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Endogenous antibody responses to mucin 1 in a large multiethnic cohort of patients with breast cancer and healthy controls: Role of immunoglobulin and Fc γ receptor genes

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Abstract

High levels of naturally occurring IgG antibodies to mucin 1 (MUC1), a membrane-bound glycoprotein that is overexpressed in patients with breast cancer, are associated with good prognosis. This suggests that endogenous anti-MUC1 antibodies have a protective effect and, through antibody-mediated host immunosurveillance mechanisms, might contribute to a cancer-free state. To test this possibility, we characterized a large number of multiethnic patients with breast cancer and matched controls for IgG antibodies to MUC1. We also aimed to determine whether the magnitude of anti-MUC1 antibody responsiveness was associated with particular immunoglobulin GM (γ marker), KM (κ marker), and Fc γ receptors (Fc γ R) genotypes. After adjusting for the confounding variables in a multivariate analysis, we found no significant difference in the levels of anti-MUC1 IgG antibodies between patients and cancer-free controls. However, in patients and controls, particular GM, KM, and Fc γ R genotypes—individually or epistatically—were significantly associated with the levels of anti-MUC1 IgG antibodies in a racially restricted manner. These findings, if confirmed in an independent investigation, could help

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Conflicts of interest

None

Author contributions

J.P.P. designed and supervised the project and wrote the manuscript. A.M.N. measured the anti-MUC1 antibodies. B.W. performed the statistical analyses. M.I., Y.K., G.S.H., and S.T. contributed to the study design, recruitment of the subjects, and data and blood collection. All authors contributed to the final draft of the manuscript.

identify individuals most likely to benefit from a MUC1-based therapeutic or prophylactic vaccine for MUC1-overexpressing malignancies.

Keywords

GM/KM allotypes; Fc γ R genes; Humoral immunity; MUC1

1. Introduction

Mucin 1 (MUC1) is a membrane-bound glycoprotein that is expressed at low levels in healthy tissues but overexpressed in the majority of adenocarcinomas, and high levels of expression are associated with a poor prognosis. Breast cancer patients as well as healthy individuals generate humoral immune responses to MUC1. Several studies have shown that high levels of naturally occurring anti-MUC1 IgG antibodies are associated with good prognosis in breast cancer (von Mensdorff-Pouilly et al., 2000; Von Mensdorff-Pouilly et al., 2011; Fremd et al., 2015), which could be due to their involvement in host immunosurveillance mechanisms, such as antibody-dependent cellular cytotoxicity (ADCC) (Moreno et al., 2007). About two-thirds of the human population remains free of cancer (Klein, 2014), and host immunosurveillance mechanisms mediated by naturally occurring antibodies against tumor-associated antigens may, at least in part, be responsible for the cancer-free state.

We hypothesized that if elevated immune responses to MUC1 contributed to the superior prognosis in breast cancer patients, healthy individuals should have higher levels of endogenous antibodies to MUC1 than patients with breast cancer. To test this hypothesis, we characterized a large number of multiethnic patients with breast cancer and matched controls for IgG antibodies to MUC1. There are inter-individual differences in the naturally occurring anti-MUC1 antibody levels in both patients and controls, but the host genetic factors that might contribute to these differences are not completely understood. MUC1 is a target of many immunotherapeutic trials (Kimura and Finn, 2013), and for a proper evaluation of the efficacy of these trials, it is necessary to identify the confounding host genetic factors that might influence the naturally occurring immune responses to MUC1. Therefore, to gain further insights into the genetic control of immunity to MUC1, we determined whether anti-MUC1 antibody levels in breast cancer patients and healthy controls were associated with particular immunoglobulin GM (γ marker), KM (κ marker), and Fc γ receptor (Fc γ R) genotypes.

