 3 Immunoelectrophoresis of 10-fold Ka concentrated urine (wells 1 and 3) employing antisera to λ chains (A) and to α chains (B). Well 2 was left empty.

tion with 0.02 M dithiothreitol and 0.05 M iodoacetamide, respectively, were performed as previously described (8). Anti-IgA antisera were made in goats by injection of three different IgA1 myeloma proteins in complete Freund's adjuvant. The antisera were rendered specific for α chains by absorption with cord serum, IgM macroglobulins, and κ and λ Bence Jones proteins. One antiserum was made specific for the Fc fragment by absorbing an aliquot with $F(ab')_2$ fragments of κ and λ IgA myeloma proteins. Radial immunodiffusion plates were prepared with the anti- α specific antiserum according to Mancini et al. (11). Two bullfrog antisera specific for IgA1 and IgA2 were the gift of Dr. John Coe.

A Beckman microzone electrophoresis apparatus was used for cellulose acetate electrophoresis. Analytical ultracentrifugation and polyacrylamide (10%) gel electrophoresis in 1% sodium dodecylsulfate, and 8 M urea of the native and reduced proteins were performed as previously described (8). The $S_{20,w}$ rate was calculated by standard methods but no correction for concentration dependency could be made because of paucity of material.

The turnover studies were performed in three rhesus monkeys according to previously described protocols (12, 13). One monkey each was injected i.v. with a ^{125}I -labeled control IgA myeloma protein and either the ^{131}I -labeled 7.0S or 4.5S IgA fraction and the third monkey received only ^{131}I -labeled 4.5S protein. Blood was obtained by venipuncture and the collected urine was cleared by centrifugation. The total and protein-bound excreted radioactivity was determined daily and expressed as percent of the average intravascular pool of that day.

RESULTS

Electrophoretic, antigenic, and ultracentrifugal analyses. The analysis of the Ka serum and concentrated urine by cellulose acetate electrophoresis is shown in Fig. 1. The serum showed a relatively broad monoclonal band of γ_1 electrophoretic mobility and the urine showed a sharply delineated slightly more anodal monoclonal band. The concentration of the myeloma protein in the serum was 1.3 g/100 ml and the monoclonal protein in the urine was 62 mg/100 ml. Immunoelectrophoresis demonstrated a λ , IgA protein in the serum, and large amounts of λ chain

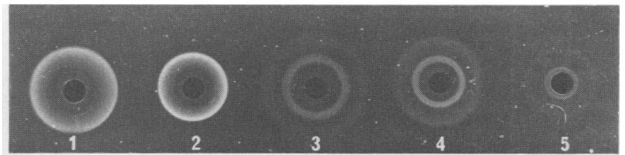


FIGURE 4 Radial immunodiffusion employing an anti- α chain antiserum. Wells 1 and 2 contained a control IgA myeloma protein at 6 and 3 mg/ml, respectively. Wells 3 and 4 contained Ka serum diluted 1:5 and 1:10. Well 5 contained 50-fold concentrated urine.

in the urine. The antigenic reaction with the anti- α anti-serum was abnormal. It showed a double precipitin arc with the serum of the patient (Fig. 2). The concentrated urine also reacted with the anti- α antiserum by showing a single arc of a more anodic mobility than the arc seen with the anti- λ antiserum (Fig. 3). When an antiserum rendered specific for the IgA Fc fragment was used, only a single precipitin line was observed with the Ka patient serum and no reaction with the urine. Double precipitin lines were also observed in radial immunodiffusion plates with both serum and urine of the patient (Fig. 4) but not with normal sera or other IgA myeloma proteins of κ or λ light chain type. Three different commercially available IgA radial immunodiffusion plates also showed double precipitin rings with serum Ka. Because of the abnormal antigenic reactions the IgA myeloma protein was isolated by Pevikon block electrophoresis. The ultracentrifuge analysis of the isolated IgA of patient Ka is shown in Fig. 5 and compared to another similarly isolated IgA myeloma

