Immunoglobulin Genes Influence the Magnitude of Humoral Immunity to Cytomegalovirus Glycoprotein B

Janardan P. Pandey,[1] Emily Kistner-Griffin,[2] Faisal F. Radwan,[1] Navtej Kaur,[1] Aryan M. Namboodiri,[1] Laurel Black,[1] Mary Ann Butler,[3] Tania Carreón,[3] and Avima M. Ruder[3]

[1]Department of Microbiology and Immunology, and [2]Department of Public Health Sciences, Medical University of South Carolina, Charleston; and [3]National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention, Cincinnati, Ohio

Human cytomegalovirus (HCMV) is a risk factor for many human diseases, but among exposed individuals, not everyone is equally likely to develop HCMV-spurred diseases, implying the presence of host genetic factors that might modulate immunity to this virus. Here, we show that antibody responsiveness to HCMV glycoprotein B (gB) is significantly associated with particular immunoglobulin GM (γ marker) genotypes. Anti-HCMV gB antibody levels were highest in GM 17/17 homozygotes, intermediate in GM 3/17 heterozygotes, and lowest in GM 3/3 homozygotes (28.2, 19.0, and 8.1 μg/mL, respectively; $P = .014$). These findings provide mechanistic insights in the etiopathogenesis of HCMV-spurred diseases.

Keywords. GM allotypes; humoral immunity; human cytomegalovirus; glycoprotein B; candidate genes.

Human cytomegalovirus (HCMV), a common herpesvirus, which is known to contribute to birth defects and to morbidity and mortality in immunocompromised individuals, has also been implicated in some autoimmune and malignant diseases [1–3]. However, there is vast divergence in HCMV seroprevalence and the prevalence of HCMV-spurred diseases—some of which are very rare—implying the presence of host genetic factors that may modulate viral disease-mediating properties.

Received 5 May 2014; accepted 21 June 2014; electronically published 27 June 2014.

Correspondence: Janardan P. Pandey, PhD, Department of Microbiology and Immunology, Medical University of South Carolina, Charleston, 173 Ashley Ave, SC 29425 (pandeyj@musc.edu).

The Journal of Infectious Diseases® 2014;210:1823–6

DOI: 10.1093/infdis/jiu367

A genome-wide association study (GWAS) has revealed no major genes associated with anti-HCMV immunoglobulin G (IgG) antibody responses [4]. As pointed out elsewhere [5], commonly used genotyping platforms in GWAS do not include immunoglobulin GM genes, highly polymorphic γ chain determinants. Furthermore, because GM genes were not typed in the HapMap and 1000 Genomes projects, they cannot be imputed.

Involvement of GM genes in immunity to several viruses—and especially their modulating influence on HCMV's immunoevasion strategies [6]—makes them ideal candidates for influencing immunity to HCMV. Immunoglobulin KM (κ marker) and Fcγ receptor (FcγR) genes are also good candidates, as they too influence immunity to some viruses. Thus, the aim of this investigation was to determine whether particular GM, KM, FcγRIIa, and FcγRIIIa genotypes were individually or epistatically associated with antibody responsiveness to the HCMV glycoprotein B (gB), which is required for viral infectivity and is a major component of the viral envelope.

MATERIALS AND METHODS

The study population comprised a subset of 131 unrelated white control participants in the Upper Midwest Health Study [7]. The study was approved by the Medical University of South Carolina Institutional Review Board for Human Research. Antibodies to HCMV gB in the sera were determined by a previously described enzyme-linked immunosorbent assay [8]. Genotyping for κ light chain determinants KM 1 and KM 3 was done by a previously described polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) method [9]. For the determination of GM 3/17 alleles, the FcγRIIa alleles histidine and arginine, and the FcγRIIIa alleles phenylalanine and valine, we used PCR-RFLP and a predesigned TaqMan genotyping assay from Applied Biosystems.

GM $23^{+/-}$ alleles were determined by a nested PCR-RFLP method. In brief, a 915-bp region of the *IGHG2* gene that incorporates the sites for the allelic substitutions was amplified, using the following primers: 5′-AAATGTTGTGTCGAGTGCCC-3′ and 5′-GGCTTGCCGGCCGTGGCAC-3′. A 197-bp segment was further amplified from this 915-bp fragment, using the following primers: 5′-GCACCACCTGTGGCAGGACC-3′and 5′-TTGAACTGCTCCTCCCGTGG-3′. After digestion of the amplified product with the restriction enzyme NlaIII, the following products corresponding to the 3 genotypes were obtained: GM 23^{+}, 90 bp, 63 bp, and 44 bp; GM 23^{-}, 134 bp and 63 bp; and GM $23^{+/-}$, 134 bp, 90 bp, 63 bp, and 44 bp.