2. Patients and methods

2.1. Archived specimens

The study population from which the specimens were obtained has been described in detail elsewhere (Iwasaki et al., 2011). Briefly, it consisted of breast cancer patients from hospitals in Nagano, Japan, and São Paulo, Brazil. Healthy controls were matched to case patients by ethnicity, residential area during the study period, and age (within 3–5 years). The protocol was approved by the IRB of the respective institutions. There were a total of 1733 subjects:

527 Caucasians (Brazil), 84 subjects of African descent (Brazil), 159 subjects of Japanese descent (Brazil), 167 subjects from the Brazilian mulatto population, 796 subjects from Nagano, Japan. Data were collected on family history of cancer, menstrual and reproductive history, anthropometric factors, physical activity, smoking habits, and estrogen and progesterone hormone receptor status.

2.2. Anti-MUC1 antibody measurements

IgG antibodies to MUC1 in sera were determined by a previously described ELISA (Silk et al., 2009; Pandey et al., 2013). The quantity was expressed as arbitrary units per μL (AU/ μL).

2.3. Determination of GM and Fc γ R alleles

GM alleles (3/f,17/z,23+/n+,23-/n-,5/b1, 21/g) were previously determined by TaqMan[®] and PCR-RFLP genotyping methods (Pandey et al., 2012). Fc γ RIIa alleles, histidine (H)/arginine (R) and Fc γ RIIIa alleles phenylalanine (F)/valine (V) were previously determined by TaqMan[®] genotyping assays (Iwasaki et al., 2011).

2.4. Determination of KM alleles

The KM 1,3 alleles were previously determined (Pandey et al., 2014), by a PCR-RFLP method (Moxley and Gibbs, 1992).

2.5. Statistical analysis

For the combined sample (1733), we used a series of linear mixed regression models for univariate associations between anti-MUC1 IgG antibody levels and the covariates (case status, hormone receptor status, ethnicity, age, menopausal status, number of births, age at first birth, body mass index, alcohol drinking, smoking status, moderate physical activity in the past 5 years, vitamin supplement use, family history of breast cancer, history of benign breast disease, breast feeding, and age at menarche). The mixed regression approach was used to account for matching between breast cancer cases and controls. A multivariable linear mixed regression model including breast cancer status ($p = 0.278$) and smoking status ($p < 0.001$) was selected using backwards selection. For linear models, anti-MUC1 antibody levels were log-transformed to meet model assumptions. Estimates of anti-MUC1 levels were calculated by back-transforming the log (anti-MUC1) from the model and thus represent geometric means.

For the stratified populations, we compared anti-MUC1 antibody levels between breast cancer patients and controls within each population using a stratified linear mixed regression model approach. Based on the results for all participants, we only adjusted for smoking status. In each population, we also evaluated differences in anti-MUC1 antibody levels by the main/marginal effects of genotypes at 6 GM, KM, and Fc γ R loci. For each test, we considered mixed regression models with no additional effects and mixed models that included the interaction between genotype and cancer status to determine if differences in anti-MUC1 antibody levels existed across cases and controls or within cases only or controls only. We considered 4 different models: genotypic, additive, dominant, and recessive.

Using a series of linear regression models, we also tested the interactive effects of GM x FcγR and GM x KM genotypes on anti-MUC1 antibody levels within each population group. The best fitting model for interactions between genotypes (e.g. GM 5/21 dominant x FcγRIIIa recessive) was chosen by using the Akaike information criterion. For all significant epistatic interactions, mean anti-MUC1 antibody level for each genotype combination was estimated from the best fitting model. All hypothesis tests were two-sided with significance set at $\alpha = 0.05$. Since these analyses are exploratory, the p values given were not adjusted for multiple testing. Therefore, these findings would need to be verified in additional studies.

3. Results

3.1. Anti-MUC1 IgG antibody levels in patients and controls

A combined analysis of all subjects showed no significant difference in the levels of anti-MUC1 IgG antibodies between patients and cancer-free controls (geometric mean $\pm$ SE: 4.94 ± 1.03 vs. 5.07 ± 1.02 arbitrary units per μL (AU/ μL), $p = 0.278$). In stratified analyses, no significant differences were observed in anti-MUC1 antibody levels between patients and controls in any population group (data not shown).

