Published in final edited form as:

Mol Immunol. 2007 July ; 44(15): 3805–3808.

Fcγ receptor-like hepatitis C virus core protein binds differentially to IgG of discordant Fc (GM) genotypes

Aryan M. Namboodiri^a, Agata Budkowska^b, Paul J. Nietert^c, and Janardan P. Pandey^{a,*}

^a Department of Microbiology and Immunology, Medical University of South Carolina, Charleston, South Carolina, USA

^b Unité Hépacivirus, Institut Pasteur, Paris, France

^c Department of Biostatistics, Bioinformatics & Epidemiology, Medical University of South Carolina, Charleston, South Carolina, USA

Abstract

Immunoglobulin GM allotypes are associated with the outcome of several infections, including hepatitis C virus (HCV) infection, but the underlying mechanisms are not known. HCV employs sophisticated strategies to evade host immunosurveillance. One such strategy might involve the scavenging of the Fcγ domain of the anti-HCV IgG antibodies by its Fcγ receptor-like site formed by HCV core protein, potentially interfering with the Fcγ-mediated host defense mechanisms. We tested the hypothesis that GM allotypes modulate this viral strategy through differential binding to the core protein. Here we show that the absorbance values for binding to the HCV core protein were significantly higher for IgG1 with GM 3 allotype than that for the allelic GM 1, 2, 17 determinants ($p=0.0003$). These results provide a mechanistic explanation for the involvement of GM allotypes in the outcome of HCV infection. These findings also shed light on the possible evolutionary selective mechanism that maintains GM polymorphism.

Keywords

FcγR; HCV core protein; GM allotypes

1. Introduction

Immunoglobulin (Ig) GM allotypes—hereditary antigenic determinants of γ chain constant regions—are encoded by three very closely linked cistrons on human chromosome 14. There are currently 18 testable GM specificities. Linkage disequilibrium in the GM system is almost absolute and the determinants are transmitted as a group called GM haplotypes. Each major race has a distinct array of haplotypes (Steinberg and Cook, 1981). The biological role and the evolutionary mechanisms for the maintenance of GM polymorphism remain unknown. The striking qualitative and quantitative differences in the distribution of these determinants among different races, strong linkage disequilibrium within races, and racially-restricted occurrence of GM haplotypes, all suggest that differential selection over many generations may have played an important role in the maintenance of polymorphism at these loci. One likely selective

*Corresponding author. Tel.: +1 843-792-4360; fax: +1 843-792-7793. E-mail address: pandeyj@musc.edu (J.P. Pandey).

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

force may be association between particular allotypes and specific immune responses to pathogens, resulting in differential immunity to infectious diseases.

HCV is a common infection and a major health problem worldwide, and there is as yet no vaccine against this pathogen. About 20% of the acutely infected individuals spontaneously clear the virus, whereas a large majority of cases develop chronic liver disease, which can lead to liver cirrhosis and hepatocellular carcinoma. The antiviral treatments available eradicate the virus in 40–80% of patients depending on the virus genotype. Understanding of the immunological mechanisms underlying the resolution of acute HCV infection would provide valuable insights in devising novel therapeutic strategies, including the designing of a vaccine, to combat this virus. Among the factors influencing the outcome of HCV infection, the host genetic factors are thought to play a predominant role. Reports from several studies documenting associations of HLA, KIR and other genes of the immune system with viral persistence and clearance support this contention (Martin and Carrington, 2005). We have previously reported that particular combinations of GM and KM (genetic markers of Ig κ chains) phenotypes are associated with HCV clearance and persistence (Pandey et al., 2004). The immunogenetic mechanisms responsible for this association are not known.

