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SHORT COMMUNICATION

Physicochemical Properties of IgD-type M Protein by Electrophoretic Analysis

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SUMMARY

Background: **We examined the physicochemical properties of IgD-type M protein from 10 patients with IgD-type M protein and those with other types of M protein.**
Methods: **Identification of the L-chain type by routine methods (Immunoelectrophoresis: IEP, Immunofixation electrophoresis; IFE), detection of IgD-IgG complexes and the changes in the mobility by treatment with acid.**
Results: **Identification of the L-chain type by both IEP and IFE was impossible. Only one patient revealed the possibility of IgD-IgG complex bands in 6 of the 10 samples. Changes in mobility by treatment with acid were observed in 8 of the 10 samples of IgD-type M protein.**
Conclusions: **These abnormalities are caused by the primary structure of IgD, which may be related to the well-known weak reactivity with L-chain antibody.**
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KEYWORDS

IgD-type M protein, multiple myeloma, physicochemical properties, immunofixation electrophoresis (IFE), immunoblotting

INTRODUCTION

IgD-type M proteins account for 2% of myeloma cases, many of which have a poor prognosis and are rarely encountered in the laboratory [1-3]. It is often difficult to identify the L-chain form of IgD-type M protein from patient serum [4]. However, as this type of M-protein is almost always associated with Bence Jones protein (BJP), it is thought that the L-chain type has been identified on the basis of the urinary BJP type. We reported the case in a Japanese Journal named " Jap J Electroph" (in English) as a preliminary case report in 2000. In our previous cases, we also encountered IgD-type M-proteins for which L-chain type determination was not possible by immunoelectrophoresis (IEP) and immunofixation electrophoresis (IFE) methods. Figure 1 shows the IEP pattern (Figure 1). Therefore, the lambda chain was identified by immunoblotting after agarose membrane

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Table 1. Object.

Case No.	L chain type	IgD (g/L)	IgG (g/L)	BJP	Total protein (g/L)
1	λ	18.1	5.2	+	78
2	λ	26.9	5.8	+	68
3	λ	5.6	6.9	+	59
4	λ	18.1	4.9	+	60
5	λ	6.0	5.7	+	64
6	λ	16.5	5.2	+	71
7	κ	1.6	4.2	+	61
8	λ	3.8	4.7	+	67
9	λ	42.1	3.7	+	78
10	λ	2.2	5.4	+	62

Table 2. Physico-chemical characteristics of IgD-type M proteins.

Case No.	(a) L chain identification with IEP	(b) L chain identification with IFE	(c) relative mobility of M-protein	(d) acid treatment	(e) complex
1	impossible	impossible	0.6	change	IgD-IgG
2	possible	possible	0.55	change	BJP-IgG
3	indistinct	indistinct	0.68	change	(-)
4	possible	possible	0.63	no change	(-)
5	possible	possible	0.6	change	IgD-IgG
6	piled up of precipitation line	possible	0.74	change	IgD-IgG
7	piled up of precipitation line	possible	0.67	no change	IgD-IgG
8	piled up of precipitation line	possible	0.7	change	(-)
9	possible	possible	0.85	change	IgD-IgG
10	indistinct	indistinct	0.53	change	IgD-IgG

electrophoresis. At the same time, we found that this M protein had IgG-binding capacity and that its mobility changed in response to acid treatment. Through the analysis of this case, it was considered that the weak L-chain antibody reactivity was due to the structure of IgD. In the present study, we compared the physico-chemical properties of 10 cases of IgD-type M protein with those of other types of M protein and discussed the cause of the weak L-chain reactivity of IgD-type M protein.