Table 1. Tests of Associations Between KM, GM, and FcγR Variants and Anti-Human Cytomegalovirus (HCMV) Glycoprotein B (gB) Immunoglobulin G (IgG) Antibody Levels in Upper Midwest Health Study Control Participants

Locus, Genotype	No.	Anti-HCMV gB IgG Antibody Level, μg/mL, Mean ± SD	*P* Values			
			Genotype Model	Additive Model	Dominant Model	Recessive Model
KM 1/3						
3/3	109	15.3 ± 37.9	.757	.516	.578	.551
1/3	21	12.7 ± 24.4				
1/1	1	1.1 ± NA				
GM 3/17						
3/3	59	8.1 ± 19.3	.028	.018	.097	.014
3/17	58	19.0 ± 47.8				
17/17	12	28.2 ± 28.6				
GM 5/21						
5/5	58	7.6 ± 19.0	.020	.010	.057	.013
5/21	59	19.0 ± 47.1				
21/21	14	26.9 ± 30.3				
GM $23^{+/-}$						
$23^{+/+}$	31	7.9 ± 11.2	.172	.206	.068	.822
$23^{+/-}$	67	21.3 ± 47.7				
$23^{-/-}$	32	8.3 ± 15.9				
FcγRIIa						
R/R	3	16.6 ± 25.0	.753	.582	.873	.453
R/H	62	17.4 ± 47.1				
H/H	36	8.7 ± 16.8				
FcγRIIIa						
F/F	52	14.1 ± 26.1	.083	.686	.555	.057
F/V	67	10.8 ± 20.2				
V/V	12	40.5 ± 93.7				

Abbreviations: F, phenylalanine; H, histidine; NA, not available; R, arginine; V, valine.

GM 5/21 alleles were determined by previously described PCR-RFLP methods [10].

Linear regression models were constructed to test associations between genotypes and anti-HCMV gB IgG antibody responses. Tests of genotype models (ie, 2-*df* tests with no assumptions about inheritance models) and 1-*df* tests of additive, dominant, and recessive models of the effects of the minor allele were considered. Anti-HCMV gB IgG antibody levels were log transformed to avoid violating model assumptions. The threshold for statistical significance was defined as a *P* value of <.05. The *P* values were not adjusted for multiple testing, because the tests were not independent, owing to the extensive linkage disequilibrium (LD) within the GM and FcγR loci. All reported *P* values are 2 sided.

RESULTS

All genotypes were in Hardy-Weinberg equilibrium ($P > .24$). The distribution of KM, GM, and FcγR genotypes among control participants in relation to the mean levels of IgG antibodies to HCMV gB is given in Table 1. The association between GM 3/17 genotypes and the level of anti-HCMV gB antibody responses was statistically significant for the genotype, additive, and recessive models but not for the dominant model of inheritance. The anti-HCMV gB antibody levels were highest in GM 17/17 homozygotes, intermediate in GM 3/17 heterozygotes, and lowest in GM 3/3 homozygotes (28.2, 19.0, and 8.1 μg/mL, respectively; $P = .014$). The genotypes at the GM 5/21 locus were similarly associated with anti-HCMV gB antibody responses: the anti-HCMV gB antibody levels were highest in GM 21/21 homozygotes, intermediate in GM 5/21 heterozygotes, and lowest in GM 5/5 homozygotes (26.9, 19.0, and 7.6 μg/mL, respectively; $P = .013$). The association between GM 3/17 genotypes and the level of anti-HCMV gB antibody responses is depicted in Figure 1. It shows clear-cut responses associated with the 3 genotypes. Because the GM 3/17 and GM 5/21 loci are tightly linked, a figure showing antibody levels by GM 5/21 would be nearly identical to Figure 1. KM and FcγR genotypes were not associated with antibody responsiveness to HCMV gB.

