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Antibodies against GM1 Gangliosides Associated with Metastatic Melanoma

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SUMMARY The aim was to analyze humoral immune response against GM1 ganglioside expressed on the surface of melanocytic cells, and the possible correlation between the level of antibodies against GM1 IgG and IgM class and melanoma progression. The study included 128 adult patients with malignant melanoma, without paraneoplastic neurologic disorders, 48 adults with dysplastic nevi and 48 healthy volunteers. The presence of IgM and IgG antibodies against GM1 was demonstrated by Immunodot method. Automatic evaluation of strips marked with GM1 antigen was performed by EUROLineScan software. Lactate dehydrogenase (LDH) activity was evaluated by spectrophotometry. Serum concentration of gangliosides was determined using the method with resorcinol-HCl. IgG antibodies against GM1 gangliosides were identified in six patients with melanoma (4.68%) and in none of the subjects from other groups. AntiGM1 IgM class were observed in 20 (15.63%) melanoma patients, three (6.25%) dysplastic nevi patients and one healthy volunteer. No statistically significant difference was observed when serum profile of GM1 IgM antibodies in patients with localized melanoma was compared with that of other study subjects. The levels of IgM antibodies varied with clinical stage of tumor and histopathologic features. Moreover, a statistically significant positive correlation was found between IgM antibodies and LDH (r=0.87; p=0.01; IC=95%). In conclusion, antibodies against GM1 ganglioside are frequent in patients with melanoma. Dysplastic nevi and early melanoma cannot be differentiated using the antiGM1 antibody profile. The synthesis of these antibodies is characteristic for advanced stages of melanoma.

KEY WORDS: malignant melanoma, lactate dehydrogenase, lipid associated sialic acid (LASA), GM1 antibodies

INTRODUCTION

The major ganglioside components isolated from nevi melanocytes were monosialogangliosides, and from melanoma disialogangliosides (1); these results corroborated previous data (2,3). These statements support the idea that sialyltransferase II is activated in melanoma cells and differentiates gangliosides biosynthesis to a-series or b-series (4). It should be noted that sialyltransferase II (GD3 synthase) and GM2 synthase (GM3: GalNAc transferase) are important enzymes which using the same substrate determine the biosynthesis of gangliosides in two series, a and b (5).

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GM1 influence growth and cell differentiation, proliferation and adhesion, by regulating signal transduction and transmembrane receptor activity. Various clinical and experimental trials suggest that GM1 modulate nerve growth factor (NGF) receptor by activating tyrosine kinase (6), mitogen-activated protein kinases and cAMP-response element-binding protein in the retina with axotomized nerve (7), stimulate dimerization of receptors for tyrosine kinase-dependent neurotrophic factors (8), and act as co-receptor for fibroblast growth factor (FGF) transmembrane receptors (9) and as a binding site or receptor for bacterial toxins (*Vibrio cholera*, toxigenic *Escherichia coli* diarrhea) (10-12). GM1 and GM2 inhibit cellular growth in a platelet-derived growth factor (PDGF) and epideremal growth factor (EGF) dependent manner (13,14). GM1 and GM2 might also have mitogenic activity (15). Stimulating the endothelial smooth cells with GM1 and GM2 determined a dose dependent increase of DNA. GM1 and GM2 stimulate extracellular signal-regulated kinases (ERKs) 1 and 2 and c-Jun N-terminal kinase (JNK) phosphorylation, but it has no effect on MAPK phosphorylation (15). GM1 is a major receptor for galectine-1, present on human neuroblastoma surface (16). GM1 might induce ERK1/2 activation and stimulation of DNA synthesis in U-1242 human glioma cells (17) and teratocarcinoma (18,19).

Other studies have demonstrated that GM1 induces apoptosis (20,21), exogenous GM1 induces apoptosis of feline thymocytes by NF-KB suppression and a decrease in interleukin-2 (IL-2) and interferon gamma (IFN-γ) production. GM1 protects dopaminergic neurons (22,23), even in case of neurotoxin exposure (24,25). Various studies evaluated the expression, distribution and evaluation of antigenic capacity of GM1 ganglioside in neoplastic cells (1,26,27).

