Immunoglobulin Class Switch From IgG to IgA in a Patient With Smoldering Multiple Myeloma

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Serum of a 67-year-old male patient with smoldering multiple myeloma was shown to contain two monoclonal immunoglobulins, IgG and IgA. For the initial seven months, monoclonal IgG was predominantly elevated. During the next one year and eight months, however, serum concentration of the monoclonal IgA increased, with a concomitant decrease of IgG. N-terminal amino acid sequences of heavy and light chains separated from monoclonal IgG and IgA were analyzed. Both light chains were λ-type and showed identical amino acid sequences of variable regions. The heavy chains also had the same N-terminal amino acid sequence between IgG and IgA. These results strongly suggest that two monoclonal proteins, IgG and IgA, in this patient were produced by B lymphocytes within a clone and that class switch from IgG to IgA in immunoglobulin production during B cell differentiation has taken place in the clinical course of this case.

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CASE REPORT

A 67-year-old male was noticed to have a bronchiectasis complicated with a monoclonal hypergammaglobulinemia in May 1975 at a local hospital. A single peak of serum monoclonal immunoglobulin was demonstrated on the γ-region of cellulose-acetate electrophoresis and IgG was over 3,000 mg/dL. In May 1977, IgG was 3,700 mg/dL and IgA was 1,900 mg/dL. The patient gradually developed dullness of the head and pain in distal parts of extremities and was admitted to the Niigata Shimin Hospital on Oct 7, 1977. Physical examination revealed the enlargement of several inguinal lymph nodes and rashes in the lung fields. No hepatosplenomegaly was observed. The hemoglobin level was 8.5 g/dL, white blood cell counts 5,800/μL, and platelet counts 277 × 10^3/μL. Bone marrow aspiration revealed that plasma cells increased to 6.2% of nucleated cells. The total serum protein was 13.0 g/dL, and two separate peaks of monoclonal components were demonstrated first on the β-γ-region of cellulose-acetate electrophoresis. Serum IgG was 11,480 mg/dL, IgA 3,600 mg/dL, and IgM 92 mg/dL. Immunoelectrophoresis showed that two monoclonal components were IgG-λ and IgA-λ. Pyroglobulin was present in the serum.

Renal dysfunction was noticed, but there were no abnormal findings on a generalized bone survey. Histologic examination of the inguinal lymph node biopsy specimen showed diffuse infiltration of lymphocytes and plasma cells. The disease was diagnosed as multiple myeloma and treated with low doses of cyclophosphamide at first, because of hyperviscosity syndrome. However, leukocytopenia due to cyclophosphamide administration had developed, and then only plasmapheresis was performed. There was no sign of deterioration of multiple myeloma during the patient’s course. He died of sepsis on Sept 19, 1979. On his admission, monoclonal IgG was remarkably greater than monoclonal IgA, but the reverse of two immunoglobulin values occurred in February 1978, and IgA increased gradually with concomitant decrease of IgG. IgG was 1,240 mg/dL and IgA was 10,080 mg/dL in April 1979 (Fig 1).

MATERIALS AND METHODS

Two monoclonal components in this patient, IgG and IgA, were isolated from the plasma of their predominant phase by a combination of ammonium sulfate precipitation (33% saturation), diethyl aminoethyl (DEAE)-cellulose (sodium phosphate buffer, pH 8.0), and DEAE-Sephadex (Tris-HCl buffer, pH 8.0) chromatography. The purity of isolated proteins was checked by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate and double immunodiffusion with specific antisera.

Purified IgG (or IgA) (150 mg) was completely reduced and alkylated by the method of Wadsworth and Konigsberg. The H- and
L-chains were separated by gel filtration on Sephadex G-200 (2.5 x 95.0 cm) in 5 mol/L guanidine-HCl/1 mol/L acetic acid. The N-terminal amino acid residue was determined by Edman's method. Before the sequence analysis, H-chain peptides from IgG and IgA were digested with pyroglutamate aminopeptidase (from calf liver; Boehringer Mannheim, FRG) by the method of Podell and Abraham. Automated Edman degradation was carried out in a JEOL JAS-47K sequence analyzer (0.5 mol/L Quadrol program; JEOL, Tokyo). Phenylthiohydantoin derivatives were separated by gel filtration on Sephadex G-200 (2.5 x 95.0 cm).

RESULTS

Amino-terminal sequence analysis. Both L-chains obtained from IgG and IgA, designated \( \lambda_\gamma \) and \( \lambda_\alpha \)-chains, respectively, had only tyrosine as the N-terminal residue. Because no amino acid was detected by the Edman degradation of both H-chains obtained from IgG and IgA, designated \( \gamma \) and \( \alpha \)-chains, respectively, these peptides were treated with pyroglutamate aminopeptidase to remove the N-terminal pyrrolidone carboxylic acid. Table 1 shows the results of the N-terminal amino acid sequence analyses. The N-terminal sequence of \( \gamma \)-chain was identical with that of \( \alpha \)-chain. The sequences of \( \lambda_\gamma \) and \( \lambda_\alpha \)-chains were also identical.

When these N-terminal sequences were compared with other known sequences of the V-regions of H- and \( \lambda \)-chains, the V-regions of H- and \( \lambda \)-chains in this patient were found to belong to \( \text{V}_H \) III and \( \text{V}_\lambda \) III subgroups, respectively (the subgrouping is based on a classification by Kabat and Wu).

DISCUSSION

Multiple myeloma is characterized by monoclonal immunoglobulin in the serum and osteolytic bone lesions associated with an increase of abnormal plasma cells in the bone marrow. Its course is generally progressive and fatal. Multiple myeloma whose course is not progressive and is stable for several years without chemotherapy was designated as smoldering multiple myeloma. The present patient had remained stable for more than four years until he died in September 1979. Since October 1977 two remarkably elevated monoclonal proteins existed in the serum, but plasma cells in the bone marrow were less than 10% and bone lesion was not identified. Therefore, this patient was diagnosed as having smoldering multiple myeloma.

