Change in Relative Mobility of M Protein Following Neuraminidase Treatment in Patients With Multiple Myeloma and Monoclonal Gammopathy of Undetermined Significance

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The aim of this study was to clarify the relationship between the relative mobility of M protein in various disease states using cellulose acetate membrane electrophoresis. To examine the carbohydrate chain of the M protein, sera from patients with multiple myeloma (MM), various cancers, and benign disease were treated with neuraminidase.

The relative mobility in benign disease and MM patient sera following neuraminidase treatment varied among individuals,

Key words: M protein; cellulose acetate membrane electrophoresis; neuraminidase treatment

while that of IgG M protein in sera from cancer patients was from 0.2 to 0.3. Thus, the relative mobility in cancer patients was narrower than in those with MM or benign disease.

However, after neuraminidase treatment, there was no significant difference between relative mobility in cancer patient's sera and those in other disease patients. *J. Clin. Lab. Anal.* 18:182–186, 2004. © 2004 Wiley-Liss, Inc.

INTRODUCTION

It has been reported (1–3) that approximately 3% of the population over 70 years of age have monoclonal gammopathy without showing evidence of multiple myeloma (MM), amyloidosis macroglobulinemia, or other related lymphoproliferative disorders. Cases of benign monoclonal gammopathy are termed monoclonal gammopathy of undetermined significance (MGUS). However, Kyle (4,5) reported that 11% of MGUS patients developed MM, macroglobulinemia, or a malignant lymphoproliferative process from 5 to 35 years later. Thus, an understanding of the properties of M proteins is considered to be very useful for diagnosing and treating MGUS-related diseases.

We previously reported that the same type of M protein showed a variety of relative mobility values using cellulose acetate membrane electrophoresis (6), with cancer patients most frequently showing the IgG M protein. We questioned why M protein bands of the same class appeared at various positions. In the present study, we summarized the changes in relative mobility following neuraminidase treatment according to the

type of M protein using sera from MM and MGUS patients, with special focus on cancer patients with the IgG M protein.

MATERIALS AND METHODS

Subjects

Serum samples were collected from 48 patients with the M protein who underwent clinical examinations at Sanraku Hospital. The 48 patients were both in- and out-patients and ranged in age from 40 to 95 years. All subjects gave informed consent and agreed to our use of their samples for this study. The samples were stored at –80°C until analysis.

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Cellulose Acetate Membrane Electrophoresis, Protein Fractions, and Immunofixation

All serum samples were analyzed using cellulose acetate membrane electrophoresis. The specimens were loaded onto a Separax-SP membrane (Fuji Photo Co. Ltd., Tokyo, Japan) and electrophoresed in 0.6 mol/l of veronal buffer (pH 8.6) at 0.7 mA/cm for 30 min. The membrane was stained with 0.8% Ponceau-3R in a 6% TCA solution then destained in a 3% acetic acid solution.

Immunofixation was carried out according to Aguzzi et al. (7) using specific antiserum (Dako, Glostrup, Denmark) to IgG, IgA, and IgM (kappa and lambda types for each) for 20 min.

Neuraminidase Treatment

Neuraminidase treatment was carried out in a microtube in which 10 μ L of serum and 10 μ L of 1 U/mL of neuraminidase III (TOYOBO Co., Osaka, Japan) diluted in 0.1 M of phosphate buffer and 5 mM of EDTA (pH 6.0) were mixed at 37°C and kept overnight.

Relative Mobility of M Protein Bands

The M protein bands showed various positions which represented M protein relative mobility. To calculate relative mobility, the electrophoretic relative mobility distance of the M protein was divided by that of albumin (6).

Statistical Analysis

ANOVA was used for testing the statistical significance of intergroup differences, taking $P < 0.05$ as the level of significance.

RESULTS

Patients

Of all 48 patients who expressed M protein, eight patients had IgA type M protein, one had MM, and seven, including a cancer patient, had MGUS. A total of 36 patients who expressed IgG M protein with an established diagnosis were grouped according to individual diseases. Six patients had MM and 30 had MGUS, which was 62.5% of all patients with M protein. Of those 30 MGUS patients, nine had cancer (prostate, alimentary, kidney, osteoncus, or lung cancer) and 21 had benign disease (bone fracture without ill changes such as osteomyelodysplasia, infection, gout, diabetes mellitus, hepatitis, hypertension, hernia, cholecystolithiasis, cataract, tinea, or gastric ulcer osteoporosis). Four patients had IgM type M protein and one of these four had macroglobulinemia.

Change in Relative Mobility of M Protein Bands Following Neuraminidase Treatment

We examined the change in relative mobility of the M protein with or without neuraminidase treatment. Fig. 1 shows serum protein fraction images before and after neuraminidase treatment of serum samples from patients with M-proteinemia. The M protein band was identified by immunofixation. Following neuraminidase treatment of patient serum samples, the position of albumin did not change, but the fractions from the α 1 globulin fraction to γ globulin fraction shifted towards the minus side. However, the position of the M protein band changed in some patients but not in others.