Recently, the authors have shown that GM1 are expressed in less than 10% of investigated melanoma cells (the study included 411 primary melanoma cases). GM1 concentration varied between 0 and 4% of the total level of ganglioside sialic acid determined in melanoma cells (1).

Taking into account these hypotheses, the aim of the study was to investigate whether GM1 ganglioside could induce antibody synthesis and whether the antibodies against GM1 could be interesting in the management of patients with melanoma. In addition, we assessed the humoral immune profile against GM1 expressed on the surface of melanoma cells and the possible association of anti GM1 IgG and IgM class of antibodies with melanoma progression.

MATERIALS AND METHODS

This prospective study included patients with melanocytic lesions with clinical, histopathologic and/or immunohistochemical diagnoses. All study subjects signed an informed consent form and the Hospital Ethics Committee approved the study protocol.

Inclusion criteria were adult patients with melanocytic lesions without initial treatment and without associated paraneoplastic neurologic disorders. Exclusion criteria were neurologic and psychiatric disorders, autoimmune diseases, malignancies, infectious diseases, other substances that interfere with the samples (triglycerides >2000 mg/dL, conjugated bilirubin >20 mg/dL, unconjugated bilirubin >20 mg/dL, serum hemoglobin >500 mg/dL), and immunostimulating treatment.

The study was conducted during the 2008-2012 period and included 128 patients with primary melanoma and 48 patients with dysplastic nevi that met the criteria for inclusion in our clinical and laboratory study. Control group included 48 healthy volunteers. The age of the patients was between 28 and 83 years, and the male to female ratio was 1:1.37 in melanoma group, 1:1.19 in dysplastic nevi group, and 1:1.29 in control group.

Hematologic studies were done on an ABX Pentra 60 automatic analyzer (ABX Diagnostics, France), biochemical assessments on a HumaStar analyzer

Table 1. Automatic evaluation of strips marked with GM1 antigens using EUROLine Scan software

Signal intensity EUROLine Scan	Result	Antibody class	Melanoma	Dysplastic nevi	Control
0-5	0 Negative	Ig G	116 (90.62%)	44 (91.67%)	48 (100%)
		IgM	93 (72.65%)	40 (88.33%)	45 (93.75%)
6-10	(+) Borderline	Ig G	6 (4.69%)	4 (8.33%)	0
		IgM	15 (11.71%)	5 (10.42%)	2 (4.17%)
11-25 or 26-50	+/++ Positive	Ig G	6 (4.68%)	0	0
		IgM	17 (13.29%)	3(6.25%)	1 (2.08%)
>50	+++ Highly positive	Ig G	0	0	0
		IgM	3 (2.34%)	0	0

Table 2. Antibodies against GM1 ganglioside IgG and IgM class in patients with melanoma, dysplastic nevi and control group

Group	Antibody class	Positive	Negative	p
Melanoma (n=128)	IgG	6	122	NS
	IgM	20	108	0.001
Dysplastic nevi (n=48)	IgG	0	48	NS
	IgM	3	45	NS
Control group (n=48)	IgG	0	48	1
	IgM	1	47	1

Cases (n) are presented with values under 10 (negative) and over 11 (positive) for every lot; NS = non significant; $p<0.05$ = statistical significance for CI=95%

(Human GmbH, Wiesbaden, Germany), and serologic parameters by ELISA method (Huma Reader HS, Germany). Lipid associated sialic acid (LASA) was extracted, isolated and purified using the procedures described elsewhere (28) and determined by the method with resorcinol-HCl (29).

Assessment of antiganglioside antibodies was performed by Immunodot method (30) (EUROIMMUN AG) and the results were quantified using the EuroLine Scan software.

Study materials were venous blood samples collected on anticoagulant for hematologic determinations and serum for humoral assessments.

