Asymptomatic (Benign) Monoclonal Gammopathy—A Study of 100 Patients

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ABSTRACT

The clinical and laboratory findings in 100 patients with asymptomatic (benign) monoclonal gammopathy (AMG), followed for a period of 3 to 14 years, are summarized and compared to similar findings in 60 patients with clinically overt multiple myeloma (MM). Owing to the difficulty in the early distinction between AMG and MM in patients without osteolytic bone lesions and Bence Jones proteinuria, the differences found in these two groups of patients were statistically evaluated.

In the absence of clinical findings such as bone pain and Bence Jones proteinuria, the following differences, in immunologic parameters, were found: (1) AMG patients had stable monoclonal components with a mean concentration of 2103 ± 803 mg per dl versus 4170 ± 2550 mg per dl for myeloma patients and (2) the polyclonal IgA and IgM concentrations were higher in AMG patients who also had a higher incidence of lambda light chains (ratio 1:1.1 versus 2:1 for MM patients). A most significant, previously unreported finding was a predominance of monoclonal components of the IgG-1 subclass in AMG patients and the IgG-3 subclass in MM patients. This finding may be an expression of different mechanisms of clonal activation in AMG and MM.

The findings distinguishing these two entities are summarized in a profile based on the differences observed in certain AMG and MM patients in the early, pre-clinical stage of their disease.
Introduction

Monoclonal gammopathy (MG)\textsuperscript{6,24,30} is a general term for diseases accompanied by serum “M” components. The “M” may stand for “myeloma”, “macroglobulinemia”, “malignant” or “monoclonal”.\textsuperscript{12} Osserman coined the term “plasma cell dyscrasia”\textsuperscript{19} which is inclusive of this group of conditions characterized by the excessive proliferation of immunoglobulin producing cells. M-components (MC) occur mainly in plasma cell myeloma (MM), primary macroglobulinemia-Waldenström (MW) and amyloidosis (A). In recent years, however, such components have also been observed in patients with a variety of other pathologic states\textsuperscript{1,11,16,17,20} as well as in apparently healthy individuals.\textsuperscript{24}

The diagnosis of MG is based on the finding of electrophoretically and antigenically homogenous serum and/or urinary immunoglobulin components or their subunits.\textsuperscript{19} This homogeneity is the result of an identical primary structure (amino-acid sequence) of all the immunoglobulin molecules constituting the monoclonal component.\textsuperscript{8} Monoclonal immunoglobulins are the product of a single clone of plasma cells.\textsuperscript{25}

The term “asymptomatic (benign) monoclonal gammopathy” (AMG) is applied to patients with no evidence of clinically overt MM, MW, A, reticular or non-reticular neoplasms, in whose serum and/or urine M-components are detected.\textsuperscript{19} A frequent association of diseases such as cholecystitis, cholelithiasis, peptic ulcer, chronic urinary tract infection, etc. has been reported in patients with AMG,\textsuperscript{11} but it is not clear if these diseases are etio-pathogenically related. The studies of Hallen, Axellson and Bachmann\textsuperscript{3,4,6} have shown that the incidence of AMG is higher than that of MM. Large series of patients with AMG have been followed and thoroughly investigated for many years, without developing clinically overt myeloma.\textsuperscript{3,6,24}

The clinical and immunological findings are reported in 100 patients with AMG, followed in our clinic. These data are compared with the results of our study of 60 patients with clinically overt MM.\textsuperscript{21}

Materials and Methods

One hundred patients aged 17 to 85 years were diagnosed as suffering from AMG and followed in our clinic for a period of 3 to 14 years. Patients were seen regularly at six months intervals, when blood and urine examinations as well as X-ray studies were repeated. All patients had monoclonal serum components, only one had a urinary Bence Jones (BJ) protein as well (less than 500 mg per 24 hrs.). When an M-component was discovered, a thorough physical examination supplemented by a skeletal survey, complete tumor work-up, bone marrow examination and blood chemistries were performed.

The monoclonal components were quantitated in serial dilutions by the radial immunodiffusion technique of Mancini and by densitometry of serum protein electrophoresis on a Beckman R-110 scanner. Light chain type, heavy chain classes and subclasses of the monoclonal components were determined by double diffusion in gel\textsuperscript{28} and immunoelectrophoresis. Screening of the sera for additional minor “M” components (“double producers”) was performed by one of us (F.S.). The concentrations of normal-polyclonal immunoglobulins were determined at the time of diagnosis and at three to six monthly intervals by the radial immunodiffusion technique of Mancini.\textsuperscript{15} Normal immunoglobulins of the same heavy chain class as the monoclonal component, IgD and IgE concentrations were not determined.