MATERIALS AND METHODS

Materials

The materials were 10 serum samples (9 samples of λ type, 1 sample of k type) collected from 10 patients who had been clinically diagnosed as having IgD-type multiple myeloma and had given consent (Table 1). Benzamidene, as the protease inhibitor, was added to the samples to a concentration of 0.1%. As the control, 17 samples of M protein serum (10 samples of IgG-type, 3 samples of IgA-type, 1 sample of IgM-type, 3 samples of BJP type) were used. Anti-human γ antibodies, anti-human δ-chain antibodies, anti-human κ-chain antibodies, anti-human λ-chain antibodies (DAKO Inc.), and anti-human free λ-chain antibodies (DAKO Inc. MBL Inc.) were used.

Methods

Identification of the L-chain type and comparisons of electrophoregrams were performed by routine methods (IEP, IFE), and the position of M protein from the anode (relative mobility) was measured. To examine the IgG-binding ability, the samples were diluted 100- to 200-fold in PBS (pH 7.5), and the presence or absence

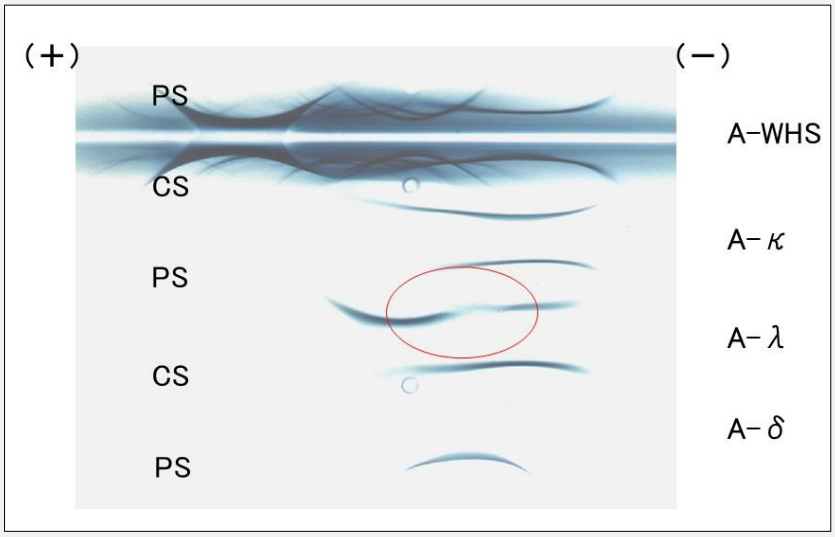

Figure 1. Immunoelectrophoresis pattern of patient's serum (Case 1).

PS - patient's serum, CS - control serum, A-WHS - anti whole human serum antibodies, A-γ - anti γ heavy chain antibodies, A-δ - anti δ heavy chain antibodies, A-λ - anti λ light chain antibodies.

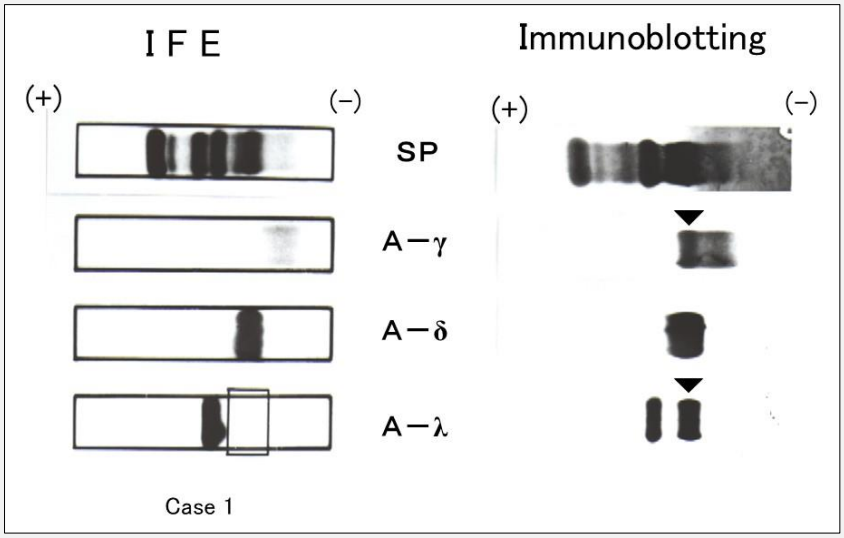

Figure 2. Immunofixation and immunoblotting patterns of the patient's serum (Case 1).