The following parameters were evaluated in all study subjects: (a) serum level of anti GM1 antibodies (IgM and IgG type), LDH activity, serum LASA; and (b) association of GM1 antibodies with histopathologic and biochemical parameters for melanoma staging.

Statistical analysis was done by use of the SPSS program. The results were expressed as mean ± standard deviation. We chose $p<0.05$ with statistical significance for CI=95%. The correlations between the variables were established by linear regression using ANOVA tests. Comparisons to the matched controls were made for the overall patient population and also by the American Joint Committee on Cancer (AJCC) staging.

RESULTS

Prevalence of autoantibodies anti GM1 in patients with melanocytic lesions

The humoral immune response antiGM1 in adult patients with malignant melanoma, in patients with dysplastic nevi and in healthy volunteers is presented in Table 1. Sampled materials were analyzed using EUROIMMUN test kit for human antibodies against gangliosides of IgG and IgM class. We established a cut-off value 10 based on the EUROLineScan software signal intensity in 48 samples from healthy volunteers and 176 patients with melanocytic lesions. Results in the range from 0 to 5 (no signal visual evaluation), or from 6 to 10 (very weak band) were considered negative; results in the range from 11 to 25 (medium band),

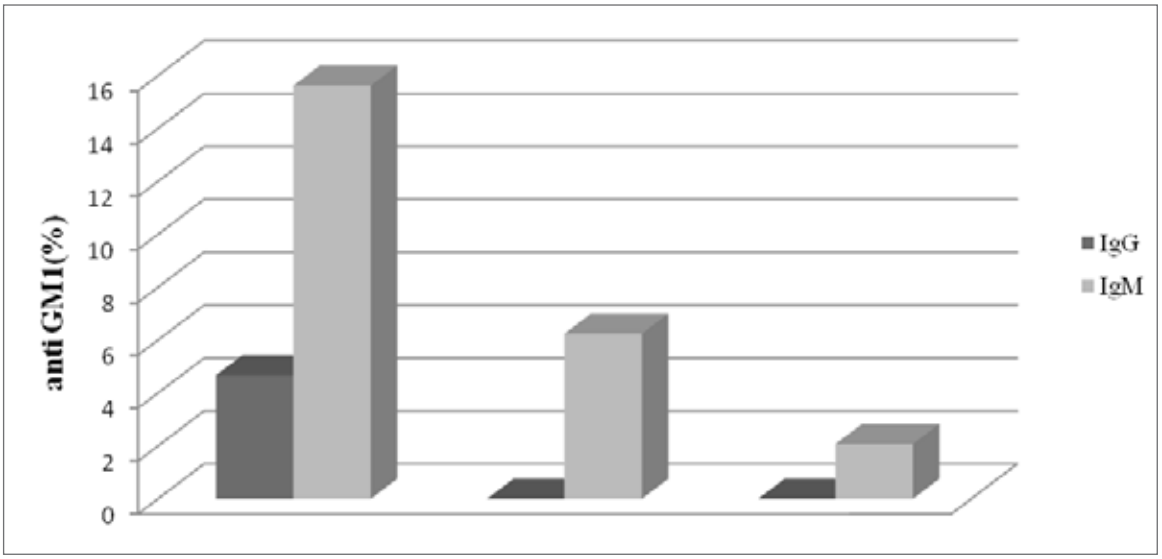

Figure 1. Prevalence of autoantibodies against GM1 in patients with melanocytic lesion and control group.

26 to 50 (strong band) and signal intensity >50 (very strong band) were considered positive.

The following results were obtained for antiGM1 antibodies: in 90.62% of patients with melanoma, anti GM1 IgG class were undetectable, while in 4.69% signal intensity was between 6 and 10, and in 4.69% between 11 and 50. Out of 48 patients with dysplastic nevi, 91.64% did not have detectable IgG antibodies, in 8.33% the signal intensity was between 6 and 10, and in 4.68% it was between 11 and 25. In control group, there were not detected antibodies of IgG class.

