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Mannose-binding lectin codon 54 genetic polymorphism and vaginal protein levels in women with gynecologic malignancies

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Abstract

Objectives—Mannose-binding lectin (MBL), an innate immune system component that binds to carbohydrates, activates the complement cascade and promotes destruction of microorganisms and abnormal cells. We determined whether a polymorphism in the MBL gene influences vaginal MBL protein concentrations and the occurrence of gynecologic malignancies.

Study design—DNA from 289 women seen in a gynecologic oncology practice and from 126 healthy women was tested for an MBL codon 54 single nucleotide polymorphism by polymerase chain reaction and endonuclease digestion. Vaginal supernatants from 282 of these women were assayed for MBL protein by ELISA.

Results—The normal (A,A) genotype was present in 84.1% of 126 healthy women and 85.3% of 95 women with a benign diagnosis as opposed to 70.0% of 70 women with ovarian cancer (p = 0.02). The MBL variant allele (allele B) frequency was 8.7% in healthy women, 8.4% in women with a benign diagnosis and 17.1% in women with ovarian cancer (p = 0.02). Vaginal MBL protein concentrations were highest in women with the A,A genotype, intermediate in A,B heterozygotes ($p < 0.0001$) and lowest in B,B homozygotes (p = .0097).

Conclusion—The MBL 54 polymorphism and reduction in vaginal MBL concentrations may be a risk factor for development of epithelial ovarian cancer.

Keywords

Mannose-binding lectin; Ovarian cancer; Gynecological malignancy; Gene polymorphism

1. Introduction

Mannose-binding lectin (MBL) is a homo-oligomeric protein composed of 3 to 6 32 kDa subunits present in the circulation [1] and in body secretions, including the vagina [2]. It binds to carbohydrate residues on the surface of microorganisms and activates the complement cascade [3]. Microbial killing occurs by complement-mediated lysis and by the

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Condensation

A polymorphism in the *MBL2* gene that leads to a reduction in vaginal mannose-binding protein concentration may be a risk factor for epithelial ovarian cancer.

ingestion by phagocytic cells of microorganisms bearing complement components or MBL on their surface.

The gene coding for MBL, gene symbol *mbl2*, on chromosome 10q11,2-q21 [4] has three functional single nucleotide polymorphisms in exon 1, with the polymorphism at codon 54 being the most common in Caucasian populations [2]. The substitution of adenosine for guanine at codon 54 leads to the insertion of aspartic acid instead of glycine in the MBL protein. This change results in inhibition of oligomerization of the MBL subunits and impairs the stability and functional activity of the protein [5]. As a consequence, circulating and vaginal MBL concentrations are greatly lowered and MBL-mediated complement activation is reduced in individuals carrying one or two copies of the codon 54 polymorphism [2,6]. The polymorphic variant at codon 54 is commonly identified as allele B, to differentiate it from the wild type allele, allele A. Codon 54 allele B is the most common cause of low MBL concentrations in the European population [7].

Oligosaccharides on the surface of mammalian cells normally terminate with sialic acid residues and therefore do not react with MBL. MBL, however, has been shown to bind to altered oligosaccharides expressed on cells undergoing apoptosis or necrosis [8] as well as on the surface of virus-infected [9] or malignantly transformed [10,11] cells. MBL binding to altered oligosaccharides exposed on the surfaces of tumor cells has been shown to induce tumor cell destruction [10].

The objective of the present study was to determine the prevalence of the MBL 54 polymorphism, as well as vaginal MBL protein levels, in women presenting to an academic gynecologic oncology practice. Our hypothesis was that possession of the variant B allele would be associated with decreased vaginal MBL levels, and therefore retard the immune response to malignantly transformed cells in the female genital tract.

2. Materials and methods

Vaginal swabs were collected from 289 women presenting to an academic gynecologic oncology practice. Healthy controls (126) of similar ages and ethnicity were selected from women being seen for a routine check-up who had a normal Pap smear and no clinical indication of disease. They did not undergo a pelvic ultrasound examination. IRB approval and written informed consent were obtained from each participant. Human experimentation was performed in accordance with The Code of Ethics of the Declaration of Helsinki.

Swabs were shaken into 1.0 ml sterile saline and the samples centrifuged to obtain supernatant and pellet fractions. The pelleted vaginal cells were lysed and DNA released, as previously described [2]. DNA was analyzed for the MBL 54 polymorphism by polymerase chain reaction using primer pairs that spanned the polymorphic region followed by endonuclease digestion and agarose gel electrophoresis, using a published procedure [2]. The vaginal supernatants were assayed for MBL by a commercial ELISA (Cell Sciences). The lower limit of sensitivity of the assay was 0.02 ng/ml. Clinical diagnoses were obtained only after completion of all laboratory testing.