3.2. Contribution of GM, FcγR, and KM genotypes to the interindividual differences in anti-MUC1 IgG antibody levels

Genotypes were in Hardy-Weinberg equilibrium in all groups, except the mulatto population, which was excluded from further analyses. In this analysis, we examined the association between anti-MUC1 antibody levels by the 3 genotypes at each locus within specific populations. We considered 4 different models of inheritance: 1) genotypic, which treats 0, 1, or 2 copies of the minor allele as categorical, 2) additive, which treats 0, 1, or 2 copies of the allele as ordinal, 3) dominant effect of the minor allele (difference in anti-MUC1 antibody levels if they have one or more copies of the minor allele), and 4) recessive effect of the minor allele (difference in anti-MUC1 antibody levels if they have two copies of the minor allele).

As shown in Table 1, we found significant associations of FcγRIIIa, GM 5/21, and KM 1/3 genotypes with anti-MUC1 antibody responsiveness in white patients with breast cancer. The patients who had two copies of the minor allele (V) at the FcγRIIIa locus had significantly lower levels of anti-MUC1 antibodies relative to those who had one or no copies of the minor allele (geometric mean $\pm$ SE: 3.08 ± 1.32 vs. 5.12 ± 1.09 AU/ μL , $p = 0.005$). At the GM 5/21 locus, patients with one or more copies of the minor allele (GM 21) had significantly higher levels of anti-MUC1 antibodies relative to those who had no copies of the minor allele (geometric mean $\pm$ SE: 5.42 vs. 4.38 AU/ μL , $p = 0.019$). Finally, patients with one or more copies of the minor allele (KM 1) at the KM 1/3 locus had significantly lower levels of anti-MUC1 antibodies relative to those who had no copies of the minor allele (geometric mean $\pm$ SE: 4.24 vs. 5.08 AU/ μL , $p = 0.047$). None of the genotypes was associated with anti-MUC1 antibody responsiveness in other groups of patients. Also, no significant associations were observed in control subjects.

3.3. Interactive effects of GM and FcγR genotypes on anti-MUC1 IgG antibody levels (AU/μL) in breast cancer patients

We found significant interactive effects of GM 5/21 and FcγRIIIa F/V genotypes on anti-MUC1 antibody levels in white patients with breast cancer (Table 2). Subjects with no copies of the minor allele (GM 21) at the GM 5/21 locus and 2 copies of the minor allele (V) at the FcγRIIIa locus had the lowest anti-MUC1 antibody levels while those with other genotypes had very similar (high) anti-MUC1 antibody levels ($p = 0.001$). A significant interaction between GM 5/21 and FcγRIIa H/R genotypes was observed in black patients with breast cancer ($p = 0.009$). As shown in Table 2, among individuals with no copies of the minor allele (GM 21) at the GM 5/21 locus, levels of anti-MUC1 were lower in those who also had one copy of the minor allele (H) for FcγRIIa compared to those subjects that had 0 or 2 copies of the minor allele at this locus. However, in individuals with at least one copy of the minor allele (GM 21) at the GM 5/21 locus, this relationship was reversed; the levels of anti-MUC1 antibodies were higher in those who also had one copy of the minor allele for FcγRIIa compared to those subjects that had 0 or 2 copies of the minor allele at this locus. No epistatic interactions were found in other group of patients.

