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Monoclonal Gammopathies and the Related Autoimmune Manifestations in Taiwan

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SUMMARY **A total of 50,000 patients were surveyed for the presence of monoclonal immunoglobulins during the past two decades. There were 411 cases of monoclonal gammopathies including 243 cases of plasma cell neoplasms and 168 cases of secondary plasma-cell dyscrasia. Among the 227 cases of multiple myeloma and Waldenström's macroglobulinemia, there were 49.3% IgG class, 22.9% IgA class, 9.7% IgM class and 13.2% light chain type. In addition, there were 1.3% of non-excretory myeloma including an IgM type. A relatively high frequency (4.8%) of IgD M-proteins was detected but heavy chain disease was not encountered in the present series. Purified M-components from patients with possible autoimmune manifestations were subjected to immunofluorescence studies. Autoimmune activity of M-proteins was found in a patient of Waldenström's macroglobulinemia with peripheral neuropathy, and another patient of cryofibrinogenemia with recurrent purpura and gangrene. In conclusion, a high frequency of IgD myeloma is found in Chinese patients of this area. M-components may have autoimmune activity resulting in unusual clinical manifestations.**

Plasma-cell dyscrasia (PCD) is usually associated with electrophoretically homogeneous immunoglobulin or its subunit, which is used interchangeably with the term monoclonal immunoglobulin or M-component.[1] From the clonal concept, spontaneous and uncontrolled proliferation of one clone of plasma cells results in monoclonal gammopathy on an electrophoregram.[2] The wide application of immunoglobulin quantitation and protein electrophoresis (PEP) has contributed greatly to the detection of M-component.[3] Since the introduction of immunoelectrophoresis (IEP), and recently immunofixation (IF), the heavy chain class and light chain type have been more precisely determined with respect to M-components and their subunits.[4,5] The relative frequency of each class and type of monoclonal gammopathy is reflected in the relative amount of each immunoglobulin in the serum of normal individuals.[6] However, genetic differences in various ethnic groups may account for relatively high percentages of some paraproteins in patients with PCD.[7] A comprehensive report of immunochemical frequency of M-components is still lacking in Chinese patients in this region.

The well-known clinical manifestations related to M-components are renal failure from tubular light-chain precipitation, hyperviscosity and cold intolerance caused by systemic paraproteinemia, and compression of surrounding tissue from extramedullary plasmacytoma.[8] In addition, there are some unusual autoimmune presentations caused by M-components against self antigens.

In the present study, M-components were surveyed by serum PEP in blood samples from 50,000 Chinese patients during the past two decades. The simultaneous urine and/or other body fluids were also screened. Heavy chain class and light chain type were determined by IEP and IF. M-components for further studies were purified from those patients with unusual clinical manifestations.

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MATERIALS AND METHODS

A total of 50,000 Chinese patients was examined per blood samples sent from more than 20 hospitals in Taiwan from 1972 to 1991. The sex ratio was approxiamtely 1 to 1 with the age range from 15 to 95 years. The specimens were screened with serum PEP and interpreted by the members of immunological section, National Taiwan University Hospital. Simultaneous PEP of urine and/or other body fluids was done in patients with M-components of blood, and in those suspected to have PCD. In blood and urine samples with monoclonal immunoglobulins or their fragments, quantitation of immunoglobulin, IEP and IF were performed. In patients with unusual clinical manifestations, monoclonal immunoglobulins were purified for further studies.

PEP

Electrophoresis was carried out on a cellulose acetate membrane, in barbital buffer 0.075 M, pH 8.6.[10] In selected specimens, agarose gels and polyacrylamide gels were used as electrophoretic media to more clearly elucidate the M-components.[11,12]

Immunoglobulin quantitation

Quantitation of immunoglobulins was done by the Mancini's single radial immunodiffusion or nephelometry (Beckman, USA).[9]

IEP/IF

IEP was carried out with 1.2% agar in Michael's buffer as previously described.[13] IF was done by Immunofixation Electrophoresis Kit (Beckman, USA) accc rding to the manufacturer's manual.

