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The decoy Fc γ receptor encoded by the cytomegalovirus *UL119-UL118* gene has differential affinity to IgG proteins expressing different GM allotypes

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Abstract

Human cytomegalovirus (HCMV) is a ubiquitous herpesvirus that has been implicated in many diseases. However, there is significant divergence between HCMV seroprevalence and the prevalence of HCMV-associated diseases, implying the presence of host genetic factors that might modulate immunity to this virus. HCMV deploys many sophisticated strategies to evade host immunosurveillance. One strategy involves encoding for proteins that have functional properties of the Fc γ receptor (Fc γ R). The aim of the present investigation was to determine whether the UL119-UL118-encoded recombinant Fc γ R ecto-domain binds differentially to genetically disparate IgG1 proteins. Results show that mean absorbance values for binding of HCMV UL119-UL118-encoded Fc γ receptor to the immunoglobulin GM (γ marker) 1,17-expressing IgG1 were significantly higher than to the IgG1 expressing the allelic GM 3 allotype (0.225 vs. 0.151; p = 0.039). These findings suggest possible mechanisms underlying the maintenance of immunoglobulin GM gene polymorphism and its putative role in the etiology of HCMV-associated diseases.

Keywords

GM allotypes; Decoy Fc γ receptor; Human cytomegalovirus; Immuno evasion

1. Introduction

Human cytomegalovirus (HCMV) is a common herpesvirus that persistently affects a majority of the world's population, causing significant morbidity and mortality in congenitally infected infants and in immunocompromised individuals. There is also increasing evidence that HCMV is somehow involved in the etiology of autoimmune and malignant diseases [1-5]. These attributes make this virus one of the most successful human

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Conflict of interest

The authors have declared they have no conflict of interest.

pathogens. The key to its success underlies in its ability to deploy sophisticated strategies to evade host immunosurveillance. One such strategy involves encoding for proteins that have functional properties of the Fc γ receptor (Fc γ R) [6,7], which may enable the virus to evade host immunosurveillance by thwarting the effector consequences of anti-HCMV IgG antibody binding, such as antibody-dependent cellular cytotoxicity (ADCC).

During the co-evolution of viruses and their hosts, the host must have evolved specific mechanisms to counter the effects of viral immunoevasion strategies and ensure our survival as a species. In a previous investigation [8], we reported that IgG1 proteins expressing the GM 3 haplotype (GM 1–, GM 3+) have significantly higher affinity for the HCMV *TRL11/IRL11*-encoded decoy Fc γ R than those expressing the GM 17 haplotype (GM 1+,2+,17+). HCMV also employs another decoy Fc γ R, encoded by its *UL119-UL118* gene, to evade Fc-mediated effector functions [6,7]. It is of interest to determine whether the two viral Fc γ Rs are functionally redundant, or, whether they evolved to target different alleles of immunoglobulin genes as a means of co-evolutionary adaptation. The aim of the present investigation was to determine whether the *UL119-UL118*-encoded recombinant Fc γ R ectodomain binds differentially to allotypically disparate IgG1 proteins.

2. Materials and methods

2.1. Study subjects

The study population consisted of 66 random blood donors who were negative for antibodies to HCMV. Anti-HCMV determinations were made by an ELISA or by a multiplex flow immunoassay, using the ToRC IgG kit (Biorad, Hercules, CA). The study protocol was approved by the Medical University of South Carolina IRB for human research.

2.2. GM allotyping

Serum samples were typed for GM allotypes 1, 3, and 17 by a standard hemagglutination-inhibition method [9]. Briefly, a mixture containing human blood group ORh+ erythrocytes coated with anti-Rh antibodies of known GM allotypes, the test sera, and monospecific anti-allotype antibodies were incubated in a microtiter plate. Test sera containing IgG of particular allotype inhibited hemagglutination by the anti-allotype antibody, whereas negative sera did not. Of 66 subjects, 33 expressed the GM 1,17 allotypes and 33 expressed the GM 3 allotype. The 3 GM alleles investigated here differ by 3 amino acid substitutions at positions 214, 356, and 358 of γ 1 chain (Table 1).

2.3. Affinity purification of IgG1 proteins expressing GM 1,17 and GM 3 allotypes

Anti-human IgG1 (Cat# HP 6069, EMD Millipore, Billerica, MA) was coupled to carbodiimide activated carboxy flexiband magnetic beads (EMD Millipore, Billerica, MA). IgG1 proteins from subjects who expressed the GM 1,17 or GM 3 allotypes were incubated with beads and washed thoroughly. Bound protein was eluted with 100 mM Glycine-HCl (pH 2.5), neutralized with Tris-HCl (pH 8.0), and concentrated over 30 kDa cut-off centrifugal filter units (EMD Millipore, Billerica, MA) and used for the binding studies.

