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CASE REPORT

Immunochemical Characteristics of Half-Molecule IgG Presumed to Lack the CH_2 Domain

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ABSTRACT

Background: **Half-molecule immunoglobulins are low-molecular-weight abnormal immunoglobulins composed of one defective heavy chain (H chain) and one light chain (L chain) of normal molecular weight.**
Methods: **The molecular weight of the low molecular weight IgG was determined using SDS-PAGE and immunoblotting with patient serum. To prove that the low molecular weight IgGs were L-chain and deficient γ-chain, SDS-PAGE was used to cut out and reduce the entire gel of unreduced, migrated proteins. Then, a second SDS-PAGE was run.**
Results**: The low molecular weight IgG component was found to consist of normal molecular weight κ chains (32 and 29 kDa) and defective γ chains (37 and 38 kDa). Low molecular weight IgG did not react with anti-γ-CH_2 domain antibodies.**
Conclusions: **The low molecular weight ɤ chain was also found to be a half-molecular IgG with a CH_2 domain deletion, as inferred from the reactivity of the antibody.**
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KEYWORDS

half-molecule, lacking the CH_2 domain, low-molecular-weight abnormal immunoglobulin

INTRODUCTION

Half-molecule immunoglobulins are low-molecular-weight abnormal immunoglobulins composed of one defective heavy chain (H chain) and one light chain (L chain) of normal molecular weight. However, in contrast to heavy chain diseases [1], reports of such half-molecule immunoglobulins are rare [2-7]. Here, we report the immunochemical characteristics of a half-molecule IgG-κ type M protein, suspected to be a half-molecule IgG based on immunoelectrophoresis (IEP) findings, in a patient with plasmacytoma. Using electrophoresis and immunoblotting techniques, we determined the molecular weight and performed structural analysis of the protein.

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CASE PRESENTATION

Present illness

A man in his 30s presented with cervical and axillary lymph node enlargement, and the biopsy confirmed plasmacytoma. Plasma cells in the bone marrow accounted for 5.8 %, with no atypia. Serum IgG and IgA levels were 25.6 g/L (8.0 - 20) and 21.5 g/L (0.7 - 4.7), respectively, with detection of an IgA-κ type M protein. Thereafter, the patient was followed on an outpatient basis. But after about 3 years, detailed examinations were performed as IEP showed an abnormal precipitation line against anti-γ chain antibody.

Laboratory findings

The white blood cell count was 6,400/μL (4,000 - 8,000). The red blood cell count was 469 x 10^4/μL (420 - 540), and hemoglobin concentration was 139 g/L (125 - 175). The serum total protein level was 95 g/L (65 - 82). On serum protein fractionation, the β fraction was increased at 34% (3.46 g/L) (Figure 1A). IgG was increased at 42.7 g/L (8.0 - 20), and IgA and IgM were reduced at 5.7 g/L (0.7 - 4.7) and < 0.2 g/L (0.7 - 4.7), respectively (nephelometry). Concerning IgG subclasses, IgG1 was 2.51 g/L, IgG2 was 5.60 g/L, IgG3 was 0.10 g/L, and IgG4 was 0.05 g/L, and the total of IgG subclasses showed a marked deviation from the original IgG level (nephelometry). The serum soluble interleukin-2 receptor was markedly increased at 1,891 U/mL (220 - 530).

MATERIALS AND METHODS

Materials

Serum and urine samples were analyzed to investigate the characteristics of the immunoglobulin. Buffer exchange for urine was performed using a PD-10 column (Pharmacia Co. Ltd., Sweden). Immunological reactions were obtained from Dako, MBL, Binding Site (for IgG subclass analysis), and Bethyl Laboratories (goat anti-human γ-Fab antibody).

Methods

IEP and IFE

IEP was performed at 2.5 mA for 90 minutes. IFE was carried out according to the manufacturer's instructions.