Genotype and allele frequencies were determined by direct counting and then dividing by the number of chromosomes to obtain allele frequency and by the number of women to determine genotype frequency. Goodness of fit to Hardy–Weinberg (H–Y) equilibrium was determined by comparing the expected genotype frequencies with the observed values, using the chi square test. The H–Y equilibrium is a test to determine whether both genotype and allele frequencies are in equilibrium. Associations between MBL genotypes and alleles and clinical diagnosis were assessed by Fisher's exact test. Odds ratios (OR) and 95% confidence intervals (CI) were also determined. The association between vaginal MBL

protein levels and MBL genotype was determined by the Mann–Whitney test. A p value <0.05 was considered significant.

3. Results

The most common diagnosis in our subject population was a benign condition (n = 95), followed by endometrial cancer (n = 88) and ovarian cancer (n = 70). Cervical cancer was diagnosed in 27 women while nine had other malignancies (Fallopian tube cancer, labial cancer, sarcoma, vaginal or vulva cancer). The benign conditions were 45.8% abnormal Pap readings (ASC-US or low grade cervical dysplasia), 15.0% ovarian cysts, 15.0% uterine fibroids, 7.8% cervical dysplasia, 6.8% post-menstrual bleeding, 3.9% endometrial polyps, 3.3% endometrial hyperplasia, and 2.6% condylomas. The median age of the subjects was 53 years with a range from 20 to 86 years. The majority of subjects were Caucasian (66.8%), followed by Asian (11.3%), Hispanics (6.5%) and Blacks (5.1%); the remainder (16.1%) were either of a different or unknown ethnicity.

The MBL codon 54 genotype and allele distributions are presented in Table 1. All genotype distributions were in Hardy–Weinberg equilibrium. The homozygous wild type genotype (A,A) was more prevalent in healthy women (p = 0.0273) as well as in those with a benign diagnosis (p = 0.0213) as compared to women with ovarian cancer. Conversely, the allele B frequency was also significantly increased in women with ovarian cancer (17.1%) as compared with healthy women (8.7%, p = 0.0209) and women with benign diagnoses (8.4%, p = 0.0255). In contrast, the MBL genotype distribution and allele frequencies were not significantly different between healthy women and those with a benign diagnosis or with endometrial or cervical cancer.

The majority of women with ovarian cancer had an epithelial cell tumor (82.9%). Analyzing this group separately (Table 2) revealed a decreased prevalence of the A,A genotype and an increased occurrence of allele B in these patients as compared to both healthy women and benign controls.

Vaginal supernatants from 282 of the women with either benign conditions or a malignancy were available for evaluation of vaginal MBL concentrations (Table 3). The median vaginal MBL level was highest in women with the A,A genotype, intermediate in women who were A,B heterozygotes (p < 0.0001 vs. A,A genotype) and lowest in those who were B,B homozygotes (p = 0.0097 vs. A,A genotype). While the MBL concentration varied depending on carriage of the A,A or A,B genotype, there were no differences in vaginal MBL levels between women with the identical genotype regardless of their clinical status (data not shown).

4. Comment

Women diagnosed with ovarian cancer had a reduced prevalence of the normal MBL codon 54 A,A genotype and an increased occurrence of the polymorphic allele B variant in comparison to healthy women and those with benign conditions. This was especially the case in women diagnosed as having epithelial ovarian cancer. These findings parallel a previous investigation that demonstrated a reduced occurrence of the MBL A,A genotype in Polish women with ovarian cancer compared to healthy controls [12]. In combination, these findings strongly suggest that MBL may function in the immune defense against ovarian cancer.

The mechanisms for this postulated biological anti-tumor activity remain a matter of speculation. It is established that the glycosylation pattern of proteins on tumor cells becomes significantly altered following the establishment of malignancy [13]. The

glycosylation changes in ovarian cancer include the appearance of Lewis-type antigens in glycoproteins and an increase in fucose [14,15]. In addition to binding to mannose and N-actylglucosamine, MBL also binds to fucose [16]. More recently, the ability of MBL to react with Lewis A and Lewis B antigens has been demonstrated [17]. Thus, the recognition of moieties that appear during ovarian carcinogenesis may promote the host's immune mediated attack against this malignancy.

Human colorectal carcinoma cells transplanted into MBL-deficient nude mice were eliminated following treatment with a recombinant vaccinia virus into which the MBL gene was inserted [10]. The mechanism was complement-independent and appeared to involve phagocytosis of the tumor cells. MBL has also been shown to bind to the extensively glycosylated zinc metalloproteinases, meprin α and β [18]. Meprins degrade components of the extracellular matrix, such as collagen type IV, gelatin, fibronectin, laminin and nidogen [19] and, thereby contribute to the migration and metastasis of tumor cells. MBL binding results in the inactivation of meprin activity [18]. The inhibition of meprin-dependent degradation of the extracellular matrix by MBL may, therefore, protect against tumor advancement. The involvement of this mechanism in ovarian cancer remains to be determined.