The average and SD results are shown in Table 1. The IgG M protein relative mobility of 0.291 ± 0.079 changed to 0.266 ± 0.075 after neuraminidase treatment.

Fig. 1. Electrophoretic pattern of M protein following neuraminidase treatment. Following neuraminidase treatment, most of M protein bands changed position to become similar to the sample application position (origin) in comparison with that prior to treatment, though some sample positions did not change, i.e. IgG (I). *M protein band.

TABLE 1. Age and relative mobility according to M protein type (mean $\pm$ SD)

	IgA(n = 8)	IgG(n = 36)	IgM(n = 4)
Age	68.5 $\pm$ 7.7	68.0 $\pm$ 11.7	68.6 $\pm$ 13.2
Relative mobility			
Untreated	0.434 $\pm$ 0.068	0.291 $\pm$ 0.073	0.327 $\pm$ 0.039
With treatment	0.378 $\pm$ 0.051	0.266 $\pm$ 0.075	0.267 $\pm$ 0.054
P value untreated vs. with treatment	0.005	0.0001	0.039

The IgG kappa:lambda ratio was 22:14, with the IgG kappa type M protein relative mobility of 0.291 ± 0.076 changed to 0.264 ± 0.075 and the IgG lambda type M protein relative mobility of 0.292 ± 0.089 changed to 0.270 ± 0.077 .

Five out of 22 (22.7%) patient sera samples with the kappa type M protein and eight out of 14 (57.1%) with the lambda type showed no change in relative mobility following neuraminidase treatment. Further, there was a higher incidence of IgG lambda type M protein than the kappa type.

The IgA M protein relative mobility was 0.434 ± 0.068 , and following neuraminidase treatment, it changed to 0.378 ± 0.051 . The IgM M protein relative mobility of 0.327 ± 0.039 changed to 0.267 ± 0.054 following neuraminidase treatment.

The average of relative mobility of IgG M protein was smaller than those of IgA or IgM. The relative mobility of IgA and IgM M protein following neuraminidase treatment was changed more than that of IgG M protein.

Change in the Relative Mobility of M Protein Bands According to Age

The relative mobility of the IgA and IgG M proteins correlated with the age of the patients with positive correlation coefficients of 0.962 and 0.169, respectively. The relative mobility of M protein following neuraminidase treatment correlated with the age of the patients with positive correlation coefficients of 0.829 and 0.219, respectively. For IgA M protein patients, the relative mobility showed a good relation to the patient's age, i.e., relative mobility increased with age.

Change in Relative Mobility According to Disease Following Neuraminidase Treatment

Next, we examined the differences between relative mobility in sera from patients with MM, various cancers, and MGUS excluding cancer (benign disease). Of all 48 patients that expressed M protein, 36 IgG type M protein with established diagnosis were grouped by individual disease. The M protein isotype identified in those patients are summarized in Table 2.

TABLE 2. Change in the relative mobility according to disease after neuraminidase treatment

Type(case)	Relative mobility neuraminidase treatment		P
	–	+	
MM			
ALL(6)	0.316 $\pm$ 0.104	0.288 $\pm$ 0.110	0.179
IgGK(3)	0.344 $\pm$ 0.108	0.301 $\pm$ 0.125	
IgGL(3)	0.261 $\pm$ 0.041	0.261 $\pm$ 0.041	
Cancer			
ALL(9)	0.298 $\pm$ 0.056	0.249 $\pm$ 0.025	0.208
IgGK(6)	0.278 $\pm$ 0.033	0.242 $\pm$ 0.028	
IgGL(3)	0.337 $\pm$ 0.080	0.262 $\pm$ 0.015	
Benign disease			
ALL(21)	0.273 $\pm$ 0.074	0.263 $\pm$ 0.071	0.253
IgGK(13)	0.272 $\pm$ 0.065	0.257 $\pm$ 0.060	
IgGL(8)	0.276 $\pm$ 0.091	0.273 $\pm$ 0.091	

The mean values and SD of the relative mobility of all M protein bands in MM, cancer, and benign disease patients are shown in Table 2. The relative mobility in MM patients and benign disease patients was not significantly different. Further, the kappa:lambda ratio was 3:3 in MM patients, 6:3 in cancer patients, and 13:8 in benign disease patients. Isotype incidence was not significantly different between MM, cancer, and benign disease.

No change in relative mobility following neuraminidase treatment in MM patients occurred in one out of three (33.3%) with the kappa type and three out of three (100%) with the lambda type. In cancer patients, no change occurred in two out of six (33.3%) with the kappa type and one out of three (33.3%) with the lambda type. In benign disease patients, the occurrence was two out of 13 (15.3%) with the kappa type and four out of eight (50%) with the lambda type. The occurrence of no change in relative mobility following neuraminidase treatment was higher in lambda type than in kappa type patients.

The relative mobility in cancer patients was narrower than in those with MM or benign disease. Following neuraminidase treatment, the relative mobility of the IgG M protein in cancer patients showed a narrow range (0.217 to 0.270), whereas in those with MM and benign disease, it showed a wide range (0.169 to 0.516 and 0.123 to 0.450, respectively) (Fig. 2).