IFE - immunofixation electrophoresis, SP - staining protein, A-γ - anti γ heavy chain antibodies, A-δ - anti δ heavy chain antibodies, A-λ - anti λ light chain antibodies.
IFE results showed that the L-chain of the IgD-type M protein was not detected, but a band of the λ type BJP was detected in the patient's serum sample (left panel, open square); immunoblotting studies after IFE agarose gel electrophoresis confirmed that the L-chain of the IgD-type M protein was the λ chain. Furthermore, the IgD-type M protein was found to react with anti γ chain antibodies at the same position as the IgD- type M protein (IgD-IgG complex (right panel, arrow)). Reprinted with permission and modified from "Jap J Electroph 2000;44:9-14".

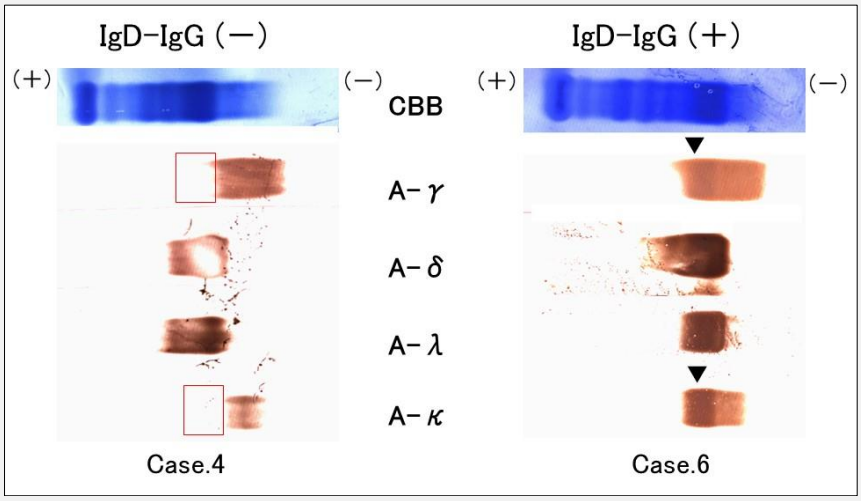

Figure 3. Examination of IgG-binding ability by immunoblotting.

CBB - 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 light chain antibodies
Left (Case 4) shows IgD-IgG complex negative case and right (Case 6) shows IgD-IgG complex positive case.

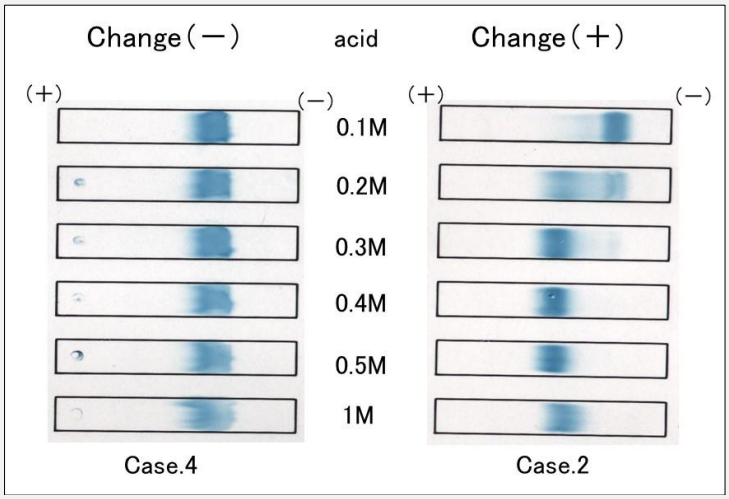

Figure 4. The mobility change by treatment with acid.