Molecular weight determination

To determine the molecular weight of abnormal IgG, SDS-PAGE and immunoblotting were performed with and without the reducing agent, and the results were compared. SDS-PAGE was carried out using polyacrylamide gels with concentration gradients of 7 - 15% and 10 - 15% using the Laemmli method [8]. A Low Molecular Weight Marker Kit (Pharmacia Co., Ltd.) was used. For immunoblotting, proteins were transferred onto PVDF (polyvinylidene difluoride) membranes using a blotting apparatus (Bio Craft Co., Ltd.) at 200 mA for 45 minutes, followed by blotting according to the method of Towbin [9] and staining by the peroxidase-labeled antibody method. The secondary antibody used was peroxidase-labeled goat anti-rabbit immunoglobulin (MBL Co., Ltd.). To further investigate the components of the abnormal IgG, two-dimensional electrophoresis was performed by our previously reported method [6]. In this procedure, the first SDS-PAGE (1st PAGE) was performed without reduction treatment. The target band was then excised from the gel, subjected to reduction treatment, and placed onto another gel for a second SDS-PAGE (2nd PAGE). The constituent components of the low-molecular-weight immunoglobulin were then analyzed.

Partial purification of abnormal IgG

Partial purification of abnormal IgG was performed. Patient serum was used as the starting material. Partial purification of the abnormal IgG was made from the unbound fraction of the patients' serum by first removing albumin using DEAE Affi-Gel Blue (Bio-Rad Laboratories, CA, USA) and then removing normal IgG using Protein A Sepharose CL-4B (Pharmacia Co., Ltd., Sweden).

N-terminal amino acid sequence analysis

The N-terminal amino acid sequences of the γ and κ chains of the abnormal IgG were determined using a previously described method [6]. The target abnormal γ and two κ chains were separated by SDS-PAGE and transferred to a PVDF membrane. After protein staining, the target bands were excised, washed overnight with redistilled water, and subjected to sequence analysis using a 477A protein sequencer (Applied Biosystems, Inc., Foster City, CA, USA).

RESULTS

Findings from IEP

In the IEP analysis of the patient's serum, an abnormal precipitation line forming an M-bow was observed near the application point (β region) inside the polyclonal anti-γ chain antibody arc. This protein reacted with anti-κ chain antibody, anti-γ-Fc antibody, and anti-γ-Fab antibody but showed no reactivity with the anti-CH_2 domain antibody or with any of the subclass antibodies (Figure 1B). Similarly, in both unconcentrated and 10-fold concentrated samples of the patient's urine, reactivity was observed with anti-κ chain antibody, anti-γ-Fc antibody, and anti-γ-Fab antibody but not with the anti-CH_2 domain antibody or any of the subclass antibodies (data not shown).

Molecular weight determination of abnormal IgG

Without reduction treatment of the samples, the main band in the normal control appeared at 150 kDa. In contrast, in the patient's serum and urine, a band that reacted with both anti-γ chain and anti-κ chain antibodies was observed at a low molecular weight of 73 kDa (→), and another band, presumed to be a free γ chain, was observed in an even lower molecular weight region (⇢)

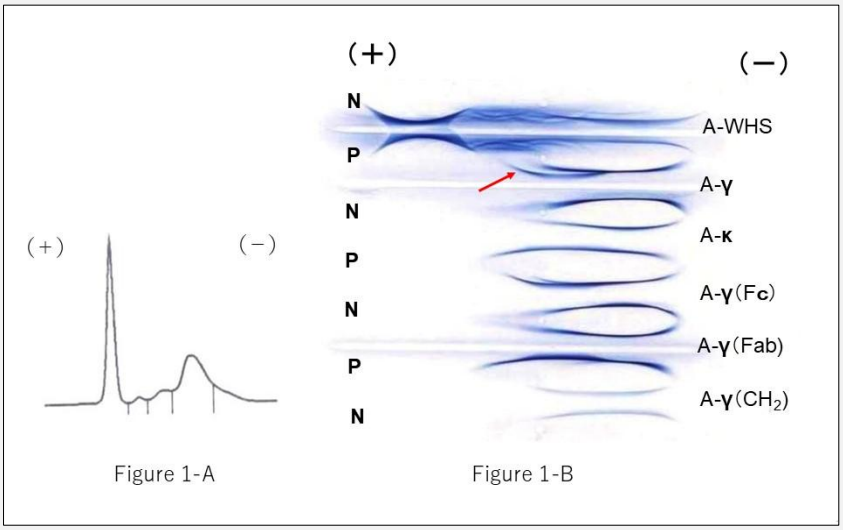

Figure 1.