DISCUSSION

We attempted to clarify the relationship between the relative mobility of M protein and carbohydrate chain

Fig. 2. Relative mobility of IgG M protein in patients according to disease. Left Y ray: Relative mobility of M protein. Relative mobility in serum from cancer patients following neuraminidase treatment ranged from 0.2 to 0.3.

structure, or disease, using cellulose acetate membrane electrophoresis, and we examined the difference in relative mobility of the IgG M protein in MM and other disease patients, especially those with cancer. Previously, we found that the relative mobility of M protein increased with age, which we suggested was due to changes in the carbohydrate chain associated with age (6). In addition, we reported that the IgG M protein was most frequently expressed in cancer patients.

IgG consists of two heavy chains and two light chains that are composed of four homologous domains (V_H , C_{H1} , C_{H2} , and C_{H3}) and two homologous domains (V_L and C_L), respectively. Further, IgG molecules possess a single conserved glycosylation site at Asn-297¹ in the C_{H2} domain of each of the heavy chains.

The neutral oligosaccharides linked to IgG are classified into three types—G0, G1, and G2—which possess 0, 1, and 2 terminal Gal residues, respectively. The proportion of carbohydrate chains of IgG bearing G0 is increased in the serum of patients with rheumatoid arthritis (RA) (8,9), juvenile chronic arthritis (10), active Crohn's disease (11,12), infection due to *Mycobacterium tuberculosis* (12), and spondyloarthropathy (13).

Neuraminidase treatment is a simple method for the detection of sialic acid binding in the carbohydrate chain. The M protein without sialic acid, i.e., G0 IgG, does not change position in serum protein fractions during cellulose acetate membrane electrophoresis following neuraminidase treatment. Based on that, we treated sera that came from IgG type M protein patients who had MM and MGUS (including those with cancer) with neuraminidase, and compared the relative mobility of M protein with or without neuraminidase treatment.

The relative mobility in MM and cancer patients was significantly different, while that of IgG MM patients showed a wide range, as well as various migrations following neuraminidase treatment. Further, the carbohydrate chain structure in MM patient serum samples showed a varied microheterogeneity.

Schipper and Prange (14) reported that MM patients with IgG type M protein had more sub-fractions extending over a wider pI range than those with MGUS and that M protein in MM patients is less stable and more likely to release charged amino groups (14). The present results using serum of MM patients with IgG M protein patients were demonstrated using cellulose acetate membrane electrophoresis, a routine test conducted in most clinical laboratories.

Following neuraminidase treatment, the M protein band in cancer patients appeared in a narrow range (0.217 to 0.270), whereas the relative mobility in benign disease and MM patient sera varied from individual to individual. In cancer patients, a peculiar immunoglobulin might be produced, and we intend to investigate the M protein structure in those patients in a future study. The relative mobility of M protein in serum following neuraminidase treatment, which changed from 0.2 to 0.3, may be a new marker to determine whether benign disease patients with the M protein will develop cancer.

Three of the benign patients had gouty arthritis and the relative mobility did not change following neuraminidase treatment in any of their samples. G0 may have been increased in the gout patients, and may increase in those with RA or infection as well, because it is known that G0 is increased in patients with these diseases (8, 9, 12).

At the point of the M protein isotype, the relative mobility according to disease was not significantly

different following neuraminidase treatment. Lambda type M protein, however, showed a higher incidence than the kappa type even though its relative mobility showed no change following neuraminidase treatment.

We examined IgA and IgM M protein in sera, even though the number of patients in this group were few in number. IgA and IgM have more carbohydrate chains than IgG, which has only two. Differences in the number of the carbohydrate chains caused changes in relative mobility following neuraminidase treatment. Crook et al. (15) suggested that the sialylation status of the immunoglobulin paraproteins may be different for IgG, IgA, and IgM in MM patient groups. We speculated that our findings were due to changes in the carbohydrate chain structure.

We previously reported that the IgA M protein in MM and MGUS patients showed changes in the electrophoretic pattern using cellulose acetate membrane isoelectric focusing (16). The width of the M protein band in MM patients with IgA M protein decreased following neuraminidase treatment, whereas that in MGUS patients increased. Further, in patients with cancer and the IgA M protein, the relative mobility after neuraminidase treatment changed to 0.273, which was similar to that of patients with cancer and the IgG M protein (data not shown). In the future, we intend to collect additional serum samples from IgA type M protein patients and perform further experiments.

In the present study, we investigated whether the IgG M protein in cancer patients was different from that in patients with MM or benign disease patients. This was done using cellulose acetate membrane electrophoresis following neuraminidase treatment, which is considered to be a simple and useful method for the detection of sialic acid in the carbohydrate chain.

The relative mobility of IgG M protein in sera from cancer patients following neuraminidase treatment was from 0.2 to 0.3. We will continue to follow the patients with benign disease, whose relative mobility after neuraminidase treatment was from 0.2 to 0.3, to determine whether they develop cancer.

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