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Association of myeloma protein with IgG and its poor reactivity against anti-human λ antibodies: A case of IgD(λ)-type multiple myeloma

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SUMMARY

We encountered a case of IgD-type multiple myeloma in which we could not identify the type of light (L) chains of the IgD M-protein in the patient's serum by conventional immunoelectrophoresis or immunofixation electrophoresis. To clarify the cause of this failure, the immunochemical and structural properties of IgD in the patient's serum were characterized. The immunochemical reactivity of IgD M-protein against anti- λ antibodies was extremely low and the IgD-IgG complex was present in the patient's serum. The complex was dissociated in the presence of SDS, indicating that the association was formed via non-covalent binding. In a reassociation test, IgG prepared from normal subjects did not form a complex with the patient's IgD pretreated with a reducing agent, but formed a complex with IgD pretreated with papain. These results suggest that IgG formed a complex with the Fab of the patient's IgD.

Key words: IgD(λ)-type multiple myeloma, conformation abnormality, epitope, IgD-IgG complex.

INTRODUCTION

IgD-type multiple myeloma is rare compared with other types of myeloma, and in most cases it is accompanied by Bence Jones protein (BJP), whose light chain is λ -type in 90% of cases. Further, L-chain typing for IgD type M-protein by immunoelectrophoresis (IEP) has been shown to be difficult.¹⁾ We report here on a patient with IgD-type multiple myeloma, whose L-chain typing of IgD by conventional IEP and immunofixation electrophoresis (IFE) was unsuccessful. However, the L-chain was identified as λ -type by performing an additional immunoblotting after electrophoresis on an IFE agarose sheet. The present study was undertaken to clarify the reason why the L-chain reactivity of the IgD M-protein was extremely low. The results described in this paper indicate that IgD forms a complex with IgG in serum, and that the L-chain separated from the parent

IgD shows poor reactivity against three different anti-human λ antibodies, possibly preventing access of antibodies to their epitope.

CASE REPORT

1. Present illness

A 54-year-old male truck driver with a chief complaint of fever consulted our hospital for a thorough medical examination, following a check-up at a local hospital which revealed anemia, the presence of paraprotein proteins, and renal hypofunction.

2. Laboratory observations

Erythrocyte sedimentation rate: >140 mm/h (reference range; <10 mm), hemoglobin concentration: 66 g/l (125-175), serum total protein: 78 g/l (65-82), blood urea nitrogen 0.56 g/l (0.08-0.2), amylase 319 IU/l (100-300). Amylase isozymes S1-S4 and P1 were detected. On serum protein electrophoresis, an M peak

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was observed (the concentration of abnormal protein band at the γ region was 14 g/l). Immunoglobulin D concentration was increased to 18.1 g/l (0.0–0.12), and immunoglobulins G, A and M were 5.16 g/l (8.0–20), 0.15 g/l (0.7–4.7) and 0.22 g/l (0.4–3.5), respectively. When serum samples were subjected to IEP and IFE, the band for λ -type BJP was detected, but that for L chain was not detected at a position corresponding to the M bow of IgD. In urine samples, λ -type BJP was also positive. Bone marrow examination was not possible due to a dry tap of the bone marrow.

MATERIALS AND METHODS

1. Materials

To inhibit proteinase activity in serum, amidino-phenylmethane sulfonyl fluoride (APMSF), benzamide, EDTA, N-tosylphenylalanyl chloromethyl ketone (TPCK), and N-tosyllysylphenylalanyl chloromethyl ketone (TLCK) were each added at a final concentration of 1 mM. Control sera were collected from healthy subjects and one other patient with ordinary IgD-type multiple myeloma. Plates for IEP and agarose sheets for IFE were purchased from Helena Lab. (TX, USA). All antisera were obtained from DAKO (Copenhagen, Denmark), and anti-human λ light chain antibodies were obtained from DAKO, Japan-Tanner Co. (Kobe, Japan), and MBL Co., Ltd. (Nagoya, Japan). Dithiothreitol (DTT) (Wako Pure Chemical, USA) was used as a reducing agent. Low and high molecular weight marker kits were obtained from Pharmacia Co., Ltd. (Sweden).