3.4. Interactive effects of GM, KM, and FcγR genotypes on anti-MUC1 IgG antibody levels (AU/μL) in healthy controls

Table 3 presents the significant epistatic interactions observed in the control population. In white controls, there were significant interactions between GM 5/21 with FcγRIIa and between GM 3/17 with KM 1/3 genotypes ($p = 0.032$ and 0.029 , respectively). For the interaction between GM 5/21 and FcγRIIa genotypes, subjects that had at least one copy of the minor allele for GM 5/21 and 2 copies of the minor allele for FcγRIIa had the lowest anti-MUC1 antibody levels, while those with no copies of the minor allele for GM 5/21 but 2 copies of the minor allele for FcγRIIa had the highest observed levels. For the interaction between GM 3/17 and KM 1/3 genotypes, in individuals with 0 or 1 copy of the minor allele (GM 17) for GM 3/17, levels of anti-MUC1 antibody levels were lower in those who also had one copy of the minor allele for KM 1/3 compared to those subjects that had 0 or 2 alleles. However, in individuals with 2 copies of the minor allele for GM 3/17, this relationship was reversed; anti-MUC1 antibody levels were higher in those who also had one copy of the minor allele for KM 1/3 compared to those subjects that had 0 or 2 copies of the minor allele at this locus.

In control subjects from Ngano, Japan, significant interactions between GM 3/17 and KM 1/3 and FcγRIIIa were observed ($p = 0.032$ and 0.029 , respectively). For the interaction between GM 3/17 and KM 1/3, the lowest observed anti-MUC1 antibody level was in the one individual who had 2 copies of the minor allele for GM 3/17 but no copies of the minor allele for KM 1/3, while the highest average value was observed in individuals who had at least one copy of the minor allele for KM 1/3 and 2 copies of the minor allele for GM 3/17. It should be noted that for both interactions, only three subjects had 2 copies of the minor allele for GM 3/17. For the interaction between GM 3/17 with FcγRIIIa, the highest observed anti-MUC1 antibody level was in the one individual who had 2 copies of the minor allele for GM 3/17 but no copies of the minor allele for FcγRIIIa, while the lowest average value was observed in individuals who had 2 copies of the minor allele for GM 3/17 and one

copy of the minor allele for Fc γ RIIIa. No other significant associations were found in any other group of controls.

4. Discussion

A combined analysis of all subjects, as well as the stratified analysis of individual groups, showed that levels of autoantibodies to MUC1 were not significantly different in cancer-free controls from that in patients with breast cancer. This would appear to refute our hypothesis that host immunity against MUC1 plays a significant role in keeping people free of breast cancer. However, to conclusively resolve this issue additional studies are needed. In some malignancies, certain IgG subclasses interfere with ADCC/ADCP of tumors mediated by other IgG subclasses (Karagiannis et al., 2013). Therefore, the role of subclass specific anti-MUC1 IgG antibodies in the Fc-mediated immunosurveillance mechanisms, such as ADCC or ADCP, needs to be investigated to shed further light on their possible contribution to the cancer-free state. We plan to do these studies in our future investigations.

These results are in agreement with a previous study that found no substantial difference in the levels of anti-MUC1 antibodies between breast cancer patients and healthy controls (von Mensdorff-Pouilly et al., 2000). This study did, however, find a significant association between high levels of anti-MUC1 antibodies and a favorable prognosis. Due to the lack of survival data, we could not address this question in the current investigation.

Results of our genetic analyses involving individual loci showed that homozygosity for the V allele at the Fc γ RIIIa locus was associated with low antibody responsiveness to MUC1 in white patients with breast cancer. One mechanism underlying this association could involve the antigen processing/presenting pathway. Antigen presenting cells (e.g. macrophages, dendritic cells) express the activating receptor Fc γ RIIIa, which could bind to the Fc region of anti-MUC1 antibodies and form immune complexes. Immune complexes are known to enhance antigen presentation (Bournazos et al., 2017). It is possible that Fc γ RIIIa-V/V expressing antigen-presenting cells are less efficient than those expressing the receptors with the F/F or F/V alleles in the uptake of opsonized MUC1 and presenting it to collaborating helper T cells, thus resulting in lower B-cell activation.