2.4. Expression of recombinant UL119-UL118-encoded ectodomain of Fc γ R in mammalian cells and purification from culture supernatant

The gene encoding the 293-amino acid sequence of the extra-cellular domain of *UL119-UL118*-encoded protein of HCMV (AD169) [6] was synthesized by GenScript (Piscataway, NJ) and inserted in a pcDNA3.1/V-His between HindIII and BamHI restriction sites. The recombinant protein was expressed in CHO-K1 cells (Chinese hamster ovary cells; ATCC, CCL-61), using standard methods. It was affinity purified by passing through Ni NTA agarose affinity matrix (Qiagen, Germantown, MD) and eluted with phosphate buffer containing 250 mM imidazole. Purity of the preparation was tested by SDS–polyacrylamide gel electrophoresis (SDS–PAGE) using 12% polyacrylamide gels followed by staining with 0.25% Coomassie Brilliant Blue (R-250). As shown in Fig. 1A, the preparation was free of impurities even when an amount as high as 30 μ g was loaded on to the gels. The molecular weight of the purified glycoprotein was estimated to be of 68 kDa. Proteins were blotted onto BioTrace PVDF membranes (Pall Life Sciences, Port Washington, NY) and probed with mouse monoclonal anti-6 $\times$ His epitope tag antibody (MA 1-213150, Thermo Scientific, Waltham, MA) to show that the expressed protein is *UL119-UL118*-encoded protein with 6 $\times$ -His tag. Fig. 1B shows the immunoblot analysis with anti 6 $\times$ -His tag antibodies.

2.5. ELISA for the binding of UL119-UL118-encoded Fc γ R to IgG1 proteins

The binding of genetically different IgG1 proteins—expressing GM 1,17 or GM 3 allotypes—to the *UL119-UL118*-encoded protein was determined by an ELISA, using the recombinant viral Fc γ R protein coated microtiter plates for binding and the HRP conjugate of an Fab-specific anti-human IgG Fab (Jackson ImmunoResearch Laboratories Inc., West Grove, PA) for detection. To determine the dilution suitable for binding studies, a full titration curve was generated for each affinity purified IgG1 preparation on sheep anti-human IgG (Sigma Chemical Co., St. Louis, MO) coated ELISA plates, and the dilution required to give the absorbance at the midpoint of the titration curve (mid-OD) was determined in a manner similar to that described by Shields et al. [10]. (The anti-human IgG, used as standard, had no specificity for any GM allotypes.) The absorbance value for the binding of each IgG1 protein to the *UL119-UL118*-encoded protein was then expressed relative to its binding to the same Fc specific sheep anti-human IgG under the same dilution. Therefore, the absorbance values are a ratio of the binding of each allotypically disparate IgG1 protein relative to the anti-human IgG used as standard. Each experiment was replicated 6 times, i.e., 198 (6 $\times$ 33) replications per group of allotypically different subjects.

2.6. Statistical analysis

For comparison of the absorbance values between the 2 groups (GM 3 vs. GM 1, 17), mixed linear regression models (SAS v9.4 Proc Mixed) were used. This type of model is ideal for handling within-subject repeated observations [11]. To account for a lack of normality, the absorbance values were log transformed (base 10) prior to the model construction; however, since there were 5 absorbance values measured to be zero, 0.005 was added to all absorbance values before the log transformation. The model included a random subject effect with a compound symmetry covariance structure to account for the intra-class correlation among individual subjects' six repeated measurements. A fixed effect for

experiment number (1–6) was also included to account for any potential systematic bias from one experiment to another. Results were back-transformed for reporting purposes. Also, a sensitivity analysis was performed to determine whether the estimated group differences differed when the 5 observations with absorbance values of 0.0 were excluded from the analysis. All tests were two-tailed, and the statistical significance was defined as $p < 0.05$.

3. Results and discussion

Fig. 2 shows the comparative binding of IgG1 proteins expressing GM 1,17 or GM 3 allotypes to the *ULI19-ULI18*-encoded Fc γ R protein. After adjusting for experiment number and accounting for the within-subject intra-class correlation, the mean absorbance values (and 95% confidence interval [CI]) for the GM 3 and GM 1,17 groups were estimated to be 0.151 (95% CI: 0.116–0.197) and 0.225 (95% CI: 0.172–0.292), respectively. In other words, the absorbance values in the GM 1,17 group were 1.5-fold higher, on average, than those in the GM 3 group, and this group difference was statistically significant ($p = 0.039$). The sensitivity analyses indicated that the observed group difference remained significant ($p = 0.026$) even when the subjects with zero absorbance values were excluded from the analysis.

