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Research paper

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

Janardan P. Pandey[a,*], Aryan M. Namboodiri[a], Bethany Wolf[b], Motoki Iwasaki[c], Yoshio Kasuga[d], Gerson S. Hamada[e], Shoichiro Tsugane[f]

[a] Department of Microbiology and Immunology, Medical University of South Carolina, Charleston, SC, United States
[b] Department of Public Health Sciences, Medical University of South Carolina, Charleston, SC, United States
[c] Division of Epidemiology, Center for Public Health Sciences, National Cancer Center, Tokyo, Japan
[d] Department of Surgery, Nagano Matsushiro General Hospital, Nagano, Japan
[e] Nikkei Disease Prevention Center, São Paulo, Brazil
[f] Center for Public Health Sciences, National Cancer Center, Tokyo, Japan

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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 Fcγ receptors (FcγR) 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 FcγR 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 identify individuals most likely to benefit from a MUC1-based therapeutic or prophylactic vaccine for MUC1-overexpressing malignancies.

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.

* Corresponding author at: Department of Microbiology and Immunology, Medical University of South Carolina, Charleston, South Carolina, SC 29425, United States.
E-mail address: pandeyj@musc.edu (J.P. Pandey).

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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 Fcγ receptor (FcγR) 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 FcγR 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). FcγRIIa alleles, histidine (H)/arginine (R) and FcγRIIIa 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 FcγR 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 ± 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 anit-MUC1 antibodies relative to those who had one or no copies of the minor allele (geometric mean ± 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 ± SE: 5.42 vs. 4.38 AU/μL,

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

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