In 72.65% of patients with melanoma, antiGM1 antibodies IgM class were negative, in 11.71% signal intensity was between 6 and 10, in 13.29% between 11 and 50, and in 2.34% it was over 50. In 88.33% of patients with dysplastic nevi, no IgM antibodies were determined, in 10.42% signal intensity was between 6 and 10, and in 6.25% between 11 and 25. In 93.75% of 48 healthy volunteers, no IgM antibodies were observed, in 4.17% signal intensity was between 6 and 10, and in 2.08% between 11 and 25. In consequence, measurable IgG antiGM1 were observed in 4.68% of cases, and IgM antiGM1 in 15.63% of patients with malignant melanoma (Fig. 1).

There was no statistically significant difference in antiGM1 antibodies of IgG class between patients with melanoma and those with dysplastic nevi *versus* control. A statistically significant difference was recorded in antiGM1 antibodies of IgM class between melanoma patients and healthy individuals (p=0.001) (Table 2).

AntiGM1 immune profile and melanoma progression

AntiGM1 antibody titer was analyzed according to predefined stratification variables: sex, histologic characteristics (Breslow index, Clark level, histologic type of tumor, site of primary tumor, and presence/absence of ulceration) and biochemical markers for disease staging (LDH activity and serum LASA concentration).

Table 3. IgM anti GM1 antibodies stratified by clinical and histopathologic characteristics in melanoma patients

Parameter	Signal intensity of IgM	p
Sex		
Male (n=54)	8.96±18.28	p=0.1427
Female (n=74)	5.14±11.09	
Site of tumor		
Head-neck (n=11)	13.28±9.86	p=0.022
Trunk (n=83)	8.87±17.30	
Limbs (n=86)	0.88±1.24	
Histologic type		
Nodular melanoma (n=61)	13.26±19.28	p=0.0001
Extensive surface melanoma (n=21)	0.82±1.11	
Lentigo malignant melanoma (n=22)	0.77±0.97	
Acral-lentiginous melanoma (n=9)	1.0±0.5	
Unclassified melanoma (n=15)	1.41±1.40	
Breslow index		
0-1.0 mm (n=22)	0.82±1.11	p=0.0004
1.01-2.0 mm (n=34)	1.23±1.15	
2.01-3.0 mm (n=16)	9.18±6.82	
3.01-4.0 mm (n=36)	9.0±15.59	
>4.01 mm (n=20)	16.5±26.73	
Clark level		
II (n=27)	1.07±1.30	p=0.0001
III (n=47)	1.0±0.97	
IV (n=36)	12.61±14.85	
V (n =18)	18.38±27.59	
Ulceration		
Ulcerated melanoma (n=17)	36.43±23.86	p=0.0001
Unulcerated melanoma (n=108)	2.46±4.39	

Results (mean ± SD) were grouped according to melanoma characteristics; p<0.05 = statistical significance for CI=95%.

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Table 3 shows the means and standard deviations of signal intensity of anti GM1 antibodies IgM class in patients with melanoma grouped according to clinical and histopathologic criteria. The signal intensity of anti GM1 antibodies IgM class did not vary with sex. In patients with head and neck melanoma, the signal intensity of IgM antibodies was higher than with other tumor sites. In patients with higher Breslow and Clark levels, the signal intensity was higher. The presence of ulceration increased the antibody signal intensity. Statistically significant differences were recorded in IgM signal intensity according to the anatomic site of tumor (p=0.022), histologic type (p=0.0001), Breslow index (p=0.0002), Clark level (p=0.0001), and presence/absence of ulceration (p=0.0001).

Table 4 summarizes the means and standard deviations of anti GM1 antibodies IgM class, LDH activity, and LASA concentration in 20 patients with measurable antibodies. Although we had a small number of patients, statistical analysis showed positive correlation between signal intensity of IgM against GM1 and LDH (r=0.87, p=0.01, IC=95%), intensity signal of IgM and LASA (r=0.86, p=0.72, CI=95%), and LDH and LASA (r=0.65, p=0.005, IC=95%).