In addition to its function in the formation of HCV nucleocapsid, the HCV core protein has multiple regulatory functions in various cell processes, including cell signaling, lipid metabolism and gene expression (McLauchlan, 2000). The HCV core protein may also interfere with host defense mechanisms by suppressing T-cell immune responses (Kittlesen et al., 2000). The HCV core protein and HCV nucleocapsids circulating in HCV infected individuals have functional properties of Fc γ receptor (Fc γ R) that may allow them to interfere with certain host defense mechanisms—such as antibody dependent cellular cytotoxicity (ADCC), cytokine release, and activation of the classical complement pathway—mediated by the Fc γ part of the anti-core IgG antibodies (Maillard et al., 2004). Thus, the core protein may scavenge the Fc γ domain of the anti-core antibodies by “bipolar bridging”: binding of the Fab (paratope) to the antigenic target (epitope) and that of the Fc to the Fc γ R-like site on the viral protein (Maillard et al., 2004). We hypothesized that GM allotypes could contribute to the outcome of HCV infection by influencing the strategies employed by this virus to evade host immunosurveillance. Aminoacid substitutions associated with GM alleles cause structural changes in the Fc and Fd regions of IgG, which may result in altered affinity of this domain to the Fc γ R-like site on the core protein. To test our hypothesis, we measured the binding of the HCV core protein to the IgG1 molecules carrying distinct GM alleles, which differ by four aminoacid residues at positions 214, 356, 358, and 431 (Table 1).

2. Materials and methods

2.1. Study subjects

The study population consisted of anti-HCV antibody negative healthy blood donors—17 South American Indians and 17 Caucasians from the U.S. The study was approved by the local institutional review boards.

2.2. GM allotyping

Serum samples were characterized for the four known IgG1 allotypes—1/a, 2/x, 3/f, and 17/z—by a standard hemagglutination-inhibition method (Vyas et al., 1968). The notation follows the international system for human gene nomenclature. The haplotype carrying the GM determinants 1,2,17 is allelic to the one carrying the GM 3 allotype.

2.3. FcγR-like HCV core protein

Recombinant HCV core protein (aa 2–122) was produced in *Escherichia coli* and purified as described elsewhere (Maillard et al., 2004). Control experiments—using various Ig isotypes, HCV protein E2, a panel of synthetic HCV core peptides, and HBV pre-S1—have established the specificity of the binding of HCV core protein to the Fcγ fragments of IgG; these experiments also showed that amino acid sequence spanning residues 3–75 was crucial for optimal activity (Maillard et al., 2004).

2.4. Purification of IgG1 proteins

IgG1 proteins were isolated from sera by subclass-specific affinity chromatography.

2.5. Binding of HCV core protein to IgG1

The binding of IgG1 proteins (GM 1,2,17 or GM 3 genotype) to the HCV core protein was quantitated by an ELISA. The absorbance value for binding of each IgG1 protein to the HCV core protein is relative to its binding to an Fc-specific sheep anti-human IgG antibody (Sigma, U.S.A.), which was used as a standard and had no specificity for any GM allotypes. For each affinity purified IgG1 preparation, a full titration curve was generated on sheep anti-human IgG 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. (2001). This dilution of IgG1 was used for measuring its binding to the core protein and to the standard. At this dilution, the mean absorbance value for the binding of GM 1,2,17 carrying IgG1 proteins to the sheep anti-human IgG standard was 0.35, and for binding to the core protein was 0.11. The mean absorbance values for the binding of GM 3 carrying IgG1 proteins to the standard and to the core protein were 0.31 and 0.13, respectively. Experiments were replicated three times, and each time in duplicate.

2.6. Statistical analysis

For comparison of the absorption values for binding of the two IgG1 proteins to the core protein, a mixed linear regression model (SAS v9.1 Proc Mixed) was used. This model included a random subject effect with a compound symmetry covariance structure to account for the intraclass correlation among individual subjects' six repeated measurements. The OR values were calculated by logistic regression (SAS v9.1 Proc Logistic). All tests were two-tailed, and the statistical significance was defined as $p < 0.05$.