Acid: the effect of different acetic acid concentrations on mobility was observed.
Left (Case 4) no change, right (Case 2) change with 0.2 M acetic acid.

of abnormal bands of IgD-IgG complexes was observed by the immunoblot method after electrophoresis on agarose membrane. To evaluate the changes in the mobility by treatment with acid (assessment of conformation abnormalities), the samples were mixed with 1 M acetic acid at a ratio of 1:1, and the mobility of IgD-IgG complex bands were examined by electrophoresis on agarose membrane. Other types of M protein as the control

were examined by the same procedures.

RESULTS

The results of the identification of the L-chain type and comparisons of electrophoregrams performed by routine methods (Table 2), identification of the L-chain type by both IEP and IFE was impossible in case 1 alone, but ambiguous results were obtained in the other 2 patients (case 3 and case 10). We have encountered no patients in whom M protein of other types (IgG-type, IgA-type, IgM-type, BJP-type) could not be identified. Figure 2 shows the images of the serum of patient 1 by IFE and the immunoblot method. The examination of abnormal bands of the IgD-IgG complexes by the immunoblot method after electrophoresis on agarose membrane revealed the possibility of IgD-IgG complex bands in 6 of the 10 samples of IgD-type M protein. Figure 3 shows the images of the complexes (-) and (+). Changes in mobility by treatment with acid were observed in 8 of the 10 samples of IgD type M protein. Figure 4 shows the images with changes in the mobility (-) and (+). The examination of the other types of M protein in the 17 control patients by the same procedures demonstrated changes in the mobility in 1 sample of BJP-λ type M protein alone. Table 2 summarizes the results. There were no correlations between the items and results.

CONCLUSION

Since IgD-type M protein is rare compared to other types of M protein, studies on the physicochemical properties of IgD-type M protein have not been advanced. It was often difficult to identify the L-chain type of M protein by IEP, and the L-chain type was determined based on the BJP type until IFE kits became widely used because IgD-type M protein accompanies BJP in almost all patients. In this study, the L-chain type could not be identified in 1 patient (Case 1) even by IFE. Further examination revealed weak L-chain reactivity and abnormalities in the IgG-binding ability and conformation (changes in the mobility by treatment with acid), showing that such a case is very rare. To evaluate whether these abnormalities were peculiar to the patient and whether they were observed in other types of M protein, we examined other types of M protein in the 17 control patients and found that these abnormalities were characteristic of IgD-type M protein alone. It was also found that IgD-IgG complexes were observed in 6 of the 10 patients, and changes in mobility by treatment with acid were observed in 8 of the 10 patients. Since there was only 1 patient in whom the L-chain type could not be identified by both IEP and IFE, the patient was considered peculiar among the patients with IgD-type M protein. In the control group, there were no patients, except for patients with H-chain disorder, in whom the L-chain type could not be identified, nor was IgG-binding ability detected. Changes in mobility by treatment with acid was observed in 1 patient of the BJP type alone. The abnormalities in the conformation were assessed by examination of the changes in mobility of M protein by treatment with acid. The 2 patients (Case 4 and Case 7) in whom abnormalities in the mobility were not detected might have had structural abnormalities, such as amino acid substitution, which could not be detected by the methods used in this study. Therefore, the absence of abnormalities in the conformation could not be determined by these methods alone. Biaz et al. reported [4] that L-chain typing was difficult in IgD multiple myeloma and that they performed L-chain typing by increasing the concentration of antiserum. We, too, were able to easily determine the L-type using immunoblotting technology in a case that was difficult to determine with IFE.

Unlike other M proteins, IgD-type M proteins have few reactive groups on the outer side, suggesting that they have an inner side that is difficult to react with and could be detected by disrupting conformation by acid treatment or other means.

Patients with IgD-type M protein are rare, and there have been no studies on the physicochemical properties of the protein. This study suggested an abnormal structure unique to IgD-type M protein. These abnormalities are caused by the primary structure of IgD, which may be related to the well-known weak reactivity with L-chain antibodies.

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Declaration of Interest:
The authors declare no conflict of interest.

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