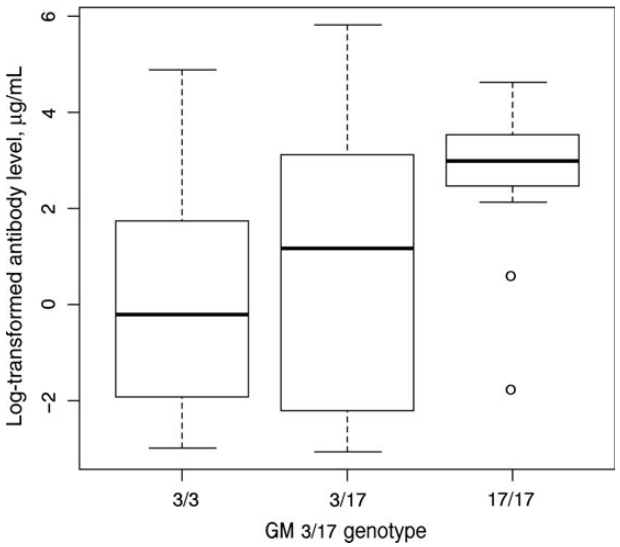

Figure 1. Box plots of anti-human cytomegalovirus glycoprotein B immunoglobulin G (IgG) antibody levels and GM 3/17 genotypes in Upper Midwest Health Study control participants. Bold horizontal lines denote median IgG antibody levels corresponding to the 3 genotypes, boxes denote interquartile ranges (IQRs), dotted lines denote 1.5 times the upper and lower limits of the IQRs, and open circles denote minima and maxima.

DISCUSSION

The results presented here show that control participants who were homozygous for the γ1 determinant GM 17 had higher levels of IgG antibodies to HCMV gB than those with the other 2 genotypes at this locus. Similar results were obtained for the γ3 determinant GM 21, which is in strong LD with GM 17 in white individuals.

Immunoglobulin GM alleles could directly affect immune responsiveness to gB through their modulating influence on viral immunoevasion strategies. The HCMV genes *TRL11/IRL11* and *UL119-UL118* encode 2 proteins that have functional properties of the FcγR [11], which the virus uses to evade the effector consequences of anti-HCMV antibody binding, such as antibody-dependent cellular cytotoxicity. Interestingly, the HCMV-encoded FcγR binds differentially to the allotypically disparate IgG1 proteins: the HCMV *TRL11/IRL11*-encoded FcγR has significantly higher affinity for the IgG1 proteins expressing the GM $3^+,1^-,2^-$ allotypes than for those expressing the allelic GM $17^+,1^+,2^+$ allotypes [6]. This implies that in people homozygous for the GM 3 allele, most of the anti-HCMV gB antibodies would form immune complexes with the virus by bipolar bridging, resulting in a lower concentration of free anti-HCMV gB antibodies circulating in the system. The opposite results would be expected in people homozygous for the GM 17 allele. Results presented here are consistent with these predictions. (GM 3–carrying haplotypes in white individuals do not express GM 1 and 2, and the GM 17 peptide is usually positive for GM 1.) The involvement of GM 5/21 alleles may be a consequence of their LD with GM 3/17, or they also may be involved in modulating the HCMV immunoevasion strategies, which needs to be investigated.

GM genes could also cause conformational changes in the antigen-binding site in the immunoglobulin variable regions associated with anti-HCMV gB antibody specificity. The allelic determinants GM 3 (arginine) and 17 (lysine) are located in the CH1 region of the γ1 chain. In mice, amino acid sequence polymorphism in this region has been shown to modulate the kinetic competence of antigen-binding sites [12]. GM allotypes could also influence antibody responsiveness to HCMV gB through the B-cell–mediated antigen processing/presentation pathway. Perhaps the B-cell membrane–bound IgG molecules expressing the GM 17/21 determinants constitute a higher-affinity receptor for the HCMV gB epitopes and are more efficient than those expressing GM 3/5 alleles in the uptake of these antigens and presenting them to collaborating helper T cells, thus resulting in higher B-cell activation.

Results presented here appear to shed light on the putative mechanisms underlying the GM gene association with breast cancer [13], a malignancy in which HCMV appears to play an oncomodulatory role [2]. If anti-HCMV antibodies were protective, subjects with the low responder genotypes—GM 3/3 and GM 5/5—would be expected to be at a higher risk of developing breast cancer than the subjects with the high responder genotypes. This is what we found in white subjects in a large matched case-control study [13]: compared with subjects who were homozygous for the GM 17 allele, the GM 3 homozygotes were over twice as likely to develop breast cancer. The GM 5 homozygotes were also associated with breast cancer at a borderline level of statistical significance.