3. Results and discussion

As shown in Table 2, the mean absorbance values for binding to the immobilized core protein were significantly higher for IgG1 with the GM 3 allotype, than that for the molecules carrying the GM 1,2,17 determinants (0.44 vs 0.32, $p = 0.0003$). These results shed new light on the HCV core-protein binding site on the IgG molecule. Previous experiments showed that the recombinant HCV core-protein binding site on the IgG molecule was located in the CH2-CH3 interface region (Maillard et al., 2004). This was based on the observation that the binding of IgG and Fcγ to the core protein was markedly inhibited (by 40%) by protein A from *Staphylococcus aureus*, whose binding site is located in the CH2-CH3 interface of IgG (Deisenhofer, 1981). Results presented here, however, suggest that the amino acid substitutions in CH1 (GM 3) and CH3 (GM 1,2,17) domains influence binding of the HCV core protein to IgG1. It is possible that the HCV core protein has two binding sites: one located in the CH2-CH3 interface and the other constituted by the simultaneous presence of particular residues in CH1 and CH3 domains of IgG. The latter is suggested by the observation that the HCV core protein does not bind to the Fab fragment of IgG, which includes the variable and the CH1 domain only. Formation of a binding site by residues that are some distance apart is not

unprecedented in the Ig gene family. For instance, anti-KM 3 antibodies recognize allotypic determinant KM 3, which is constituted by residues at positions 153 and 191 of the κ chain.

The results of the HCV core-protein binding site on the IgG molecule are reminiscent of the findings in herpes simplex virus 1 (HSV1). Indeed, the earlier studies showed that histidine at position 435 at the CH2-CH3 domain interface of IgG was a critical residue for the formation of HSV1-Fc γ R-like binding site (Chapman et al., 1999), but studies involving GM allotypes clearly established that residues in CH1 (especially arginine at position 214) and CH3 domains, outside of the CH2-CH3 interface, influence the binding of the viral Fc γ receptor to IgG (Atherton et al., 2000).

The results presented here could, at least in part, explain the involvement of GM allotypes in the outcome of HCV infection. Since binding of IgG1 of GM 3 allotype to HCV core protein was significantly higher than that of other allotypes, IgG antibodies directed to the core protein in subjects with this determinant are more likely to have their Fc domains scavenged, thereby reducing their immunological competence to eliminate the virus or circulating nucleocapsids through ADCC and other Fc-mediated effector mechanisms. This would suggest a higher prevalence of GM 3 in subjects with persistent HCV infection compared to those who have cleared the virus. This appears to be the case in our study population reported earlier (Pandey et al., 2004). Among subjects with persistent HCV infection ($n = 196$), there were 72 subjects (36.7%) with a relatively frequent GM phenotype carrying the GM 3 allele, while among patients that cleared the virus ($n = 99$), there were 25 subjects (25.3%) with a relatively frequent GM phenotype carrying the GM 3 allele (OR, 1.72; 95% CI, 1.00 to 2.94). Considering all GM phenotypes found in this population, including extremely unusual and rare phenotypes, the trend for higher prevalence of GM 3 in the group with persistent HCV infection persists. As GM 3 is in significant linkage disequilibrium with certain determinants in IgG2 and IgG3 subclasses (Steinberg and Cook, 1981), HCV core-protein binding studies involving these determinants, as well as simultaneous examination of other candidate genes (e.g. KM, Fc γ R, HLA, and KIR) in a large study population would be necessary to better understand the genetic mechanisms underlying the persistence or clearance of HCV infection.

The reported similarities between the HCV core protein and the neonatal Fc receptor (FcRn) suggest additional mechanism for the involvement of GM allotypes in HCV clearance and persistence (Maillard et al., 2004). Studies by Schilling et al. (2003) suggest that FcRn mediates the cellular uptake of neutralizing IgG antibodies to the hepatitis B virus in hepatocytes and interferes with the secretion of the virions from the cells. Similar mechanisms may be applicable to the HCV as well. Thus, anti-HCV IgG antibodies with high affinity Fc (GM) determinants may efficiently bind the core protein in the acidic intracellular environment and prevent the release of the HCV virions into the extracellular fluids, where they can be cleared by various effector mechanisms. This will lead to the persistence of HCV infection, and conversely, anti-HCV core IgG antibodies with low affinity Fc (GM) determinants will lead to HCV clearance.