Normal control immunoglobulin concentrations were determined by the same
methods in 500 sera from normal subjects. All determinations were performed in duplicate with an error not exceeding ± 5 percent. The normal concentrations were in mg per dl: IgG, 1040 ± 240; IgA, 260 ± 80; and IgM, 125 ± 35. Antibody (Ab.) activity was assayed by double diffusion in gel in the presence of ethylene diamine tetraacetic acid (EDTA), using the following antigens: Salmonella tranarella, Bacillus proteus, Levan-Bacillus-Circulans, Salmonella westlaco, Arabinogalactan and DNP-BSA.

Urinary proteins were quantitated by the biuret method and by densitometry of electrophoretic patterns of urine concentrated in Minicon* chambers.

The anti-sera used were monospecific anti-\(\gamma\), anti-\(\delta\) and anti-\(\mu\)-chain and purchased from Hyland Ltd; anti-Kappa and anti-Lambda monospecific antibodies were prepared in rabbits in our laboratory. IgG\(_1\) and IgG\(_3\) subclass specific reference sera and anti-sera were obtained from F.S. The antigens used for antibody activity testing were provided by Dr. M. Potter, N.I.H., Bethesda, MD.

Owing to the fact that the group of patients reported here numbers 100, the data relating to AMG, in the various tables, can also be read as percent. Statistical significance was determined for differences between means by the student’s-“t”-test and for differences between proportions by the \(X^2\) test.

Results

A quantity of 15,000 consecutive serum and urine samples, from a general hospital population, were screened for MC during a period of seven years. A total of 222 (1.5 percent) serum MC and 55 (0.4 percent) urinary BJ proteins were detected. One hundred of these 222 MC were found in patients classified as AMG, 60 were patients with MM,\(^{21}\) 30 were patients with MM,\(^{22}\) 20 were patients with malignant epithelial tumors\(^{20}\) and 12 were patients with amyloidosis.

Age and Sex

Fifty-seven patients were males and 43 females (M/F = 1.32). The mean age of the 100 AMG patients was 63.4 yr. The respective mean ± S.D. of the age was 66.8 ± 11.2 for males and 59.7 ± 13.7 for females. Two way analysis of variance showed that the females were significantly younger than the males (p (F) < 0.01).

Symptoms

The most frequently associated disorders were arteriosclerotic cardiovascular disease, duodenal ulcer, diabetes mellitus and chronic infections, particularly urinary tract infections. Only a single patient had confirmed gall bladder disease. Clinical signs of the hyperviscosity syndrome were not observed in our patients. In certain patients, the symptoms were those of the associated diseases. In others who were asymptomatic, the MC was detected during periodic “check-up examinations.” None of the patients had bone pain.

Course

The “follow-up period” is defined as the time interval between the discovery of the “M” component and the last subsequent examination. Seventy-eight patients were followed for more than three yr and 22 for more than five yr. Two additional patients were eliminated during follow-up, one developed clinically overt myeloma and the second one developed, after six yr, chronic lymphocytic leukemia. The mean follow-up period was four yr and 87 patients are still alive. Out of the 13 patients who expired, three died of sepsis, five of renal failure and five patients died of other, presumably unre-
lated, causes. Clinical, laboratory and X-ray investigations, repeated periodically over several years, did not reveal an underlying malignancy.

**General Laboratory Findings**

In 75 patients, the erythrocyte sedimentation rate (Westergren) was accelerated (more than 30 mm in the first hr).

A hemoglobin concentration below 12.0 g per dl and hematocrit of less than 40 percent were considered as abnormal in patients of this series. Twenty-one patients had anemia of the normochromic, normocytic type, 14 of these had hemoglobin concentrations of less than 9 g per dl, the lowest concentration being 6.9 g per dl. No patients with pancytopenia were seen.

Five patients were uremic (BUN over 40 mg per dl) and had a high serum uric acid concentration (over 8 mg per dl). Only one patient had hyper-calcemia (12.2 mg per dl).

The serum total protein concentration ranged from 5.8 to 9.5 g per dl with a mean of 8.0 g per dl, the serum albumin concentration ranged from 3.1 to 4.3 g per dl with a mean of 3.7 g per dl and the total globulin concentration ranged from 2.9 to 5.5 g per dl with a mean of 4.2 g per dl. These concentrations remained stable during follow-up periods from three to over 10 yr (table I).

In 63 patients, a bone marrow plasmacytosis of 3 to 10 percent was found. Most plasma cells showed a prominent Golgi region; "immature", multinucleated forms were seen only rarely. In the bone marrow of one patient with an IgA/ MC, typical "flame" cells were seen; in four others, the morphologic appearance of the bone marrow could not be distinguished from MM. In 32 patients, the bone marrow was apparently normal.

Complete skeletal surveys were performed yearly in all patients. No osteolytic lesions, vertebral body wedging or collapse, pathological fractures or osteoporo-
sis incompatible with the patient's age, were found.