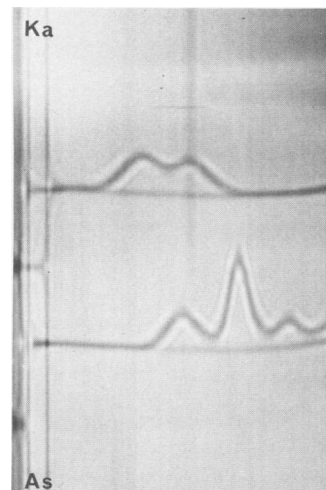


FIGURE 5 Ultracentrifugal analysis of IgA fractions isolated by Pevikon block electrophoresis of Ka and control patient (As). Picture was taken 48 min after reaching two-thirds of maximum speed (52,600 rpm) by using a double sector cell.

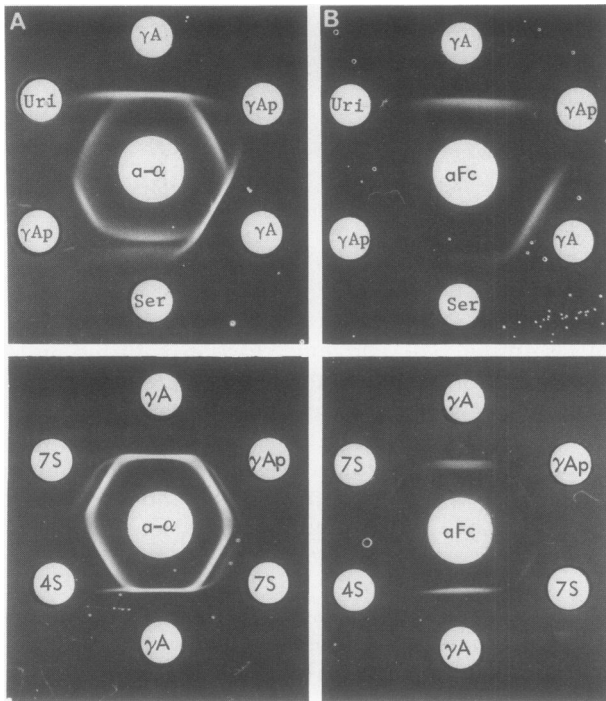


FIGURE 6 Double gel diffusion employing anti- α and anti-IgA Fc fragment specific antisera. γ A: control IgA myeloma protein and its F(ab')₂ fragment obtained by pepsin digestion (γ Ap). Ser (serum) and uri (urine): serum diluted 1:20 and 50-fold concentrated urine of Ka. 7S and 4S: 7.0S and 4.5S fraction of Ka protein. All isolated protein preparations were tested at 1 mg/ml.

protein. The protein Ka sedimented in two peaks, one having a sedimentation rate of 7.0S and the other of 4.5S. The control IgA myeloma analyzed simultaneously in the double sector cell showed three major protein peaks of sedimentation rates of 7.2, 9.9, and 13.0S.

The 7.0S and 4.5S IgA proteins of patient Ka were isolated by Sephadex G-200 gel filtration. Both showed monoclonal bands of an electrophoretic mobility similar to the myeloma protein band in the serum (Fig. 1). When the isolated 7.0S and 4.5S myeloma protein fractions were analyzed by double gel diffusion employing antisera to α chain, both the 7.0S and 4.5S fractions were antigenically deficient when compared to control IgA myeloma proteins as indicated by a spur formation (Fig. 6). The 7.0S and urinary protein that reacted with the anti- α chain antiserum showed a line of identity to pepsin digested IgA myeloma proteins. A second weaker precipitin line formed with the 7.0S fraction and diluted serum. This line showed a line of identity to the spur formed by the control IgA myeloma protein and it most likely represented residual normal IgA. The pepsin digested IgA also showed a double

precipitin line and the second faint line was due to traces of undigested myeloma protein. The antiserum specific for the IgA Fc fragment reacted strongly only with the control IgA myeloma protein. The faint precipitin lines observed with the diluted serum, the 7.0S fraction and the IgA F(ab')₂ fragments represented reactions with the residual normal and undigested IgA in these preparations.