Figure 1-A. Pattern of the patient's serum protein fraction. Abnormal protein bands were detected from β to γ position.
Figure 1-B. Immunoelectrophoresis pattern of patient's serum. A-WHS: anti whole human serum, A-ɤ: anti-γ chain antibodies, A-k: anti-k chain antibodies, A-ɤ (Fc): anti-γ-Fc antibodies, A-γ (Fab): anti-γ-Fab antibodies, A-γ (CH_2): anti-γ-CH_2 domain antibodies. A double precipitation line was detected in the anti γ heavy chain antibodies (⇢).

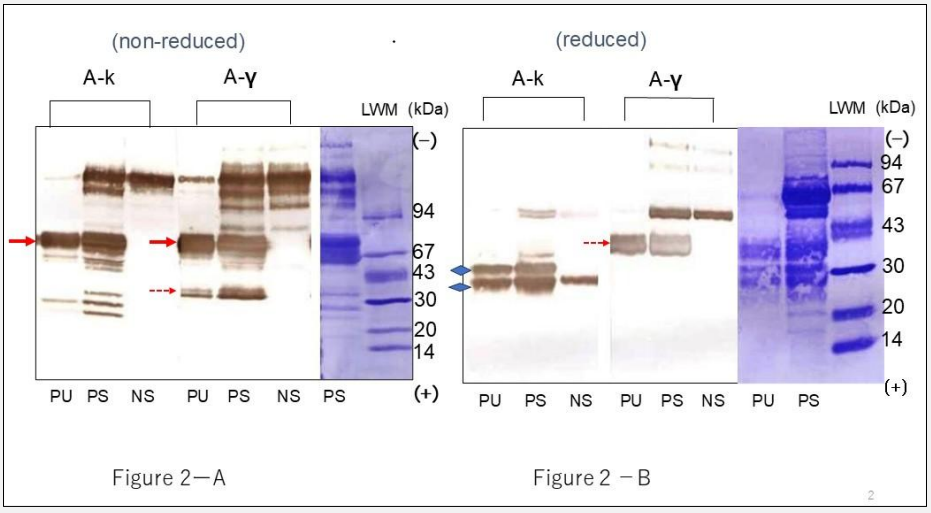

Figure 2.

Figure 2-A. Immunoblotting pattern of the patient's serum and urine after SDS-PAGE (non-reduced). PS: patient's serum, PU: patient's urine, NS: control serum, LWM: low weight marker, A-ɤ: anti-γ chain antibodies, A-k: anti- k chain antibodies. Bands reacting with anti-γ heavy chain antibodies and anti-k light chain antibodies were detected at the 73 kDa position (→). Furthermore, low molecular weight free γ chain reactions were observed at around 35 kDa in patient's serum and urine (⇢).
Figure 2-B. Immunoblotting pattern of the patient's serum and urine after SDS-PAGE (reduced) PS: patient's serum, PU: patient's urine, NS: control serum, LWM: low weight marker, A-ɤ: anti-γ chain antibodies, A-k: anti-k chain antibodies. In patient serum, bands of 36 to 38 kDa (⇢) were observed in addition to the normal molecular weight of 50 kDa for anti-γ chain antibodies, and bands of 32 kDa and 29 kDa were observed in normal samples for anti-κ chain antibodies, which normally have a band of 30 kDa.