**IMMUNOLOGIC INVESTIGATION**

Eighty-six patients had MC of the IgG and 14 of the IgA class. The overall \( \kappa/\lambda \) ratio of the serum M.C. was 48/52. This ratio was 40/46 for the IgG components and 8/6 for the IgA components. The percentage of AMG patients with IgG-MC did not differ significantly from MM patients. Among the AMG patients with IgG or IgA-MC, the \( \kappa/\lambda \) ratio was close to 1:1. Among MM patients with IgG-MC-kappa light chains predominated and the ratio was 3:1. This ratio was found to be significantly elevated when compared to the other groups.

The heavy chain subclass distribution, among AMG patients with IgG-MC, showed a significant reduction in the proportion of IgG-3 subclass in favour of IgG-1 as compared to MM. With regard to IgG-2 and IgG-4, no meaningful comparison was possible due to the small number of cases (table II).

The mean concentration of the 100 MC (IgG + IgA) was 2103 mg per dl. The mean concentration for IgG components was 2131 mg per dl and for IgA components 1700 mg per dl. The mean MC concentrations showed marked stability during follow-up periods of three to 10 years and more. The fluctuation did not exceed the range of the laboratory error. No spontaneous decrease in the serum MC concentration occurred during the period of follow-up.

In patients with IgG-MC, the mean normal polyclonal IgA concentration was 165 mg per dl and the mean normal polyclonal IgM concentration 63 mg per dl. These concentrations remained stable throughout the follow-up period. In patients with IgA-MC, the mean normal polyclonal IgG concentration was 888 mg per dl and the mean normal polyclonal IgM concentration 34 mg per dl. These concentrations also remained stable (table I).

Among AMG patients with IgG-MC, 23.2 percent had normal polyclonal immunoglobulin (Ig) levels in both IgA and IgM while in the MM group, patients with normal polyclonal Ig levels were not found. This difference is highly significant. In AMG patients, only 27.9 percent (24/66) were low in both polyclonal IgA and IgM, while within MM patients with low polyclonal Ig levels, 75 percent were low in both. Among patients with IgA-MC, in both AMG and MM, none had normal polyclonal Ig levels of both IgG and IgM. In both groups, all had low IgM, but in MM, none had low IgG while in AMG, in 35.7 percent this immunoglobulin was low.

In 9 patients, a second minor MC was detected ("double producers"). The typing resulted in five patients with IgG-1/

<table>
<thead>
<tr>
<th>TABLE II</th>
<th>Distribution of Light and Heavy Chain Classes in 100 Asymptomatic Monoclonal Gammopathy and 44* Multiple Myeloma Patients with Serum Monoclonal Components</th>
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<tr>
<td></td>
<td>Asymptomatic Monoclonal Gammopathy</td>
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<tr>
<td>Distribution</td>
<td>No.</td>
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<td>100</td>
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</tbody>
</table>

*An additional 16 patients with multiple myeloma had Bence Jones proteinuria only.

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In 9 patients, a second minor MC was detected ("double producers"). The typing resulted in five patients with IgG-1/
IgG-2, one with an IgG-1/IgG-3, two with IgG-1/IgG-4 and one with IgG-1/IgG-1 double components.

The MC of five patients had “antibody acitivity”, when examined by gel diffusion; three patients with IgG-λ components had anti-DNP activity* and one patient with an IgG-K component had antibody activity against B. proteus mirabilis. One IgG-K component had anti β-lipoprotein activity. The interaction with the ligand of the three MC with anti-DNP activity was weak, the association constants, being lower than 10^4 M^-1 (determined by equilibrium dialysis).

Discussion

Refinement of diagnostic approaches, relying on serum protein electrophoresis, immunoelectrophoresis and gel diffusion, has added a new dimension to the area of monoclonal gammopathies. Among these, AMG and MM are two distinct entities which share a great number of diagnostic features. Numerous reports9,24,26,31,32 emphasize the importance of an early distinction between these two entities, especially when BJ proteinuria and osteolytic lesions are absent.

Many attempts were made to establish better diagnostic criteria for the classification of patients with monoclonal gammopathy for early identification of those who require chemotherapy. A bone marrow plasma cell concentration of over 20 percent is generally considered as a criteria of neoplastic plasmacytic proliferation.5,16,32 However, there is a wide variation of bone marrow plasma cells in patients with MG, and a reliable differential diagnosis cannot be based on morphologic criteria alone.24 Various “MC threshold levels” were also suggested for the distinction between AMG and MM9,16

Ritzmann24 considers 3000 mg per dl for IgG and IgA to be a realistic value for most, but not all, patients with AMG. Lindström concluded14 that “a clear difference” exists between peripheral blood B-cell counts in AMG and MM, but, according to this author, serial studies of individual subjects are still needed to establish the clinical value of this diagnostic test. Mundy’s report18 that “osteolytic bone lesions and hypercalemia in myeloma are due to the secretion of a soluble factor, by myeloma cells, that in turn stimulates osteoclastic activity in adjacent bone”, focuses on a new plasma cell factor. This soluble factor, if proved to be specific for MM, may represent one of the most valuable markers for the distinction of AMG and MM plasma cells.