The isolated 7.0S Ka protein produced weak double precipitin lines with an anti-IgA1 antiserum and failed to react with an anti-IgA2 antiserum.

Typing for genetic markers of the half-molecule, kindly performed by Dr. Erna van Loghem, showed absence of α 2 and presence of α and α 1 determinants and clearly n A2m(2), the isoallotype occurring on IgA1 and A2m(1) proteins. The Gm-Km haplotype is prevalent in Caucasians: γ 1,f; γ 2,n; γ 3,b⁰,b¹,b³,b⁵; α 2,1 and κ ,3.

Structural analyses. The Ka Pevikon, 7.0S and 4.5S fractions were analyzed in both native and reduced states by polyacrylamide gel electrophoresis in 1% sodium dodecyl sulfate (Fig. 7). The native Pevikon fraction showed two major and one minor band. The two major bands corresponded to the 7.0S and 4.5S fractions and the minor band had a mobility

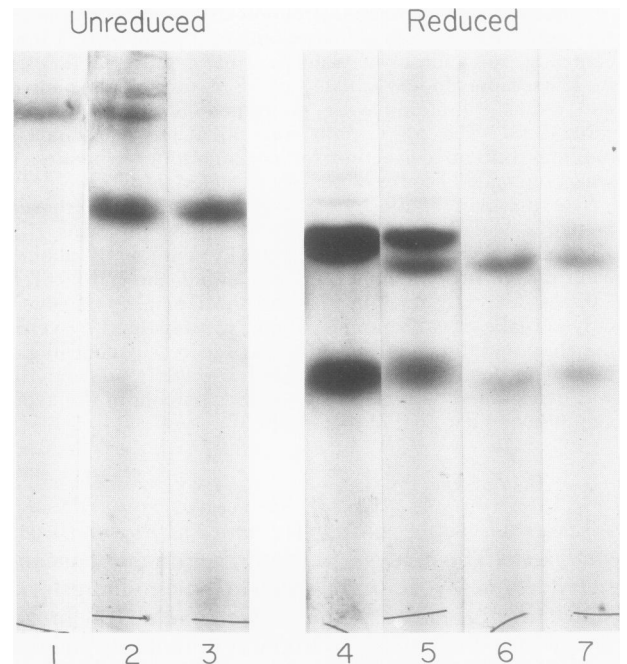


FIGURE 7 Polyacrylamide gel electrophoresis in 1% sodium dodecylsulfate analysis of unreduced 7.0S (1), Pevikon (2), and 4.5S (3) Ka IgA protein fractions and reduced control IgA myeloma protein (4), 4.5S (6), and 7.0S (7), and mixture of control IgA and 4.5S fraction (5). A wire was inserted into the bottom of the gel to show the position of the dye marker.

SEPHADEX G-200, PBS

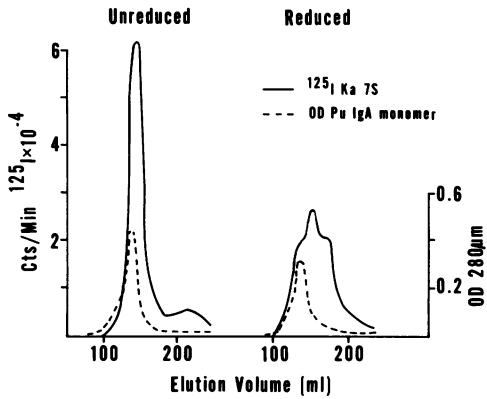


FIGURE 8 Elution from a Sephadex G-200 column of mixtures containing 50 µg ¹²⁵I-labeled Ka 7.0S and 20 mg control IgA Pu in either native (unreduced) or reduced and alkylated states. Solvent was phosphate buffered saline (PBS).

common to monomeric control IgA myeloma proteins. After reduction, both the 7.0S and 4.5S fractions showed two bands, one having a slightly faster mobility than control α chains and the other comparable to normal light chains. An average mol wt of 46,500 ± 1,500 was calculated for the Ka α chain from three analyses and 55,000 ± 2,200 for control α chains assuming mol wt of 70,000 and 23,000 for control μ and light chains, respectively.