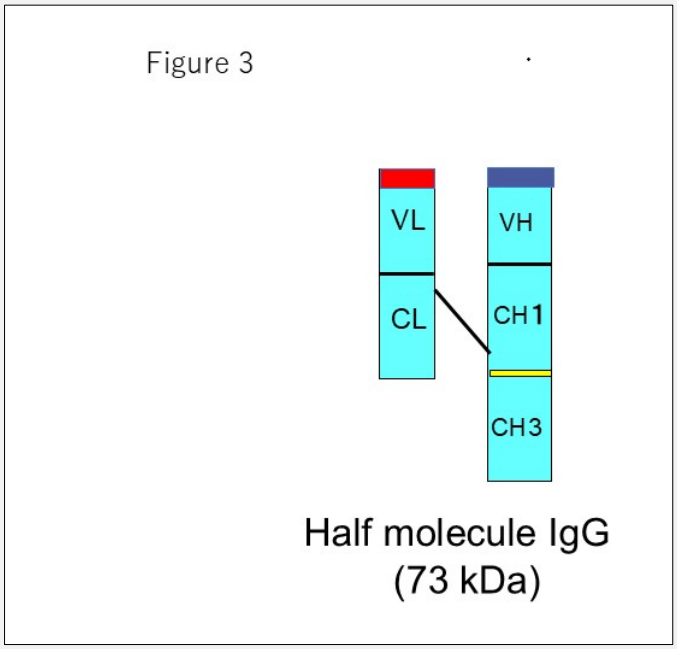

Figure 3. Schematic model of half molecule IgG.

(Figure 2A). After reduction treatment, in the normal control, a main band that reacted with the anti-γ chain antibody was observed only at 50 kDa. However, in the patient's serum, reactivity was also observed at 36 - 38 kDa (⇢) in addition to the band at 50 kDa, in the patient's urine, only bands at 36 - 38 kDa were present. Also, while only one band at 30 kDa reacted with anti-κ chain antibody in the normal control, the patient's serum and urine showed two bands at 32 kDa and 29 kDa (Figure 2B) (🔷). In addition, from two-dimensional electrophoresis (data not shown), these findings indicated that the 73 kDa low-molecular-weight protein consisted of a 37 - 38 kDa γ chain (corresponding to a deletion of approximately 12 - 13 kDa from the normal γ chain) and two κ chains of different molecular weights, confirming that the proteins were so-called IgG half molecules.

N-terminal amino acid sequence analysis

The N-terminal amino acid sequence (15 residues) of the abnormal γ chain did not match any known sequences in existing databases. Similarly, the N-terminal sequences of the two κ chains were also not found in known databases, and the two κ chains had the same N-terminal sequence.

DISCUSSION

Half-molecule immunoglobulins are abnormal, low-molecular-weight immunoglobulins consisting of a single defective H chain and one L chain. Regarding half-molecule IgG, the first case was reported by Hobbs et al. [4], and, in Japan, a case was reported by Nemoto et al. [2]. For other immunoglobulin classes, Sakurabayashi et al. reported a case of half-molecule IgA [5], and we previously reported two cases of half-molecule IgM in 1999 and 2017 [6,7]. In the present case, a low-molecular-weight IgG was detected in both the serum and urine of a plasmacytoma patient. Through a series of analyses, this abnormal protein was confirmed to be half-molecule IgG, consisting of a single γ chain with a 13 kDa deletion (normal γ chain: 50 kDa) and a single κ chain (32 kDa and 29 kDa). Regarding the abnormal structure, the following findings were derived from 1) two-dimensional electrophoresis and 2) reactivity to antibodies:

1) In two-dimensional SDS-PAGE, the 73 kDa band that reacted with both anti-γ chain and anti-κ chain antibodies under non-reducing conditions was excised from the gel and subjected to a second SDS-PAGE after reduction with DTT. As a result, the 73 kDa protein was separated into a single 37 kDa γ chain (compared to 50 kDa in the normal control) and two κ chains (32 kDa and 29 kDa). 2) The crude purified abnormal protein

and the abnormal protein detected in the urine showed no reactivity with anti-CH_2 domain antibodies, suggesting a complete deletion of the CH_2 domain from the hinge region, which binds H chains.

Figure 3 shows a schematic diagram inferred from these findings. Various other reports have also described the structural features of half-molecule immunoglobulins [10 - 12].