Multiple myeloma is usually associated with single monoclonal protein. Although the prevalence of double monoclonal components in the serum of patients with multiple myeloma is uncommon, it has been recognized in 1% of 6,141 recorded cases of multiple myeloma.

Among 141 cases with double monoclonal proteins, frequencies of different associations were reported as follows: IgG and IgA, 32.6%; IgM and IgG, 24.1%; IgG and IgG, 17.0%; IgM and IgA, 8.5%; IgM and IgM, 7.8%. In 1970, Wang et al. first indicated that L-chains and N-terminal amino acid sequences of H-chains were identical between two monoclonal proteins (IgG-\( \gamma \) and IgM-\( \alpha \)) from a single patient. They speculated that during immunoglobulin synthesis different cells of a single clone synthesized immunoglobulins M and G and that the L-chains and the \( \text{V}_H \) of the two proteins were identical within the clone.

The idea of class switch in immunoglobulin production was brought up by Nossal et al. more than 20 years ago. They suggested that antibody-producing cells go through a sequence by which each synthesizes first 19S and later 7S antibody. This idea was developed and the concept of an intraclassal class switch was established by Cooper et al. This was supported by the demonstration of coexistence of double monoclonal proteins originated from cells within a clone in patients with myeloma. In some of the cases with IgG and IgA double monoclonal proteins, two monocomponents were demonstrated to be the products of common clonal cells by immunofluorescent study, idiotypic antibody study, both of them, or analysis of amino acid sequences. These findings suggested the possible sequence from IgG to IgA in a class switch. However, Gearhart et al. observing a clone of cells developed from an antigen-stimulated mouse B lymphocyte, indicated two pathways to IgA production. One is a direct switch from IgM to IgA, and the other consists of two successive switches from

| Table 1. N-terminal Amino Acid Sequences of H- and L-chains of IgG and IgA from the Patient |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
|                                | 1     | 2     | 3     | 4     | 5     | 6     | 7     | 8     | 9     | 10    | 11    | 12    | 13    | 14    | 15    | 16    | 17    | 18    | 19    | 20    | 21    | 22    |
| \( \gamma \) chain             | PCA   | Val   | Gin   | Leu   | Val   | Leu   | Val   | Val   | Val   | Lys   | Pro   | Gly   | Gly   | Gly   | Gly   | Pro   | Gly   | Ser   | Lys   | Pro   | Gly   | Ser   | Cys   |
| \( \alpha \) chain             | PCA   | Val   | Gin   | Leu   | Val   | Glu   | Ser   | Gly   | Gly   | Gly   | Val   | Val   | Val   | Val   | Val   | Val   | Lys   | Pro   | Gly   | Gly   | Ser   | Lys   | Leu   | Leu   | Ser   | Cys   |
| \( \lambda_\gamma \) chain     | Tyr   | Glu   | Leu   | Thr   | Gin   | Pro   | Pro   | Ser   | Val   | Val   | Ser   | Val   | Ser   | Ala   | Gly   | Thr   | Ala   | Thr   | Ile   | Thr   | Cys   | Ser   |
| \( \lambda_\alpha \) chain     | Tyr   | Glu   | Leu   | Thr   | Gin   | Pro   | Pro   | Ser   | Val   | Val   | Ser   | Val   | Ser   | Ala   | Gly   | Thr   | Ala   | Thr   | Ile   | Thr   | Cys   | Ser   |

PCA, pyrrolidone carboxylic acid.
IgM to IgG and IgA during clonal expansion of antigen-stimulated B cells.

The concept of intraclonal class switch was perfectly compatible to an allelic deletion model for mouse V_{H}-C_{H} gene recombination. Recently, the entire region of mouse C_{H} gene family was cloned by Shimizu et al., the organization of which was shown as C_{2-4}C_{2-4}C_{2-4}C_{2-4}C_{2-4}C_{2-4}C_{2-4}C_{2-4}. As to the human study, C_{H} genes were identified on chromosome 14q32 in the order of μ, δ, γ, γ, α, γ, γ, η, and α. These results indicate that during differentiation of a single B lymphocyte, a given V_{H} gene is first expressed in combination with the C_{μ} gene and then expressed with a different C_{H} gene in the order described earlier.

The patient described here was observed to have monoclonal protein of IgG more than two years before admission to the hospital and then showed remarkable double monoclonal immunoglobulins on admission. They were identified as IgG_{κ} and IgA_{κ} by immunoelectrophoresis. IgG was extremely more predominant than IgA in October 1977. However, the IgG value declined progressively during his clinical course. In contrast, IgA value kept increasing through his terminal stage, with crossing IgG in February 1978. The amino acid sequence analysis of variable regions in H- and L-chains from the patient's monoclonal IgG and IgA has been performed. The N-terminal amino acid sequence of γ-chain of IgG predominant phase was identical with that of α-chain of IgA predominant phase. In addition, the N-terminal sequences of λ_{γ} and λ_{α}-chains were also identical.

These results suggest that the variable regions of H-chains and the L-chains of IgG and IgA, respectively, are identical, which gives us a strong evidence that IgG and IgA were derived from a common clonal immunoglobulin-producing cell lineage. These results, together with the clinical finding that IgA in the patient plasma increased with a concomitant decrease of IgG, give enough evidence to conclude that neoplastic B lymphocytes within a clone in this case have undergone a class switch in C_{H} gene expression from γ to α with cell differentiation in vivo. This is a novel case of myeloma in which an immunoglobulin class switch from IgG to IgA was observed during the clinical course.

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