To determine if the Ka 7.0S myeloma protein dissociated into half-molecules after mild reduction in aqueous solution, it was trace labeled with ¹²⁵I and mixed with 40 mg of a monomeric control IgA myeloma protein. Half of the mixture was reduced and alkylated in aqueous solution and the two preparations applied separately onto Sephadex G-200 columns. As can be seen in Fig. 8, the radioactivity representing the native Ka 7.0S fraction eluted slightly after the monomeric IgA protein. By contrast, most of the reduced protein Ka eluted after the control IgA protein which remained a 7.0S protein as all structurally intact Ig do after reduction in aqueous solutions.

Turnover studies. The Ka 7.0S and 4.5S fractions were labeled with ¹³¹I and injected together with ¹²⁵I-labeled control IgA myeloma proteins into rhesus monkeys. As can be seen in Fig. 9, all preparations were eliminated at a comparable rate from the circulation and were excreted in part into the urine. The serum half-lives varied from 1.5 to 2.7 days, the fractional turnover rates from 87 to 127% of the intravascular pool and 3.5 to 8.9% of the intravascular pool was recovered in the urine as trichloroacetic acid precipitable radioactivity. The amount of trichloroacetic acid precipitable radioactivity excreted into the

urine of the 4.5S protein did not differ significantly from that of either the 7.0S myeloma protein or control IgA myeloma proteins.

DISCUSSION

The Ka IgA myeloma protein existed in the patient's serum in two molecular species, one being of the classical 7.0S covalent four chain structure and the other a 4.5S two chain half-molecule. Both species were antigenically deficient in the Fc fragment and their α chains had a deletion of about 8,500 daltons. The deletion probably caused the absence or at least great diminution of the noncovalent interactions between the α chains and also was most likely responsible for an incomplete assembly of all myeloma proteins to 7.0S four chain Ig molecules. These findings strikingly parallel the characteristics of a human IgG myeloma protein forming half-molecules that was recently studied in our laboratory (8). Like the IgA myeloma protein, the IgG myeloma protein had a deletion in the Fc fragment, which in the latter was localized as an internal deletion of the third constant domain of the γ chain. The 7.0S IgG myeloma molecules also dissociated into half-molecules after mild reduction and alkylation in aqueous solutions, indicative of a lack of noncovalent interactions between the γ chains. It is conceivable that deletions of analogous structures in both α and γ chains are responsible for half-molecule formation.

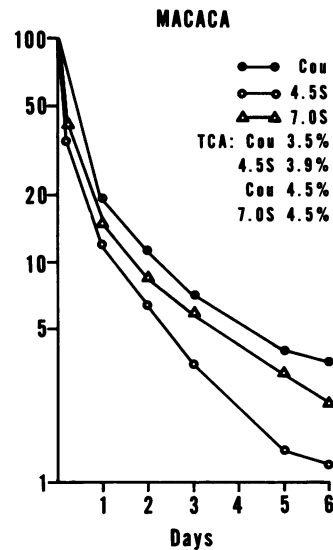


FIGURE 9 Elimination from the circulation of rhesus monkeys of monomeric control IgA myeloma protein (Cou) and Ka 4.5S and 7.0S fractions. Trichloroacetic acid (TCA): percent protein-bound radioactivity of intravascular pool excreted per day in two different monkeys. Abscissa represents percent of either ¹²⁵I or ¹³¹I protein-bound radioactivity in the plasma.