Purification of M-components

For IgG and IgA M-components, ammonium sulphate salt fraction was first adopted and followed by DEAE-cellulose ion-exchange chromatography as previously described.[14,15] Separations of IgM M-components were done initially by sepharose 4B gel filtration column, then recycled with Sephadex G-200 gel column to obtain pure IgM.[14,16]

Immunofluorescence study

Sections of healthy human sciatic nerve tissue, cut at 5 μm, were incubated with appropriate dilution of purified M-components.[16] In addition to 5 M-components derived from patients with peripheral neuropathy, other 3 M-components derived from control patients without peripheral neuropathy were also purified and used in immunofluorescence staining. Skin sections from the cryofibrinogenemia were stained with various FITC-conjugated antisera specific to either heavy chains, light chains, complements or fibrinogen. The purified IgG component was studied by double diffusion in agarose gel to analyze its specificity with fibrinogen. The cold saline-washed cryoprecipitate was also subjected to double diffusion to determine its compositions.

Table 1. Distribution and frequency of M—proteins in 243 cases of plasma cell neoplasms

	IgG		IgA		IgM		IgD		LC		Total
	κ	λ	κ	λ	κ	λ	κ	λ	κ	λ	
MM	62	50	30	22	0	0	2	9	17	13	205
WM	0	0	0	0	11	11	0	0	0	0	22
EMP	2	3	2	0	0	0	0	0	1	0	8
SOP	0	1	1	0	0	0	0	0	0	0	2
PA	0	1	0	1	0	0	0	0	0	2	4
PCL	0	0	0	0	0	0	0	0	2	0	2
Total	64	55	33	23	11	11	2	9	20	15	243
Frequency	49.0%		23.0%		9.1%		4.5%		14.4%		100%

LC = Light chain, MM = Multiple myeloma, WM = Waldenström's macroglobulinemia, EMP = Extramedullary plasmacytoma, PA = Primary amyloidosis, SOP = Solitary osseous plasmacytoma, PCL = Plasma cell leukemia.

RESULTS

There was a total of 411 cases of monoclonal gammopathy including 243 cases of plasma cell neoplasms and 168 cases of secondary PCD among the 50,000 patients. The distribution and frequency of M-components in plasma cell neoplasms are summarized in Table 1. Among the 227 cases of multiple myeloma (MM) and Waldenstrom's macroglobulinemia (WM), there were 49.3% IgG class, 22.9% IgA class, 9.7% IgM class, 4.8% IgD class, and 13.2% light chain type. In addition, there were 3 cases (1.3%) of nonexcretory myeloma; among them one case was proved to be IgM class. Presence of intracytoplasmic IgM M-components was demonstrated by immunofluorescence and immunoenzymatic staining of plasma cells obtained from bone marrow.[17] The other 168 cases with various diseases and associated M-proteins are listed in Table 2. They were classified into four types, including neoplasms other than plasma-cell origin, autoimmune and rheumatic diseases, inflammatory and infectious diseases, and monoclonal gammopathy of unknown significance. The most commonly encountered disease was malignant lymphoma, accounting for 13.7% of secondary PCD.

Table 3 shows the unusual clinical manifestations associated with M-components in the immunofluorescence study. A female patient presenting with recurrent purpura and gangrene on the distal extremities had cryofibrinogenemia and IgG monoclonal gammopathy. There were 5 cases of monoclonal gammopathy associated with peripheral neuropathy, including 1 WM, 2 solitary osseous plasmacytomas (SOP) and 2 secondary PCD without other diseases except neuropathy. The associated M-components were 1 IgM, 2 IgG and 2 IgA, respectively. Among the 5 cases with neuropathy and 3 cases without neuropathy, only

Table 2. Diseases associated with 168 cases of secondary plasma cell dyscrasia

Classification	No. of cases
Neoplasm:	56
Malignant lymphoma	23
Chronic lymphocytic leukemia	3
Other hematological neoplasm	12
Solid tumor	18
Autoimmune:	31
Systemic lupus erythematosus	10
Rheumatoid arthritis	6
Primary Sjogren syndrome	6
Essential mixed cryoglobulinemia	2
Other	7
Miscellaneous inflammation or infection[1]	41
Monoclonal gammopathy of unknown significance	40

1= Including one IgG λ lichen myxedematosus and one IgA κ pyogenic granuloma.