Regarding the mechanism underlying the generation of half-molecule IgG, gene deletion or mutation at the DNA level, abnormal RNA splicing after transcription, and degradation after protein synthesis by proteases have been proposed. In the present case, N-terminal amino acid sequencing revealed that the N-terminal sequence of the γ chain did not match any known sequences in existing databases. Similarly, in our previously reported cases with structurally abnormal immunoglobulins, we observed N-terminal sequences that were either blocked or did not show homology to known sequences. Regarding half-molecule IgM [6], we described an N-terminal of a low-molecular-weight μ chain followed by a sequence of 20 amino acids identical to the patient's κ chain with a unique, previously unreported sequence beginning at the 21st residue. Such abnormal amino acid sequences are considered to be caused frequently by somatic mutations [13,14]. None of the reported cases of structurally abnormal immunoglobulins with molecular weight deficiencies exhibited normal sequences. Although direct identification of abnormal IgG from bone marrow cells was not attempted in the present case, abnormal IgG production is considered likely as in the previous cases. In addition, regarding the two κ chains with different molecular weights (32 kDa and 29 kDa) that reacted with anti-κ chain antibody, we suspected microheterogeneity, but no change was observed before and after sialidase treatment. Although not investigated in the present study, glycosylation remains a possible explanation [15]. The lack of reactivity with the anti-γ CH_2 domain antibody suggests a defect of the CH_2 domain in the low-molecular-weight γ chain of the patient's half-molecule IgG.

Declaration of Generative AI in Scientific Writing:

The authors declare that they received assistance from AI for the purpose of checking English grammar during the preparation of this paper.

Declaration of Interest:

The authors state that they have no conflicts of interest related to this work.

References:

1. Franklin EC, Lowenstein J, Bigelow B, Meltzer M. Heavy chain Disease - A new disorder of serum gamma-globulins: report of the first case. Am J Med 1964;37:332-50. (PMID: 14209281)
2. Hobbs JR, Jacobs A. A half-molecule GK plasmacytoma. Clin Exp Immunol 1969;5:199-207. (PMID: 4984125)
3. Nemoto K, Nakanishi T, Hiramatsu M. et al. [Human myeloma IgG-Kappa half-molecules: a case study (author's transl)]. Rinshou Ketsueki 1979;20(7):762-7. (PMID: 116015)
4. Spiegelberg HL, Fishkin BG. Human Myeloma IgA half-molecules. J Clin Invest 1976; 58:1259-65. (PMID: 993344)
5. Sakurabayashi I, Kin K, Kawai T. Human IgA1 half-molecules: clinical and immunologic features in a patient with multiple myeloma. Blood 1979;53:269-78. (PMID: 367466)
6. Imoto M, Ishikawa K, Yamamoto K, et al. Occurrence of heavy chain of 7S IgM half-molecule whose NH_2-terminal sequence is identical with that of κ light chain sequence in patients with Waldenstrom macroglobulinemia. Clin Chim Acta 1999;282:77-88. (PMID:10340436)
7. Imoto M, Yoshida K, Maeda Y, et al. A case of Waldenstrom Macroglobulinemia with Temporary Appearance of 7S IgM Half Molecule. Clin Lab 2017;63:983-9. (PMID: 28627827)
8. Laemmli UK. Cleavage of structural proteins during the assembly the head of bacteriophage T4. Nature 1970;227:680-5. (PMID: 5432063)
9. Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci USA 1979;76:4350-4. (PMID: 388439)
10. Spiegelberg HL, Heath VC, Lang JE. Human myeloma IgG half-molecules. Structural and antigenic analyses. Biochemistry. 1975; 14:2157-63. (PMID: 50083)
11. Kang YS, Shim BS. An abnormal human IgA1 half-molecules. Biochem Biophys Acta 1977;494:326-31. (PMID: 410452)
12. Alexander A, Anicito I, Buxbaum J. Gamma heavy chain disease in man: genomic sequence reveals two non-contiguous deletions in a single gene. J Clin Invest 1988;82:1244-52. (PMID: 3139711)
13. Imoto M, Ishikawa K, Yamamoto K, et al. A case of multiple myeloma IgG(λ) type with low molecular weight κ light chain. Clin Chim Acta 1998;277:99-105. (PMID: 9776049)
14. Tonegawa S. Somatic generation of antibody diversity. Nature 1983;302:575-81. (PMID: 6300689)
15. Imoto M, Watanabe K, Yoshida K, Nakae KI, Kamisako T, Yamada T. Glycosylated Bence Jones Protein with Poor Thermal Reactivity in Heat Coagulation Tests. Clin Lab 2020;66:2363-69. (PMID: 33180428)