The genotypes at GM 5/21 and KM 1/3 loci were also associated with the levels of anti-MUC1 antibodies. The homozygosity for the GM 5 allele was associated with low antibody responsiveness. Perhaps membrane-bound IgG molecules expressing two copies of the GM 5 allele are not as efficient as those with other genotypic combinations in the uptake, processing and subsequent presentation of MUC1 epitopes to the collaborating T cells, resulting in weaker humoral immunity. In a previous study, we found a significant association between the γ 2 determinant GM 23 and the levels of IgG antibodies to MUC1 in a Caucasian population from Estonia (Pandey et al., 2009). We did not observe this association in the current investigation. The two studies, however, are not comparable. The Estonians are likely to be genetically more homogeneous than the whites from Brazil in the current investigation.

We found a significant (albeit not very strong) association between KM genotypes and anti-MUC1 antibody levels. The homozygotes for KM 3, the major allele, had higher antibody levels than those with the other two genotypes. This association is noteworthy in view of the results from a very large and comprehensive genetic study involving several solid tumors, including breast cancer (Schmidt et al., 2012). It reported that the immunoglobulin κ constant (IGKC) gene, which encodes the KM allotypes, was a strong prognostic marker. The IGKC was as prognostic as the entire 60-gene B-cell metagene. Identification of tumor-infiltrating plasma cells as the source of IGKC expression strongly suggests a role for humoral immunity in breast cancer. The mechanisms underlying the IGKC signature are not known, but could involve KM (like GM) being part of the recognition structure on the membrane-bound B cells for the MUC1 epitopes.

Several significant epistatic interactions between GM and KM genotypes and between particular GM and Fc γ R genotypes were noted. Some likely mechanisms, which have not as yet received adequate attention in immunology, could explain these interactions. It is commonly assumed that heavy and light chains combine randomly to form an immunoglobulin molecule. However, results from some murine studies, showing nonrandom/preferential pairing of heavy and light chains, contradict this belief (Czerwinski et al., 1998; Primi et al., 1987). Thus, significant interaction between GM and KM genotypes observed in the current study may be a reflection of preferential association of heavy (γ) and light (κ) chains expressing particular GM and KM allotypes, respectively, in the synthesis of anti-MUC1 IgG antibodies.

Simultaneous involvement of GM and Fc γ R alleles in endogenous anti-MUC1 antibody responsiveness could be explained by preferential binding of the Fc region of anti-MUC1 IgG antibodies to the Fc γ Rs expressed on antigen presenting cells. Studies involving Fc γ -Fc γ R binding have treated the Fc γ as if it were monomorphic. In fact, Fc γ is highly polymorphic, with at least 16 GM alleles segregating with differing frequencies in different population groups. The Fc γ -Fc γ R binding studies have established that Fc γ RIIa H allele binds IgG2 better than the one expressing the R allele (Bruhns et al., 2009), but the contribution of IgG2 alleles (GM 23+/-) in this interaction has not been investigated. Likewise, it is well known that Fc γ RIIIa V allele binds to human IgG1 and IgG3 better than the F allele (Bruhns et al., 2009), but the putative contribution of several Fc γ 1 and Fc γ 3 alleles in this interaction is not known. In view of the epistatic GM and Fc γ R interactions observed in this investigation, it is possible that Fc of particular GM genotype preferentially binds to the Fc γ R of a particular genotype and differentially contributes to immunity to MUC1 through the antigen processing/presentation pathway.

We found racial differences in the associations between the genetic markers and the magnitude of anti-MUC1 antibody responsiveness. The reasons for these differences are not clear. One contributory factor could be the divergent allele frequencies at GM, KM, and Fc γ R loci among the racial groups examined in this investigation. Additionally, linkage disequilibrium between GM alleles in the Japanese is different from that in whites or blacks, resulting in distinct arrays of GM haplotypes in these groups (Oxelius and Pandey, 2013; Pandey and Li, 2013). It follows that linkage disequilibrium between any putative immune

response genes for the MUC1 epitopes might also be different in these groups, contributing to the ethnic differences in genetic associations with antibody responses.