Results presented here shed new light on the mechanisms underlying the involvement of GM allotypes in the outcome of HCV infection. They also underscore the importance of CH1 and CH3 domains of IgG in the binding of the Fc γ R-like site on the HCV core protein. It is interesting to note that GM markers are also involved in immunity to other viruses—HSV1, human cytomegalovirus, and Epstein-Barr virus—which also encode Fc γ R-like proteins that bind the Fc region of IgG (Atherton et al., 2000; Tortorella et al., 2000; Pandey, 2004; Biggar et al., 1984)

As pointed out elsewhere, most studies concerning the interaction of Fc and Fc γ R have not appreciated the inherent diversity of the Fc domain (Pandey, 2006). Concerted effort is

currently being directed at engineering Fc variants with optimized affinity for activating and inhibiting FcγRs. Together with this effort, it is important to evaluate the affinity and specificity of naturally occurring Fc (GM) variants that may have contributed to our survival during past epidemics. Indeed, GM allotypes appear to have influenced the chance for survival in typhoid and yellow fever epidemics in Surinam (de Vries et al., 1979). The differential binding of Fc (GM) and the FcγR-like molecule reported here might spur studies involving both cellular and virally encoded FcγRs to further delineate the role of Fc gene polymorphism as a modulator of Fc-FcγR interaction. Results of such investigations may aid in engineering the next generation of humanized monoclonal antibodies with enhanced effector functions, resulting in maximum clinical efficacy against infectious and malignant diseases. These findings also shed light on the possible evolutionary mechanism that maintains GM polymorphism. They suggest that natural selection due to infectious agents like HCV, rather than neutral mutations (Kimura, 1983) may be responsible for the maintenance of extensive GM gene polymorphism.

Acknowledgements

This work was supported in part by NIH grant R01 DK070877.

References

- Atherton A, Armour KL, Bell S, Minson AC, Clark MR. The herpes simplex virus type 1 Fc receptor discriminates between IgG1 allotypes. *Eur J Immunol* 2000;30:2540–2547. [PubMed: 11009087]
- Biggar RJ, Pandey JP, Henle W, Nkrumah FK, Levine PH. Humoral immune response to Epstein-Barr virus antigens and immunoglobulin allotypes in African Burkitt lymphoma patients. *Int J Cancer* 1984;33:577–580. [PubMed: 6327537]
- Chapman TL, You I, Joseph IM, Bjorkman PJ, Morrison SL, Raghavan M. Characterization of the interaction between the herpes simplex virus type 1 Fc receptor and immunoglobulin G. *J Biol Chem* 1999;274:6911–6919. [PubMed: 10066744]
- de Vries RRP, Meera Khan P, Bernini LF, van Loghem E, van Rood J. Genetic control of survival in epidemics. *J Immunogenet* 1979;6:271–287. [PubMed: 521665]
- Deisenhofer J. Crystallographic refinement and atomic models of a human Fc fragment and its complex with fragment B of protein A from *Staphylococcus aureus* at 2.9- and 2.8-Å resolution. *Biochemistry* 1981;20:2361–2370. [PubMed: 7236608]
- Kimura, M. *The Neutral Theory of Molecular Evolution*. Cambridge University Press; Cambridge, UK: 1983.
- Kittleson DJ, Chianese-Bullock KA, Yao ZQ, Braciale TJ, Hahn YS. Interaction between complement receptor gC1qR and hepatitis C virus core protein inhibits T-lymphocyte proliferation. *J Clin Invest* 2000;106:1239–1249. [PubMed: 11086025]
- Maillard P, Lavergne JP, Siberil S, Faure G, Roohvand F, Petres S, Teillaud JL, Budkowska A. Fcγ receptor-like activity of hepatitis C virus core protein. *J Biol Chem* 2004;279:2430–2437. [PubMed: 14610077]
- Martin MP, Carrington M. Immunogenetics of viral infections. *Curr Opin Immunol* 2005;17:510–516. [PubMed: 16084708]
- McLauchlan J. Properties of the hepatitis C virus core protein: a structural protein that modulates cellular processes. *J Viral Hepat* 2000;7:2–14. [PubMed: 10718937]
- Pandey JP. Immunoglobulin GM genes and IgG antibodies to cytomegalovirus in patients with systemic sclerosis. *Clin Exp Rheumatol* 2004;22:S35–S37. [PubMed: 15344596]
- Pandey JP, Astemborski J, Thomas DL. Epistatic effects of immunoglobulin GM and KM allotypes on outcome of infection with hepatitis C virus. *J Virol* 2004;78:4561–4565. [PubMed: 15078937]
- Pandey JP. Genetic polymorphism of Fc. *Science* 2006;311:1376–1377. [PubMed: 16527951]
- Schilling R, Ijaz S, Davidoff M, Lee JY, Locarnini S, Williams R, Naoumov NV. Endocytosis of hepatitis B immune globulin into hepatocytes inhibits the secretion of hepatitis B virus surface antigen and virions. *J Virol* 2003;77:8882–8892. [PubMed: 12885906]