2. Methods

(1) L-chain typing by IFE and immunoblotting after electrophoreses on IFE agarose sheet

IFE was performed as previously described.²⁾ The optimal concentrations of serum samples from the patient for immunoreactivity to each antibody were determined by stepwise dilution up to 30-times with physiological saline solution. The immunochemical activities of rabbit anti-human λ light chain antibodies from three suppliers were compared before use. For the determination of immunochemical reactivity of the patient's antibodies, aliquots of serum fractions diluted 100 times with PBS (pH 7.5) and mixed with an equal volume of 1 M acetic acid were electrophoresed on an agarose sheet, transferred to a polyvinylidene difluoride membrane (Millipore Co., MA, USA) by placing the membrane onto the agarose sheet for 30 min, and immunochemically detected by the conventional method.³⁾

(2) Molecular mass determination by native and SDS-PAGE

Serum samples from the patient were electrophoresed on a 4–10% gradient polyacrylamide gel under non-denaturing conditions followed by immunoblotting for the detection of an IgD-IgG complex using antibodies against δ chains, λ chains, and γ chains.

IgD was purified by chromatography on a column of DEAE Affi-Gelblue (BioRad, CA, USA) and then on a column of protein A Sepharose CL-4B (Pharmacia Co., Ltd., Sweden). The molecular mass of IgD was determined according to the method previously described.⁴⁾

(3) Detection of IgD-IgG complex in blood

1) Gel filtration⁵⁾: Serum samples were subjected to gel filtration on a Superose 12 HR column (Pharmacia Co., Ltd., Sweden). Eluate fractions were immunochemically assayed for δ and γ heavy chains using rabbit antibodies against the respective chains.

2) Protein A affinity chromatography: Serum samples were loaded on a column of protein A Sepharose CL-4B, and adsorbed materials were eluted with 1 M acetic acid. Each fraction of the eluate was subjected to IFE and assayed for δ and γ chains by immunoblotting.

3) Reassociation test: After a 10 μ l aliquot of IgD solution (1000 mg/l) purified from the patient's blood was mixed with the same volume of PBS solution containing 48 μ g, 24 μ g, 12 μ g, or 6 μ g purified human IgG prepared from normal subjects,⁶⁾ the formation of an IgD-IgG complex was assessed by immunochemical reactivity of the mixture to rabbit antibodies against the γ heavy chain and κ light chain.

4) Pretreatment of IgD samples with 1 M acetic acid, DTT and papain: To determine the chemical nature of the binding responsible for the IgD-IgG complex formation, aliquots of IgD solution (1000 mg/l) were mixed with the same volume of 1 M acetic acid, or 30 mg/dl DTT, and incubated at 37°C for 2 h. Another aliquot of IgD solution was mixed with 1/10 volume of papain solution (SIGMA, MO, USA, 0.03 U/mg) and incubated at 4°C overnight. BJP fractions obtained from the patient's serum samples by gel filtration were also tested for complex formation with IgG.

(4) Determination of the N-terminal amino acid sequence

The N-terminal amino acid sequence of δ chains purified from patient's serum was determined according to the method previously described.⁷⁾

RESULTS

1. Immunochemical analysis of patient's serum by IFE and immunoblotting after electrophoresis on IFE agarose sheet

As shown in Fig. 1, IFE revealed that L-chains of IgD-type M proteins were not detected, although a band for λ -type BJP was detected in serum samples from the patient, (Fig. 1, left panel, □). A series of dilutions (1, 1/2, 1/5, 1/10, 1/20, and 1/30) of the patient's serum also failed to show an optimal concentration at which IgD proteins reacted with appropriately diluted anti- λ chain antibodies. Although anti- λ chains antibodies (purchased from Tanner) at 1:5 dilution gave faint signals in the patient's serum fractions, the signals were not

Fig. 1. Immunofixation and immunoblotting patterns of the patient's serum.

IFE, immunofixation; SP, stain protein; A- γ , anti γ heavy chain antibodies; A- δ , anti δ heavy chain antibodies; A- λ , anti λ light chain antibodies. IFE revealed that L chains of IgD-type M proteins were not detected although a band for λ type BJP was detected in the patient's serum samples (left panel, open square). The use of immunoblotting successfully detected IgD- λ type M proteins in the patient's serum. When antibodies against γ chains were used, an M protein band was detected in coincidence with the band for IgD-type M proteins (right panel, arrow $\uparrow$).

Fig. 2. Immunofixation and immunoblotting patterns of the patient's serum treated with 1M acetic acid.