The absence of an observed difference from controls in the MBL genotype distribution in women with cervical and endometrial cancers may reflect differences in the specific immune regulatory mechanisms responsible for the control of ovarian cancer vs. these other gynecological malignancies. While low MBL concentrations may increase susceptibility to human papillomavirus infection, a woman's MBL genotype has been shown to not influence the onset or development of cervical cancer [20]. Although this is consistent with our observations, verification by further analysis of a larger sample size of women with endometrial and cervical cancer is necessary.

Our demonstration that possession of one or two MBL B alleles is associated with reduced MBL protein concentrations in the vagina reinforces the functional nature of this polymorphism and adds additional support to the hypothesis that a reduction in MBL protein due to this genetic polymorphism may increase susceptibility to development of ovarian cancer. The vaginal MBL concentration observed in our study and its variation with the *mbl2* polymorphism mirrored that found in healthy women by other investigators [21,22]. Furthermore, the lack of variation in vaginal MBL level between women with a benign or malignant condition who carry the identical *mbl2* genotype suggests that the production of MBL protein is probably not altered by any of the studied malignancies.

Many questions remain regarding the role of MBL in development of ovarian cancer. Evaluation of intraperitoneal production of MBL may better define the relationship between the development of epithelial ovarian cancer and MBL deficiency. It would be of considerable interest to evaluate whether the MBL polymorphism is also associated with more aggressive progression and/or recurrence of this malignancy. Serial levels of MBL before, during and after anti-tumor therapy may help to better define the utility of MBL as a biomarker for disease development and progression. Additional investigations of other single nucleotide polymorphisms present in *mbl2*, especially in women of different ethnicities, will further lead to a more complete characterization of MBL-mediated susceptibility to ovarian cancer.

As with any gene polymorphism study, the possibility exists that the MBL B allele is in linkage disequilibrium with a second unidentified gene that is, in fact, responsible for the observed differences between MBL genotype and ovarian cancer. Additional studies are necessary to verify and further specify the putative role of MBL in this malignancy.

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Table 1

MBL codon 54 genotypes and alleles in women with gynecologic malignancies.

Diagnosis	No. women			Allele frequency (%)	
	Genotype			Allele	
	AA	AB	BB	A	B
Healthy	106[a]	18	2	230	22 (8.7)
Benign	81[b]	12	2	174	16 (8.4)
Ovarian cancer	49	18	3	116	24[c] (17.1)
Endometrial cancer	66	20	2	152	24 (13.6)
Cervical cancer	20	7	0	47	7 (13.0)
Other cancers	8	1	0	17	1 (5.6)

[a] $p = 0.0273$ vs. ovarian cancer; odds ratio (OR) = 2.271; 95% confidence interval (CI) = 1.128, 4.574.

[b] $p = 0.0213$ vs. ovarian cancer; OR = 2.480, 95% CI = 1.155, 5.322.

[c] $p = 0.0209$ vs. healthy; OR = 0.4623, 95% CI = 0.2486, 0.8596; $p = 0.0255$ vs. benign, OR = 0.4444, 95% CI = 0.2263, 0.8729.

Table 2

MBL genotypes and alleles in ovarian cancer patients.

Diagnosis	No. women			Allele frequency (%)	
	AA	AB	BB	Allele A	Allele B
Healthy	106[a]	18	2	230	22 (8.7)
Benign	81[b]	12	2	174	16 (8.4)
Epithelial cancer	39	16	3	94	22[c] (19.0)
Germ cell tumors	4	0	0	8	0 (0)
Stromal cell tumors	4	1	0	9	1 (10.0)
Other	2	1	0	5	1 (20.0)

[a] $p = 0.0119$ vs. epithelial tumors; OR = 2.582, 95% CI = 1.247, 5.345.

[b] $p = 0.0142$ vs. epithelial tumors, OR = 2.819, 95% CI = 1.280, 6.206.

[c] $p = 0.0087$ vs. healthy; OR = 0.4087, 95% CI = 0.2159, 0.7735; $p = 0.0115$ vs. benign, OR = 0.3929, 95% CI = 0.1968, 0.7843.

Table 3

Association between MBL vaginal protein concentration and MBL genotype.

MBL genotype	No. women	Median ng/ml MBL (range)
AA	217	1.66 (<.02–75.4)[a]
AB	58	0.79 (<.02–7.99)
BB	7	0.41 (<.02–2.20)

[a] $p < 0.0001$ vs. AB, $p = .0097$ vs. BB.