Table 3. Unusual clinical manifestations associated with monoclonal gammopathy and immunofluorescence study

Clinical manifestations	Associated PCD (No.)	M–component	Immunofluorescence
Cryofibrinogenemia	Secondary (1)	IgGκ	Strong positive
Peripheral neuropathy	WM (1)	IgMκ	Strong positive
	Secondary (2)	IgGλ ,IgGλ	Negative
	Secondary (2)	IgAλ ,IgAλ	Negative
Control patients	WM (1)	IgMκ	Negative
	MM (2)	IgAλ ,IgAλ	Negative

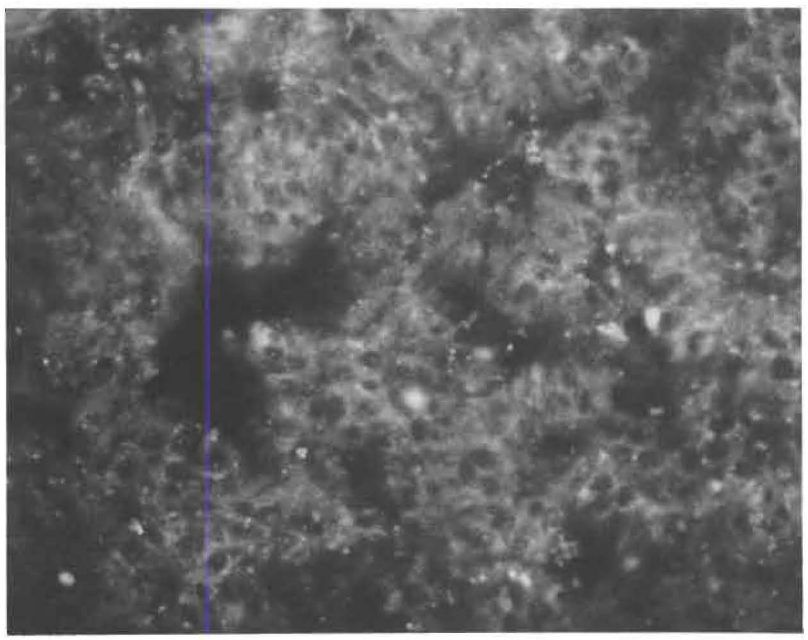

Fig. 1 Indirect immunofluorescence of human sciatic nerve shows positive staining on the myelin sheaths.

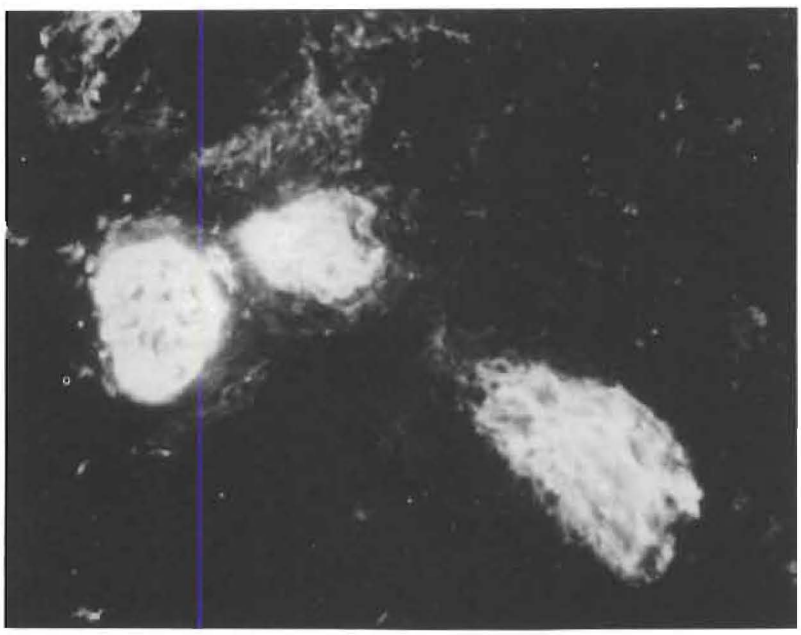

Fig. 2 Direct immunofluorescence of precipitates within vessel lumen of the dermis shows positive staining with anti–gamma chain antibody.

the IgM from a patient with neuropathy had a strong immunofluorescence staining with myelin sheaths of nerve sections (Fig. 1). Strong immunofluorescence was also detected in precipitates within blood vessel lumen of the dermis when stained with anti-kappa, anti-gamma and anti-fibrinogen antisera (Fig. 2). By double diffusion, the cryoprecipitate only reacted with anti-fibrinogen, anti-kappa and anti-gamma antisera. A positive precipitating line also formed between the purified IgG and fibrinogen.