Results presented here clearly show that *ULI19-ULI18*-encoded Fc γ R had higher affinity for IgG1 proteins expressing the GM 1,17 allotypes than those expressing the allelic GM 3 allotype. The amino acid substitutions characterizing these GM allotypes are in the CH1 and CH3 regions of the γ chain (Table 1). Although the *ULI19-ULI18*-encoded Fc γ R has been shown to bind the CH2–CH3 interface of the γ chain [12], it is possible that amino acid substitutions distant from the binding site itself could influence the conformation and thus indirectly affect the binding affinity. Importance of the GM allotypes expressed in the CH1 region of γ chain for the viral Fc γ R binding has been conclusively shown for the herpes simplex virus type 1 [13].

Higher affinity of GM 1,17-expressing IgG1 to the viral Fc γ R would imply that subjects with the GM 1,17 allotypes would be more likely to have the Fc γ domains of their anti-HCMV IgG antibodies scavenged, thereby reducing their immunological competence to eliminate the virus through ADCC and other Fc-mediated effector mechanisms. Consequently, subjects possessing the GM 1,17 haplotype would be expected to be at an increased risk—while those carrying the GM 3 haplotype (because of the lower affinity to the viral Fc γ R) at a reduced risk (protective)—of developing HCMV-associated diseases. Some data from hepatocellular carcinoma (HCC) appear to support this prediction.

Significantly higher HCMV seroprevalence in HCC patients than in patients without HCC has been reported [2]. Interestingly, many years ago particular GM haplotypes were shown to be risk factors for HCC. Nakao et al. [14] reported a dramatically increased frequency of the GM 1,2,21 haplotype in HCC patients, as compared to controls, in a large study population from Japan. Subjects in this study were not typed for the GM 17 allotype. When typed for this determinant, the relevant haplotype is GM 1,2,17,21 in this population group [15]. Although the results presented here appear to unify putative viral and genetic etiology

of HCC, to gain a deeper insight into this relationship, other GM alleles (e.g. 2 and 21) must also be examined for their possible modulatory effect on HCMV immunoevasion strategies. The GM 21 allele, expressed on IgG3, is in almost absolute linkage disequilibrium with GM 1,2, and 17 alleles, expressed on IgG1, in the Japanese population [15]. The reasons for the strong linkage disequilibrium in the GM gene complex, resulting in unique arrays of racially associated haplotypes, are not known. There might be a co-evolutionary (virus-host) selective mechanism underlying this phenomenon. Additional studies are warranted.

The results presented here, together with those involving the *TRL11/IRL11*-encoded Fc γ R [8], suggest a mechanism for the maintenance of allelic diversity at the IgG1 locus. The 2 Fc γ Rs have contrasting binding patterns: the *TRL11/IRL11*-encoded Fc γ R has higher affinity for the GM 3 haplotype, while the *UL119-UL118*-encoded Fc γ R has higher affinity for the GM 1,17 haplotype. Since the Fc regions expressing both alleles/haplotypes would be expected to be protective factors for certain HCMV-spurred diseases, the heterozygotes at this locus would have advantage over homozygotes, resulting in the persistence of both alleles/haplo-types in the population. Their relative frequency in the population would depend on the intensity of the evolutionary selective pressure exerted by the particular HCMV-associated diseases. (It might be relevant to point out that the same allele that confers a risk for one disease might also be protective against a disease with a greater influence on our fitness, thus resulting in evolutionary selective advantage.)

These results shed light on the question as to why not all equally exposed people are equally likely to develop HCMV-spurred diseases. It is hoped that these findings would inspire further investigations on the contribution of gene-environment (virus) interactions to human diseases in general, and particularly, the role of the highly polymorphic GM gene complex in the immunobiology of HCMV and in the etiopathogenesis of HCMV-associated diseases.

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Fig. 1. SDS–polyacrylamide gel electrophoresis (SDS–PAGE) of *UL119-UL118*-encoded protein, affinity purified by passing through Ni–NTA agarose column. (A) Coomassie staining of *UL119-UL118*-encoded protein. (B) Immunoblot analysis of *UL119-UL118*-encoded protein, using a mouse monoclonal antibody to 6 $\times$ his tag.

Fig. 2. Absorbance values (450 nm) for the binding of IgG1 proteins to the *ULI19-ULI18*-encoded Fc γ R protein in subjects with GM 1-,3+ ($n = 33$) and GM 1+,17+ allotypes ($n = 33$). The bars and whiskers represent the means and standard errors, respectively, which were obtained using a general linear mixed model with a random subject effect to control for within-subject correlation.

Table 1

Amino acids associated with IgG1 allotypes at key positions on the γ 1 chain.

GM allotypes	CH1 (214) GM 17/GM 3	CH3 (356) GM 1/GM -1	CH3 (358) GM 1/GM -1
GM 1,17	Lys	Asp	Leu
GM 3	Arg	Glu	Met