IFE, immunofixation; SP, stain protein; A- γ , anti γ heavy chain antibodies; A- δ , anti δ heavy chain antibodies; A- λ , anti λ light chain antibodies. Conventional IFE was able to detect IgD- λ (left panel, arrow $\uparrow$) in the 1M-acetic acid-treated patient's serum. Immunoblotting revealed an IgG-type M-protein band in coincidence with the band for IgD type M-protein (right panel, arrow $\uparrow$).

detected when the serum fractions were pretreated with protein A, which eliminates IgG in the serum. The use of immunoblotting after electrophoresis on an IFE agarose sheet successfully detected IgD- λ type M proteins in the patient's serum (Fig. 1). When antibodies against γ -chains were used, an M protein band was detected in coincidence with the band for IgD-type M proteins (Fig. 1 right panel, arrow $\uparrow$). Conventional IFE was able to detect IgD- λ type M-protein against anti- λ chain anti-

Fig. 3. Immunoblotting pattern of the patient's serum by native-PAGE.

M, standard molecular weight marker; SP, protein stained with coomassie brilliant blue; A- γ , anti γ heavy chain antibodies; A- δ , anti δ heavy chain antibodies; A- λ , anti λ light chain antibodies; A- κ , anti κ heavy chain antibodies; A-alb, anti human albumin antibodies. An IgD-IgG complex band that reacted against δ chains and γ chains was detected by native PAGE. The molecular mass of the protein was 669 to 440 kDa.

bodies in the 1M-acetic acid-treated patient's serum (Fig. 2, left panel, arrow $\uparrow$). Also, immunoblotting revealed an IgG-type M-protein band in coincidence with the band for IgD-type M protein (Fig. 2, right panel, arrow $\uparrow$).

2. Characterization by PAGE

Upon PAGE of the patient's serum samples under non-denaturing conditions, proteins reactive with antibodies against δ and γ chains were detected on immunoblots. The band revealed a molecular mass of 669 to 440 kDa (Fig. 3, arrow $\uparrow$). SDS-PAGE of proteins extracted from this gel region⁴⁾ revealed the presence of IgD (184 kDa) and IgG (150 kDa).

The purified IgD revealed a molecular mass of 184 kDa under non-reducing conditions. After incubation under reducing conditions, δ and λ chains showed molecular masses of 78 and 28 kDa, respectively, indicating that the IgD M-protein in the patient was normal in molecular mass (data not shown).

3. Characterization of IgD-IgG complex in the blood

(1) Characterization by gel filtration on a column

Blood samples were fractionated by gel filtration on a column, and materials in fractions F-1 to F-6 (Fig. 4) were subjected to native-PAGE. Samples were also fractionated by immunoblotting after agarose electrophoresis on an IFE sheet for the detection of IgD-IgG complexes. Proteins in F-2 reacted with antibodies against δ chains and γ chains. (Figs. 4 and 5).

(2) Characterization by protein A Sepharose CL-4B affinity chromatography

Serum proteins absorbed to protein A were eluted

with 1 M acetic acid, and the eluates were immunochemically characterized by immunoblotting. In the eluate of serum from a control patient with ordinary IgD-type myeloma, only IgG and albumin were detected. In contrast, in serum from the present patient, a large amount of IgD was detected in addition to IgG and albumin. On the other hand, IgD in the flow-through decreased from 28.0 g/l to 14.0 g/l.

(3) Reassociation tests

When a constant amount of IgD from the patient was

mixed with varying concentrations of normal IgG, a constant amount of IgD-IgG complex was observed independent of IgG concentrations (Figs. 6 A and 6 B).

(4) Pretreatment of IgD samples with 1M acetic acid, DTT and papain

When IgD was pretreated with either 1 M acetic acid, DTT, or papain, only papain-treated IgD formed a complex with IgG (Fig. 7, arrow ↑). Monomeric λ chains derived from BJP, which were obtained by gel filtration, did not form a complex with IgG (Fig. 7).

Fig. 4. Elution profile of the patient's serum on gel filtration column.

F-2 reacted with antibodies against δ chains and γ chains. IgD and IgG concentrations were 0.92 g/l and 0.017 g/l respectively. In F-3, IgD and IgG concentrations were 0.12 g/l and 0.17 g/l respectively. Column: Superose 12 HR (5 mm×200 mm), Absorbance measured at 280 nm, sample: 200 μl, eluent: 0.05 M Tris-HCl, 0.15 M NaCl pH 7.5, Flow rate: 0.4 ml/min, Fraction volume: 0.5 ml

Fig. 6. Immunoblotting patterns of the reassociation test by electrophoresis on IFE agarose sheet.

A-δ, anti δ heavy chain antibodies; A-γ, anti γ heavy chain antibodies; A-κ, anti κ light chain antibodies. IgD: IgG 1.100 μg: 48 μg, 2.100 μg: 24 μg, 3.100 μg: 12 μg, 4.100 μg: 6 μg (A) IgD-IgG complex was detected with anti γ heavy chain antibodies; they were observed independent of IgG concentrations. (B) IgD-IgG complex was detected with anti κ light chain antibodies.