DISCUSSION

The results of the study demonstrated that the assessment of GM1 antibodies IgM and IgG type in healthy volunteers (n=48) yielded negative findings, which is consistent with data reported by other authors (30). We did not observe any important values of GM1 antibodies in patients with dysplastic nevi. In patients with malignant melanoma without initial treatment or paraneoplastic neurologic disorders, a significant level of GM1 antibodies was detected (Tables 1 and 2). The main antibodies identified were of IgM type (Fig. 1). We have to mention that patients with dysplastic nevi could not be distinguished from patients with early melanoma. The incidence of anti-GM1 antibodies was higher in metastatic melanoma (Table 3). The clinical and paraclinical data were corroborated to exclude the false-positive cases due to cross-reaction with microbial antigens or the possible neurologic paraneoplastic manifestations. The changes in anti-GM1 synthesis associated with oncogenic transformation generated several experimental approaches concerning the link between antibody level and neoplastic disease activity. However, in patients with melanoma, IgM anti-GM1 showed correlation with clinical stage of the disease and histopathologic markers Breslow index and Clark level, anatomic site of tumor, histologic type, and presence of ulceration (Table 3). We observed a significant positive correlation (Table 4) between IgM anti-GM1 level and LDH (a marker for melanoma staging accepted by the American Joint Committee on Cancer), using ANOVA test.

Table 4. Correlation between IgM anti-GM1 antibodies, LDH and LASA in melanoma samples

Variable	Signal intensity IgM	LASA
LDH (U/L) (533.25-246.26)	r=0.87 p=0.01	r=0.65 p=0.05
LASA (mg/dL) (50.25±30.81)	r=0.86 p=0.72	1
Signal intensity IgM (33.0±22.7)	1	

LDH = lactate dehydrogenase; LASA = lipid associated sialic acid; results were considered statistically significant when p<0.05 for CI=95%.

Previous studies in patients with prostate cancer (31) or sarcoma (32) showed that anti-GM1 antibodies had no diagnostic or prognostic value in these pathologies. In patients with differentiated thyroid cancer, anti-GM1 IgM and IgG types correlated with carcinogenesis, but the lack of correlation between antibody level and clinical status indicated that anti-GM1 antibodies had no diagnostic value in differentiated thyroid cancer (33).

In a large cohort of patients with systemic lupus erythematosus, anti-GM1 ganglioside antibodies were associated with neuropsychiatric disorders, migraine, organic brain syndrome and peripheral neuropathy (34). Anti-GM1 antibodies were identified in serum of patients with idiopathic hepatitis (35), systemic infections, autoimmune diseases with neurologic attacks (encephalopathy, neurologic AIDS), and after parenteral administration of gangliosides (36-41).

Structures similar to gangliosides appear on the surface of some microorganisms like *Campylobacter jejuni*, cytomegalovirus, Epstein-Barr virus, *Mycoplasma pneumoniae*, and *Haemophilus influenzae*. The antibodies against these structures can cross-react with gangliosides in myelin structure and nerve fibers, inducing inflammation with consecutive demyelination. IgM antibodies against gangliosides were identified in IgM monoclonal gammopathies, paraneoplastic neuropathies, multifocal motor neuropathy, and Guillain-Barré syndrome (30,42-44).

The authors have initiated a study on the impact of antineoplastic agents and antiganglioside immune response in patients with melanoma, based on the above findings of humoral immune profile towards specific gangliosides expressed on the surface of tumor cells and their association with progression of melanoma (results are not finalized). There are no

available data to analyze the effect of antineoplastic agents inducing or inhibiting the synthesis of GM1 antibodies. It is conceivable that anticancer agents can induce changes in antiganglioside humoral response or antiganglioside antibodies mediated interactions, events that may be useful in managing patients with metastatic melanoma.

CONCLUSION

This study documented the immunogenic capacity of GM1 gangliosides localized on melanoma cell surface. The endogenous response against GM1 of IgM type is an immune event associated with advanced phases of melanoma. It is necessary to test a larger number of patients over a longer period of time to show the clinical importance of autoantibodies against GM1.

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