These results also suggest a putative explanation for how a common virus (HCMV seroprevalence, 80%) could spur an uncommon disease like neuroblastoma (prevalence, 0.025%) [3]. Almost 4 decades ago, 2 very uncommon GM genotypes—GM 1,3,5 and GM 1,3,21—were reported to be highly significantly associated with susceptibility to neuroblastoma [14], which could have resulted from the inability of the subjects with these genotypes to clear the virus by means of anti-HCMV antibody-mediated immunosurveillance mechanisms. It is noteworthy that the low responder allele, GM 3, is present in both uncommon genotypes associated with this malignancy. This allele has also been recently implicated in glioma risk in a study population from Portugal [15].

It is hoped that the results presented here would inspire further investigations on the role of the GM gene complex in the etiopathogenesis of other HCMV-spurred diseases, such as hepatocellular carcinoma, medulloblastoma, prostate cancer, and HCMV retinitis.

Notes

Acknowledgment. We thank John Clark of NIOSH for assistance in assembling the specimens.

Disclaimer. The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

Financial support. This work was supported by the National Institute of Neurological Disorders and Stroke, National Institutes of Health (NIH; grant NS078545); the Avon Foundation; and the National Institute for Occupational Safety and Health, CDC.

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Lunardi C, Bason C, Navone R, et al. Systemic sclerosis immunoglobulin G autoantibodies bind the human cytomegalovirus late protein UL94 and induce apoptosis in human endothelial cells. Nat Med **2000**; 6:1183–6.
2. Taher C, de Boniface J, Mohammad AA, et al. High prevalence of human cytomegalovirus proteins and nucleic acids in primary breast cancer and metastatic sentinel lymph nodes. PLoS One **2013**; 8:e56795.
3. Wolmer-Solberg N, Baryawno N, Rahbar A, et al. Frequent detection of human cytomegalovirus in neuroblastoma: A novel therapeutic target? Int J Cancer **2013**; 133:2351–61.
4. Kuparinen T, Seppälä I, Jylhävä J, et al. Genome-wide association study does not reveal major genetic determinants for anti-cytomegalovirus antibody response. Genes Immun **2012**; 13:184–90.
5. Pandey JP. Genomewide association studies and assessment of the risk of disease. N Engl J Med **2010**; 363:2076–7.
6. Namboodiri AM, Pandey JP. The human cytomegalovirus TRL11/IRL11-encoded FcγR binds differentially to allelic variants of immunoglobulin G1. Arch Virol **2011**; 156:907–10.
7. Ruder AM, Waters MA, Carreón T, et al. The Upper Midwest Health Study: a case-control study of primary intracranial gliomas in farm and rural residents. J Agric Saf Health **2006**; 12:255–74.
8. Hackett DJ, Zhang C, Stefanescu C, Pass RF. Enzyme-linked immunosorbent assay for measurement of cytomegalovirus glycoprotein B antibody in serum. Clin Vaccine Immunol **2010**; 17:836–9.
9. Moxley G, Gibbs RS. Polymerase chain reaction-based genotyping for allotypic markers of immunoglobulin kappa shows allelic association of Km with kappa variable segment. Genomics **1992**; 13:104–8.
10. Balbín M, Grubb A, de Lange GG, Grubb R. DNA sequences specific for Caucasian G3 m(b) and (g) allotypes: allotyping at the genomic level. Immunogenetics **1994**; 39:187–93.
11. Atalay R, Zimmermann A, Wagner M, et al. Identification and expression of human cytomegalovirus transcription units coding for two distinct Fcgamma receptor homologs. J Virol **2002**; 76:8596–608.
12. Torres M, Fernandez-Fuentes N, Fiser A, Casadevall A. The immunoglobulin heavy chain constant region affects kinetic and thermodynamic parameters of antibody variable region interactions with antigen. J Biol Chem **2007**; 282:13917–27.
13. Pandey JP, Kistner-Griffin E, Iwasaki M, et al. Genetic markers of immunoglobulin G and susceptibility to breast cancer. Hum Immunol **2012**; 73:1155–8.
14. Morell A, Scherz R, Käser H, Skvaril F. Evidence for an association between uncommon Gm phenotypes and neuroblastoma. Lancet **1977**; 1:23–4.
15. Pandey JP, Kaur N, Costa S, et al. Immunoglobulin genes implicated in glioma risk. OncoImmunology **2014**; 3:e28609.