As long as the specificity of some of these distinguishing features and of their clinical applicability are not shown conclusively, fine differences in clinical symptoms and signs as well as laboratory parameters will have to continue to be carefully analysed and interpreted to make an early distinction between AMG and MM possible.

When immunologic parameters were compared in the 100 AMG patients described in this paper and in the MM patients described by us previously21 different patterns of MC, H and L-chain class and subclass distribution were observed. AMG patients had a higher incidence of MC of the IgG-1 H-chain subclass and a lower incidence of the IgG-3 H-chain subclass. MM patients had a relatively higher incidence of MC components of the IgG-3 H-chain subclass (table II).

Our findings differ from the ones reported by Schui27 and Skvaril28 who found 77 percent IgG-1, 6 percent IgG-3 and 79 percent IgG-1 and 6.5 percent IgG-3, respectively, but these authors did not make a clear distinction between AMG, clinically overt MM and other related conditions. While the κ/λ ratio was for our MM

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* This was confirmed by equilibrium dialysis and DNP-Sepharose gel filtration of isolated Fab fragments produced by papain digestion of these three MC.
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patients 2:1, a ratio of 1:1.1, was found in AMG patients, and a similar reversion was also reported by Ritzmann.24 No significant difference in the frequency distribution of IgG and IgA MC was found in our two groups of patients. However, the differences in the concentrations and stability of the MC as well as in the concentrations of the normal polyclonal immunoglobulins and of hemoglobin were found to be statistically significant in the two groups of patients. Similar observations were previously reported by several investigators.6,9,12,24

The presence of a second MC as well as the demonstration of “antibody activity” of the MC were not found to be significant for the differential diagnosis. Our study of “double producers” and MC with antibody activity showed that 9 percent of AMG and 7 percent of MM patients were double producers and 4 percent of AMG and 2 percent of MM patients had MC with “antibody activity.” No major difference was found by the present authors in the distribution of H-chain classes and subclasses in AMG and MM double producers, and our data closely correlate with those reported by Skvaril.29

Age, sex, total serum protein, serum albumin and serum globulin concentrations as well as the erythrocyte sedimentation ratio (ESR) were also of no significance for the distinction between the two entities. The albumin/globulin ratio, being an indirect expression of the MC concentration, constituted a rather misleading parameter, especially in patients with MC concentrations below 2000 mg per dl. Therefore, albumin/globulin ratios should be considered of historic interest only.

In the absence of BJ proteinuria and osteolysis, owing to the considerable overlap and the non-exclusive nature of most parameters, the early distinction between AMG and MM remain difficult. In recent years, a number of publications discussed ways for the introduction of science into medical decision making, and certain authors were optimistic enough to state that these methods may become as simple as looking up “the correct standard decision tables of decision graphs.”7 This optimism cannot fully be shared by us as our experience shows that in the early phases of the disease, a significant number of patients remain diagnostically unclassifiable. Therefore, it is our belief that in AMG and MM, the evaluation of specific disease factors, in a large series of consecutive patients, permits the construction of characteristic “profiles” which apply to most patients in each diagnostic category. Such an approach was adopted, for MM patients, by Salmon26 and

| TABLE III |
| Profiles Based on Accumulated Data |

<table>
<thead>
<tr>
<th>Monoclonal Components</th>
<th>Normal Polyclonal Ig Concentrations</th>
<th>H &amp; L-chain Typing</th>
<th>Hemoglobin Concentration</th>
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</thead>
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<td>Stability</td>
<td>Concentration</td>
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<td>IgM</td>
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<td>AMG*</td>
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<tr>
<td>p&lt;</td>
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<td>MM†</td>
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<tr>
<td></td>
<td>&lt;100 mg/dl</td>
<td>&lt;50 mg/dl</td>
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</tbody>
</table>

*Asymptomatic (benign) monoclonal gammopathy. †Multiple myeloma (clinically overt).
Acknowledgments

could be an expression of different mechanisms of clonal activation in these two entities.

The profiles based on our accumulated data are shown in table III. From recent evidence, it can be surmised that MM plasma cells differ, from those found in AMG, in their growth kinetics and most probably also by the elaboration of certain substances such as the osteoclast stimulating factor. The data presented in this paper are in good agreement with previous observations and point, in addition, to an interesting difference in \( \gamma \)-chain subclass distribution of MM and AMG monoclonal components which could be an expression of different mechanisms of clonal activation in these two entities.

Acknowledgments

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References