DISCUSSION

Although the relative frequency of heavy chain class and light chain type was approximately reflected to the relative serum concentration in normal individuals, there was a high frequency of IgD monoclonal immunoglobulins and absence of heavy chain M-components in the present study. A high frequency of IgD myeloma, more than 5% among myeloma patients, has been reported in Japanese, as compared with less than 3% reported in Caucasians.[18,19] Approximately 5% of myeloma patients were IgD type in Chinese from the present study. There is a tendency of Orientals to develop undifferentiated forms of B-cell neoplasms, and IgD excretion involves a less-differentiated plasma cells.[7] A role of genetic factors is suggested by the high frequency of IgD myeloma in Orientals. Alpha heavy chain disease is frequently encountered in young adults around the Mediterranean, but it is not seen in this region in the present study.[20]

M-components may have autoimmune activity against self antigens, and the most well known phenomenon is WM with peripheral neuropathy.[16,21] The mechanism by which M-components cause peripheral neuropathy remains controversial, postulated roles including toxin injury, amyloid deposits, lymphoid cell infiltration, serum hyperviscosity and

autoimmunity.[16,22] An immunological mechanism has been suggested by the results of the present IF study, positive staining of the nerve by using purified M-component from the WM patient with neuropathy and negative staining from the WM patient without neuropathy. In addition, IgM from different WM patients with neuropathy has been shown to share cross-idiotypic antigenic determinants located at Fab fragments.[23] Using immunoblot techniques, target antigens attacked by M-components have been found in WM patients with peripheral neuropathy.[24] Thus, autoimmune mechanisms are believed to play a pathogenetic role in peripheral neuropathy of WM.[23,24] For IgG and IgA monoclonal gammopathies associated with peripheral neuropathy, nerve staining was not detected by the use of purified M-components in the present study. This has suggested that the pathogenesis probably differs from that of WM associated peripheral neuropathy. Some toxic substances released by malignant B-cells may have a role in SOP associated neuropathy.[25]

A case with purpura, cryofibrinogenemia and monoclonal gammopathy, similar to the case in our series had been reported, but there were negative pathological findings and no other studies performed.[26] In some patients, there is a tendency for circulating fibrinogen and fibrin to form complexes while exposing to cold temperature.[27] Interaction of gamma globulin with fibrinogen was reported to be responsible for the formation of cryofibrinogen.[28] In the present study, it has been proven that the M-component had immunological reactions with fibrinogen. It is possible that autoimmune mechanism play a role in cryofibrinogenemia associated with M-component.

Monoclonal immunoglobulins have many special physiochemical properties which may escape detection or defy proper interpretation; however, they represent one of the hallmarks of the diagnosis of MM. There is an exceptional variant in which myeloma cells fail to synthesize or to excrete immunoglobulin or its fragments in serum or urine samples.[29] The so-called non-secretory or non-excretory type consists of about 1% of patients with MM and WM according to Western reports.[17,30] In the present series, a similar frequency (1.3%) of patients had such type of myeloma including a rare IgM class. However, patients with non-excretory MM may show evidence of excretion as the disease progresses, which may result from increased synthesis of M-proteins with a limited capacity of extracellular degradation.[17] Further follow-up of these patients with periodic examinations of M-components is necessary to elucidate such possibility.

In conclusion, a total of 411 cases of monoclonal gammopathy were encountered in 50,000 Chinese patients during the past 2 decades. Generally speaking, the relative frequency of various M-proteins corresponded to their physiologic concentrations in normal serum, but a high frequency of IgD myeloma was detected in Taiwan. Autoimmune activity of M-components may exist in a case of WM with peripheral neuropathy and a case of cryofibrinogenemia with recurrent purpura and gangrene. Further studies, including immunoblot analysis are needed to more clearly elucidate the target antigens of these M-proteins.

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