IgA half-molecules have previously only been found in mice bearing mineral oil-induced plasmacytomas (3, 9, 14). The α chains of one of these aberrant proteins were shown to have a deletion of about 12,000 daltons and involved the entire third constant domain (9). This deletion may be larger than that of the Ka protein whose α chain lacked approximately 8,500 daltons; however, the possibility exists that the presence of additional carbohydrate side chains could have obscured a larger deletion in the Ka α chain. Four chain molecules have not been reported to be present in the serum of mice with the IgA half-molecule forming tumors. However, in vitro studies on the synthesis and assembly of IgA molecules by these tumor cells have demonstrated intracellular four chain molecules (15) indicating that they also could synthesize 7.0S IgA. A human IgA myeloma protein that had a deletion in the α chain involving the entire third constant domain was reported by Despont et al. (16). Half-molecules were not detected in this patient's serum (Dr. C. Abel, personal communication). This suggests that the deletion in the Ka α chain involved structures other than the entire third constant domain, conceivably an internal deletion similar to that demonstrated for the myeloma IgG half-molecule (8).

Myeloma Ig half-molecule formation does not seem to be associated with a distinct clinical syndrome. Patient Ka had an aggressive course of multiple myeloma, and the other patients had extramedullary plasmacytomas (4, 5), plasma cell leukemia (6), and typical multiple myeloma (Dr. M. Seligmann, personal communication). All IgG patients excreted large quantities of half-molecules into the urine. In contrast, patient Ka excreted predominantly Bence Jones protein and only scanty IgA myeloma protein. The catabolism of IgA half-molecules clearly differs from IgG half-molecules. IgG half-molecules were shown to be more rapidly catabolized than normal IgG and excreted in part into the urine when tested in one normal person and rhesus monkeys (13). In contrast, turnover studies of the Ka IgA proteins in monkeys showed no significant difference in rate of elimination from the circulation and urinary excretion among 4.5S, 7.0S, and control IgA proteins. The absence of excretion of more IgA half-molecules than normal monomeric IgA in monkeys suggests that IgA half-molecules are usually not excreted into the urine; this is also probably true in man. This may be related to the different structural and functional parameters controlling the turnover of IgA and IgG (17).

The origin of the Ka, IgA half-molecule synthesizing myeloma cells remains to be determined. It is possible but unlikely that their products represented a rare IgA subclass as the half-molecule reacted with an

anti-IgA1 antiserum. Its definitive classification as an IgA1 protein would have required amino acid sequence analyses of the α chain. The myeloma protein could have represented an IgA1 allotypic variant. This also appears unlikely because genetically controlled differences within a class or subclass have been shown to be the result of either a single or a few amino acid interchanges (18) and not of large deletions. It is more likely that the myeloma protein was formed by an IgA1 mutant clone of cells which either evolved during the malignant transformation of the plasma cells or is an aberrant form of an IgA1 line which existed as a component of the normal genome. At present, one cannot differentiate between these possibilities. It has been shown that murine myeloma plasma cells mutate in vitro at a relatively high frequency (19, 20) and that the murine tumors forming IgA half-molecules arose as mutants from tumor cell lines producing intact IgA (3, 14). An originally intact IgA-producing tumor could have been present in the patient and later was replaced by a mutant. This seems unlikely to have happened in the short time of clinical disease, as one would have expected to find complete IgA molecules formed by the initial tumor in the serum. It appears probable that the myeloma cells arose from a natural clone of cells forming IgA half-molecules. The presence of IgA half-molecules in normal human serum would support this premise. To our knowledge, IgA half-molecules have not been reported in man, but a careful search has not been made. In rabbits, nonprecipitating antibodies of a 4.3S rate similar to the half-molecule have been described (21) and the serum of colostrum deprived piglets contains IgG half-molecules (1, 2). It is conceivable that a careful search will subsequently disclose IgA half-molecules in normal human serum and this finding will support the notion that the Ka IgA half-molecule is derived from an IgA1 mutant that is carried in the human genome.

ADDENDUM

After completion of this manuscript a patient forming IgA half-molecules was reported (G. M. Bernier, J. H. Berman, and M. W. Fanger. 1976. IgA/2: Plasma cell leukemia with urinary excretion of IgA half-molecules. *Clin Res.* 24: 444A. Abstr.). This patient differed from ours in that she excreted significant quantities of IgA half-molecules into the urine besides λ Bence Jones protein. The α chain of this patient also had a deletion and its mol wt was 45,000 daltons. It was postulated that the deletion affected the third constant domain of the α chain because it lacked methionine residues.

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