- Shields RL, Namenuk AK, Hong K, Meng YG, Rae J, Briggs J, Xie D, Lai J, Stadlen A, Li B, Fox JA, Presta LG. High resolution mapping of the binding site on human IgG1 for Fc gamma RI, Fc gamma RII, Fc gamma RIII, and FcRn and design of IgG1 variants with improved binding to the Fc gamma R. *J Biol Chem* 2001;276:6591–6604. [PubMed: 11096108]
- Steinberg, AG.; Cook, CE. *The Distribution of Human Immunoglobulin Allotypes*. Oxford University Press; New York: 1981.
- Tortorella D, Gewurz BE, Furman MH, Schust DJ, Ploegh HL. Viral subversion of the immune system. *Annu Rev Immunol* 2000;18:861–926. [PubMed: 10837078]
- Vyas GN, Fudenberg HH, Pretty HM, Gold ER. A new rapid method for genetic typing of human immunoglobulins. *J Immunol* 1968;100:274–279. [PubMed: 4170633]

Abbreviations

GM	Ig γ chain marker
KM	Ig κ chain marker
CH	Ig heavy chain constant region
HCV	hepatitis C virus
OR	odds ratio

Table 1

Amino acid residues at key positions in IgG1

GM Alleles	CH1 (214) GM 17/GM 3	CH3 (356) GM 1/GM -1	CH3 (358) GM 1/GM -1	CH3 (431) GM 2/GM -2
GM 1,2,17 GM 3	Lys Arg	Asp Glu	Leu Met	Gly Ala

Table 2

Absorbance values^a (450 nm) for binding of IgG1 proteins to the immobilized HCV core protein in subjects with GM 1,2,17 or GM 3 genotypes

IgG1 (GM 1,2,17) Mean (SE) ^b	IgG1 (GM 3) Mean (SE)
0.23 (0.01)	0.35 (0.02)
0.27 (0.02)	0.38 (0.02)
0.35 (0.04)	0.40 (0.01)
0.34 (0.03)	0.33 (0.02)
0.42 (0.02)	0.60 (0.01)
0.40 (0.02)	0.61 (0.00)
0.25 (0.01)	0.69 (0.02)
0.25 (0.01)	0.43 (0.00)
0.31 (0.02)	0.36 (0.01)
0.32 (0.02)	0.42 (0.03)
0.39 (0.02)	0.41 (0.01)
0.37 (0.01)	0.48 (0.01)
0.32 (0.01)	0.37 (0.01)
0.24 (0.01)	0.33 (0.02)
0.29 (0.01)	0.44 (0.02)
0.31 (0.01)	0.43 (0.01)
0.37 (0.02)	0.40 (0.01)
0.32 (0.01)	0.44 (0.01) ^c

^a The absorbance values are a ratio of the binding of IgG1 to the core protein relative to the anti-human IgG standard.

^b Standard errors were obtained via experiment replication (i.e. 6 replications per subject, 102 replications per group).

^c $p = 0.0003$ by mixed linear regression.