Abnormal N-Glycosylation of the Immunoglobulin G \( \kappa \) Chain in a Multiple Myeloma Patient with Crystalglobulinemia: Case Report

Ryo Hashimoto,\textsuperscript{a} Tosifusa Toda,\textsuperscript{b} Hisashi Tsutsumi,\textsuperscript{a} Masatsugu Ohta,\textsuperscript{a} Mayumi Mori\textsuperscript{a}

\textsuperscript{a}Department of Hematology, Tokyo Metropolitan Geriatric Hospital, Tokyo, Japan; \textsuperscript{b}Research Team for Molecular Biomarkers, Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan

Received March 29, 2006; received in revised form November 20, 2006; accepted December 4, 2006

Abstract

Spontaneous crystallization of monoclonal immunoglobulins (crystalglobulin) is a rare complication of multiple myeloma. We describe a 64-year-old Japanese man with skin ulcers and renal failure associated with immunoglobulin G \( \kappa \) multiple myeloma. Crystallized immunoglobulin was detected in his serum at room temperature. Analysis of the patient's crystalglobulin by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and mass spectrometry suggested that the crystallization was due to abnormal glycosylation of the immunoglobulin light chain. Treatment with thalidomide and dexamethasone improved the severe skin ulcers on the patient's extremities and partially reversed his renal failure. This report is the first of abnormal glycosylation of immunoglobulin possibly caused by modification of \( N \)-glycans in the light chain. We concluded that abnormal glycosylation of the immunoglobulin light chain might be the cause of the patient's skin ulcers and renal dysfunction.

© 2007 The Japanese Society of Hematology

Key words: Crystalglobulin; Multiple myeloma; Immunoglobulin light chain; Abnormal glycosylation

1. Introduction

Crystalglobulinemia is characterized by crystallization of monoclonal immunoglobulin, with the crystal deposits affecting various tissues, especially in the kidneys [1]. Crystalglobulinemia is a rare complication of multiple myeloma [2] that has been reported in 8 series of 35 cases between 1972 and 1993 [3-10]. Although crystalglobulinemia has been treated with plasmapheresis and chemotherapy for the underlying myeloma, a standard treatment has not been established. Successful management appears to depend on early recognition and a diagnosis of crystalglobulinemia [7].

We report a case of multiple myeloma with crystalglobulinemia that was successfully controlled by thalidomide treatment in combination with dexamethasone 4 years after the symptoms first appeared.

2. Case Report

A 64-year-old Japanese man with recurrent cutaneous ulcers in both his hands and his feet was admitted to the Department of Hematology at Tokyo Metropolitan Geriatric Hospital in March 2005. Four years had elapsed since the first emergence of his painful recurrent ulcers. Although the patient had received regular supportive treatment for his ulcerous lesions in several hospitals during this period, the cause of the cutaneous ulcers was not clear.

Physical examination showed pale conjunctiva and severe cutaneous ulcers on the patient's hands, feet, and ears. His height was 161 cm, and he weighed 58 kg. All peripheral pulses were checked, and the patient's blood pressure was 146/66 mm Hg. His leukocyte count was 4.1 × 10^9/L, the hemoglobin concentration was 7.9 g/dL, and the platelet count was 159 × 10^9/L. Blood tests revealed the following: total protein, 7.1 g/dL; albumin, 3.7 g/dL; blood urea nitrogen, 46 mg/dL; creatinine, 3.5 mg/dL; uric acid, 7.7 mg/dL; potassium, 5.5 mEq/L; calcium, 8.2 mg/dL; and \( \beta \)-microglobulin, 10.6 mg/mL. The results of serum liver function and coagulation tests were normal. A urinalysis showed proteinuria (0.57 g/day), and the patient's creatinine...
clearance was 15.1 mL/min. Serum immunoelectrophoresis revealed immunoglobulin G (IgG) κ monoclonal paraprotein with a serum concentration of 1685 mg/dL. IgG (normal range, 1070-2310 mg/dL), 25 mg/dL IgA (normal range, 111-557 mg/dL), and 19 mg/dL IgM (normal range, 35-107 mg/dL). When the serum was separated and transferred to another tube for the measurement of cryoglobulin, an opaque, bulky precipitate with spontaneously crystallized from the serum at room temperature, and it did not melt to a clear liquid at 40°C (Figure 1). After removal of the precipitate, the serum was stored at 4°C overnight, and the usual cryoglobulin level was detected. Immunoelectrophoresis of the urine identified the free κ light chain. An osteolytic examination revealed no apparent osteolytic lesions; however, a bone scintigraphy showed abnormal accumulations in multiple vertebrae, the right acromion, and the femoral bones. His bone marrow was hypocellular, with 20% atypical plasma cells. From these results, we diagnosed this case as asymptomatic multiple myeloma according to the 2003 diagnostic criteria of the International Myeloma Working Group. This case was considered stage III B by the Durie-Salmon clinical staging system with atypical cryoglobulinemia (crystalglobulinemia). The patient underwent 2 cycles of chemotherapy with MCNU-VMP (50 mg/m² ranimistone on day 1 by drip intravenous infusion, 2 mg/m² vindesine sulfate on days 1 and 22 by drip intravenous infusion, 8 mg/m² melfalan on days 1-4 and days 22-25 by oral administration, and 40 mg/m² prednisolone on days 1-4 and days 22-25 by oral administration). The patient’s cutaneous ulcers and renal dysfunction improved for a short period with a decrease in the crystalglobulin concentration but then exacerbated when the crystalglobulin concentration increased. After obtaining informed consent and an institutional review board review, we started daily oral administration of thalidomide (200 mg/day) and dexamethasone (4 mg/day) in September 2005. This therapy improved the patient’s severe skin ulcers on the extremities and improved his anemia, proteinuria, and renal dysfunction. These results were accompanied by a disappearance of crystalglobulin in his serum within 8 weeks following the initiation of therapy. Following thalidomide and dexamethasone therapy, the creatinine value improved from 6.9 to 1.9 mg/dL, the urine protein value improved from 0.57 to <0.1 g/day, and the hemoglobin value changed from 7.9 to 10.1 g/dL. The patient’s bone marrow has not been evaluated after chemotherapy to confirm the decrease of myeloma cells in response to the therapy.