Fig. 5. Immunoblotting patterns of the F-2 by native-PAGE and electrophoresis on IFE agarose sheet.

IFE immunoblotting, immunoblotting after electrophoresis on IFE agarose sheet; M, standard molecular weight marker; SP, protein stain with coomassie brilliant blue; A-γ, anti γ heavy chain antibodies; A-δ, anti δ heavy chain antibodies; A-λ, anti λ light chain antibodies; A-κ, anti κ light chain antibodies. IgD-IgG complexes proteins in F-2 that reacted with antibodies against δ chains as well as γ chains (arrow ↑) were detected by immunoblotting on native-PAGE and agarose electrophoresis.

Fig. 7. Immunoblotting patterns of the reassociation test by electrophoresis on IFE agarose sheet.

A- δ , anti δ heavy chain antibodies; A- κ , anti κ light chain antibodies; A- λ , anti λ light chain antibodies. After a 10 μ l aliquot of pretreatment IgD and BJP fraction sample were mixed with the same volume of purified human IgG (1, 48 μ g, 2, 24 μ g, 3, 12 μ g or 4, 6 μ g), only papain-treated IgD formed a complex with IgG (arrow $\uparrow$).

4. Determination of the N-terminal amino acid sequence

The N-terminal amino acid sequence of the δ chain of IgD from the patient was determined to be EVRLAESG-GGLVQPGRLRL•CAGAGFTVP, indicating that the δ chain belonged to the VH subgroup III. This amino acid sequence showed a 73% homology with that of BRO-proteins.⁹⁾ The N-terminal sequencing of the IgD- λ chain and BJP- λ chain was not possible due to a blocked modification of the N-terminal amino acid residues in these proteins.

DISCUSSION

We encountered a case of IgD-type multiple myeloma, in which we failed to type the L chains of IgD in a patient's serum samples when conventional IEP and IFE were adopted. However, we were able to solve this problem by adopting the immunoblotting method, and identified the L chains of the patient's IgD as λ -chain type. Furthermore, immunoblotting revealed an IgG-type M protein band in coincidence with the band for IgD-type M proteins, implying that IgD formed a complex with IgG in the patient's blood. Indeed, we experimentally demonstrated the chemical nature of IgD-IgG complex as below. Neither the λ chain alone nor the patient's IgD pretreated with reducing agent or acetic acid formed a complex with IgG. When the patient's serum was subjected to SDS-PAGE, the expected band for the IgD-IgG complex was not detected. The reassociation test revealed that the δ chain or λ chain alone did not form a complex, and that IgD pretreated with papain formed a complex, indicating that Fc did not

participate in the complex formation. Thus, an abnormal feature of IgD in the present patient was the low reactivity of the L chain against its antibodies. This was not due to complexing of IgD with IgG since the reactivity was low after removal of IgG from the complex. After acid pretreatment, the electrophoretic mobility of IgD moved to the α_2 region from fast γ region, and the L chain of IgD and IgD-IgG complex was detected by conventional IFE. It was suggested that the conformational change of the IgD-M protein was caused by a change in the primary amino acid sequence of the δ chain of IgD. The ability of IgG molecules to form homologous complexes was recently proposed by Kaveri et al.⁹⁾ This chemical nature could also apply to IgD-IgG complex formation in the present study, although it is an interaction between different species of immunoglobulin molecules. It may be speculated that abnormal conformation of the δ chain of IgD allowed to form a heterologous complex with IgG. Since this heterologous association was shown to be mediated by weak non-covalent bindings, the associations of IgD and IgG may be similar to that of apolipoprotein and triglycerides in which the stoichiometry of components is not rigidly established.

Since IgD is very susceptible to partial proteolysis,¹⁾ all the experiments were carried out in the presence of several proteinase inhibitors. It is not likely that the phenomena described in this paper was due to the partial hydrolysis of myeloma IgD. No change in molecular mass was observed after acid treatment (data not shown).

L-chain typing for IgD type M-proteins has been

difficult in many cases¹⁾ with plasmacytomas including multiple myeloma. Even in these difficult cases, physicians could have indirectly determined L-chain type IgD by L-chain typing of BJP since IgD-type M proteins are usually accompanied by the appearance of BJP. However, the IgD-type multiple myeloma in the present study was a rare case in which L-chain typing was not possible even with the use of a highly sensitive method such as IFE.

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