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Table 1

Tests of associations between Fc γ RIIIa F/V, GM 5/21, and KM 1/3 genotypes and anti-MUC1 IgG antibody levels (AU/ μ L) in white patients with breast cancer.

Locus	Genotype	N	Mean $\pm$ SE	P-value
Fc γ RIIIa	F/F or F/V	232	5.12 $\pm$ 1.09	0.005
	V/V	25	3.08 $\pm$ 1.32	
GM 5/21	5/5	143	4.38 $\pm$ 1.13	0.019
	5/21 or 21/21	115	5.42 $\pm$ 1.15	
KM 1/3	3/3	185	5.08 $\pm$ 1.11	0.047
	1/3 or 1/1	75	4.24 $\pm$ 1.18	

Table 2

Interactive effects of GM 5/21, FcγRIIa R/H, and FcγRIIIa F/V genotypes on anti-MUC1 IgG antibody levels (AU/μL) in patients with breast cancer.

Loci	Genotype combination	N	Mean ± SE	P-value
Whites				
GM 5/21 x FcγRIIIa	(5/5); (F/F, F/V)	130	4.83 ± 1.08	0.001
	(5/5); (V/V)	9	1.26 ± 1.34	
	(5/21, 21/21); (F/F, F/V)	97	5.49 ± 1.09	
	(5/21, 21/21); (V/V)	16	5.07 ± 1.24	
Blacks				
GM 5/21 x FcγRIIa	(5/5); (H/H)	6	7.44 ± 1.20	0.009
	(5/5); (H/R)	12	4.48 ± 1.14	
	(5/5); (R/R)	6	7.98 ± 1.20	
	(5/21, 21/21); (H/H)	1	3.40	
	(5/21, 21/21); (H/R)	9	6.45 ± 1.16	
	(5/21, 21/21); (R/R)	5	4.34 ± 1.22	

Table 3

Interactive effects of GM 5/21, GM 3/17, KM 1/3, FcγRIIa R/H, FcγRIIIa F/V genotypes on anti-MUC1 IgG antibody levels (AU/μL) in healthy controls.

Loci	Genotype combination	N	Mean ± SE	P-value
Whites				
GM 5/21 x FcγRIIa	(5/5); (H/R, R/R)	92	5.25 ± 1.05	0.032
	(5/5); (H/H)	34	5.87 ± 1.09	
	(5/21, 21/21); (H/R, R/R)	101	5.24 ± 1.05	
	(5/21, 21/21); (H/H)	33	4.34 ± 1.09	
GM 3/17 x KM 1/3	(3/17, 3/3); (KM 1/1)	5	7.16 ± 1.24	0.029
	(3/17, 3/3); (KM 1/3)	46	4.93 ± 1.07	
	(3/17, 3/3); (KM 3/3)	137	5.07 ± 1.04	
	(17/17); (KM 1/1)	2	5.40 ± 1.40	
	(17/17); (KM 1/3)	17	7.31 ± 1.12	
	(17/17); (KM 3/3)	54	5.07 ± 1.07	
Japanese (Nagano)				
GM 3/17 x KM 1/3	(3/17, 17/17); (KM 3/3)	190	5.25 ± 1.03	0.032
	(3/17, 17/17); (KM 1/1, KM 1/3)	204	5.19 ± 1.03	
	(17/17); (KM 3/3)	1	2.40	
	(17/17); (KM 1/1, KM 1/3)	2	6.08 ± 1.29	
GM 3/17 x FcγRIIIa	(3/17, 17/17); (F/F)	220	5.23 ± 1.02	0.029
	(3/17, 17/17); (F/V)	142	5.25 ± 1.03	
	(3/17, 17/17); (V/V)	23	4.87 ± 1.08	
	(17/17); (F/F)	1	8.40	
	(17/17); (F/V)	2	3.25 ± 1.28	
	(17/17); (V/V)	0	NA	