An analysis of the crystalglobulin by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and mass spectrometry revealed it to be monoclonal IgG κ produced by the patient’s myeloma cells. The molecular weights of both the light and the heavy chains were increased compared with standard IgG1 samples. To elucidate the cause of this phenomenon, we conducted SDS-polyacrylamide gel electrophoresis of the crystalglobulin before and after peptide N-glycosidase F (PNGase F) treatment. Cryocrystalglobulin precipitated at room temperature was dissolved in reaction buffer (0.5 M Tris-HCl, pH 8.6, 0.5% SDS, 0.1 M 2-mercaptoethanol) and heated at 100°C for 3 minutes. After cooling the cryocrystalglobulin solution to room temperature, we mixed a 10 μL aliquot of the sample solution with 10 μL of 5% Nonidet P-40, 26 μL water purified with a Milli-Q water-purification system (Millipore, Billerica, MA, USA), and 4 μL of 0.5 U/mL PNGase F (Takara Bio, Otsu, Japan) in a microcentrifuge tube. As an enzyme-free control, we added reaction buffer instead of the enzyme solution. The deglycosylation reaction was carried out overnight at 37°C. The shift of bands observed for the patient but not for the control patient, who did not have crystalglobulinemia, indicated that the increased molecular weights of the IgG κ and γ light chains were caused by the modification of N-glycans, especially in the κ chain (Figure 2).

3. Discussion

Among the various complications associated with multiple myeloma, crystalglobulinemia has been reported for only 30 to 40 cases. Ball et al [3] reviewed the characteristics of crystalglobulinemia. Various types of multiple myeloma have similar frequencies of crystalglobulinemia. It occurs more often in male patients (male-female ratio, 2.4:1) and at a median age of 55 years (range, 26-82 years). Although crystalglobulinemia is usually associated with multiple myeloma at the time of diagnosis, it also occurs as essential crystalglobulinemia not associated with a lymphoreticular malignancy in 26% of reported cases [3-10]. Among multiple myeloma patients, multiple myeloma did not develop during follow-up periods until 3 to 20 years after the initial diagnosis of crystalglobulinemia (median, 7.75 years) [3]. Powell et al reported 8 cases of crystalglobulinemia associated with pyoderma gangrenosum and monoclonal gammopathy [11]. In 7 of these cases, the pyoderma gangrenosum preceded the detection of monoclonal gammopathy. In the present case, the clinical manifestation
Crystalglobulinemia with Myeloma

of skin ulcers similarly preceded the detection of monoclonal gammopathy, and this fact may explain why multiple myeloma was not diagnosed in our patient for 4 years. Another reason for the delay in his diagnosis may be that the monoclonal immunoglobulin in the serum was not detected because almost all of the monoclonal immunoglobulin had precipitated in a crystalline form. In fact, the patient’s serum IgG concentration was only 1685 mg/dL, despite the large amount that was present as an insoluble fraction. Crystallization has generally been supposed to occur because of interactions between IgG paraproteins, with contacts either between their Fe fragments [12] or with albumin [6], and may be facilitated by local cooling and stasis in the microvasculature. In the present case, abnormal glycosylation of immunoglobulin may be closely related to the crystallization of this patient’s IgG.

A significant missense mutation that might be the direct cause of the abnormal N-glycosylation was detected by mass spectrometry in the variable region of the κ chain. Details of the glycoproteomic analysis will be reported elsewhere. Foss et al [13] reported a similar case of abnormal glycosylation of the κ chain in both urinary Bence Jones protein and amyloid fibrils deposited in a patient with primary amyloidosis. Furthermore, Omvstved et al [14] has referred to the association of immunoglobulin light chain glycosylation with amyloidosis. This glycosylation has generally been believed to make serum proteins more soluble in serum, because carbohydrate side chains are very hydrophilic. However, abnormal glycosylation of the κ chain actually produced fibril formation at room temperature in the serum of a myeloma patient. We speculate that abnormal glycosylation may cause protein misfolding to give a β-sheet–rich conformation during processing in the endoplasmic reticulum and the Golgi apparatus. Such a glycosylated light chain may be solely amyloidogenic; however, when the abnormally glycosylated light chain binds to a heavy chain, such as a γ chain, the complete immunoglobulin molecule with an extra sugar side chain becomes less soluble in serum at room temperature.

To our knowledge, our report is the first of abnormal immunoglobulin light chain glycosylation in a patient’s serum. Our patient’s cutaneous ulcers and renal dysfunction were improved by thalidomide and dexamethasone therapy but not by the MCNU-VMP regimen. We cannot rule out the possibility that dexamethasone acted directly to decrease crystalglobulin formation. The precise mechanism of dexamethasone’s action is uncertain. Does it act to repair abnormal glycosylation or to remove deposited crystalglobulin, leading to the recovery of skin lesions or renal dysfunction? Further studies are needed to clarify the relationships of the mechanism of crystalglobulin formation to the abnormal glycosylation of the immunoglobulin light chain.

References

