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Interactive effects of immunoglobulin gamma and human leucocyte antigen genotypes on clearance and persistence of infection with hepatitis C virus

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Summary

Particular alleles of human leucocyte antigen (HLA) and immunoglobulin gamma (GM) and immunoglobulin kappa (KM) allotypes (polymorphic determinants of IgG heavy chains and κ-type light chains, respectively) are associated with the outcome of several infections. To examine their role in the outcome of hepatitis C virus (HCV) infection, we genotyped 50 individuals with resolved and 117 with persistent HCV infection. None of the GM, KM or HLA-C genotypes by themselves were associated with the resolution or persistence of HCV infection. However, particular combinations of HLA and GM genotypes were associated significantly with the outcome of HCV infection. Subjects with the HLA C1C1 genotype, in the absence of GM ff, were more than seven times [odds ratio (OR) 7·15] as likely to have persistent infection as the subjects who lacked both these genotypes. The presence of GM ff, in the absence of HLA C1C2, was associated with the resolution of infection (OR 0·27). The absence of GM fz, in the presence of HLA C2C2, was also associated with the resolution of infection (OR 0·27). Compared to the subjects who lacked both these genotypes, subjects with GM fz, in the absence of HLA C1C2, were almost four times as likely to have persistent infection (OR 3·91); similarly, subjects with HLA C1C2, in the absence of GM fz, were almost three times as likely to have persistent infection (OR 2·80). These results show, for the first time, interactive effects of GM and HLA genotypes in the outcome of HCV infection.

Keywords: allotypes, GM, HCV, HLA, KM

Introduction

Hepatitis C virus (HCV) is a common infection, affecting over 170 million people worldwide [1]. Of people acutely infected with HCV, about 15% clear the virus spontaneously. Among the factors influencing the outcome of HCV infection, the host genetic factors play a major role [2,3]. We have reported previously that particular combinations of immunoglobulin gamma (GM) and immunoglobulin kappa (KM) allotypes are associated with HCV clearance and persistence in African Americans [4]. We have also shown an association of particular GM alleles with HCV infection in a population from Thailand [5]. To our knowledge, the role of these genes in the outcome of HCV infection in a Caucasian population has not been examined. Every major race is characterized by a distinct array of GM haplotypes. In fact, unless there is genetic admixture, Negroid and Caucasoid populations do not share any haplotypes [6]. KM gene frequencies, too, vary significantly among various ethnic groups [6]. There are marked racial differences in rates of clearance of HCV and in responses to interferon-based therapies which may, at least in part, be due to the host genetic differences [2,7,8]. Therefore, it is important to examine the role of GM, KM and other candidate genes in various ethnic groups.

The aim of the present investigation was to examine the role of GM and KM genes in the outcome of HCV infection in a Caucasian population. We also wished to study the role of genetic interaction, as particular GM and HLA genes have been shown to have an interactive effect on susceptibility to some infectious diseases [9,10]. In this investigation we wished to determine possible interactive effects of GM and HLA-C genotypes, which are known to contribute to the outcome of HCV infection [4,5,11].

Materials and methods

Subjects

A total of 117 Caucasian Spanish patients (72 males and 45 females) suffering from biopsy-proven chronic hepatitis C

Table 1. Distribution of human leucocyte antigen (HLA) and immunoglobulin gamma (GM) genotypes in relation to persistence and resolution of hepatitis C virus (HCV) infection.

	Subjects with HCV infection				
	Persistent	Resolved			
Genotypes	No. (%)	No. (%)	OR	95% CI	*P*-value
HLA-C1C1	36 (30·8)	12 (24·0)	1·41	0·62–3·32	0·4
HLA-C1C2	67 (57·3)	27 (54·0)	1·14	0·56–2·43	0·7
HLA-C2C2	14 (12·0)	11 (22·0)	0·48	0·19–1·25	0·09
GM-ff	57 (48·7)	25 (50·0)	0·95	0·46–1·94	0·9
GM-fz	51 (43·6)	20 (40·0)	1·16	0·56–2·40	0·8
GM-zz	9 (7·7)	5 (10·0)	0·75	0·21–3·02	0·8

with compensated liver disease were enrolled in this study. All patients were hepatitis B surface antigen (HBsAg) and human immunodeficiency virus (HIV) negative, anti-HCV positive with raised alanine aminotransferase (ALT) levels and positive HCV RNA in serum. Anti-HCV, HBsAg and HIV were determined by commercially available methods. Fifty Caucasian Spanish patients (22 males and 28 females), who were anti-HCV positive and negative for HCV RNA, constituted the group with spontaneous viral clearance. Our cohort was > 86% HCV genotype 1. Patients agreed to a blood examination according to the guidelines of the Hospital Bioethics Committee.

HLA genotyping

HLA-C locus was genotyped using the RELI™ SSO HLA-C SSP typing kit (Dynal Biotech, Bromborough, UK). Ambiguities were resolved by sequencing analysis. Individuals from the different groups (persistent and resolved) were categorized in HLA-C1 and HLA-C2 according to their genotyping data. HLA-C1 and -C2 molecules possess asparagine and lysine at residue 80, respectively.

GM and KM genotyping

For the determination of GM f/3 and z/17 alleles (a C to T substitution in the CH1 region of the γ1 gene), a direct DNA sequencing method was used. The DNA segment encoding the CH1 region of γ1 chain was amplified by polymerase chain reaction (PCR) according to Balbìn *et al.* [12], using the following primers: 5′-CCCCTGGCACCCTCCTCCAA-3′ and 5′-GCCCTGGACTGGGGCTGCAT-3′. The purified double-stranded PCR product [364 base pairs (bp)] was subjected to an automated DNA sequencing on an ABI PRISM 377. (Because of complete linkage disequilibrium between particular alleles of γ loci within a race, it is not necessary to type for all markers to postulate the most probable haplotype segregating in a population. For instance, Caucasian subjects with GMz/17 on γ1 would have GM g/21 on γ3.) Kappa chain determinants KM 1 and 3 were characterized by a PCR-restriction fragment length polymorphism (RFLP) technique using the following primers: 5′-TAG GGG GAA GCT AGG AAG AAA-3′ and 5′-AAA AAG GGT CAG AGG CCA AA-3′. After digestion of the amplified product with the restriction enzyme Acc1, the following products corresponding to the two alleles were detected: KM 1 (a 538 bp fragment) and KM 3 (a 390 bp and a 148 bp fragment).

Statistical analysis

A fit to Hardy–Weinberg equilibrium was determined using the χ^2 test for goodness of fit. Odds ratio (OR) and *P*-values for the main and interactive effects of particular genotypes on the outcome of HCV infection were calculated by logistic regression, the most common method of analysing predictors of a dichotomous (yes/no) outcome variable. In the present investigation, the dichotomous outcome was defined as resolution or persistence of HCV infection, and the prognostic factors were GM, KM and HLA genotypes. All tests were two-tailed, and the statistical significance was defined as $P < 0{\cdot}05$.

Results

Hardy–Weinberg equilibrium for the genotypes studied is an important prerequisite for a sound population association study. A significant departure from the equilibrium could result from many factors, including genotyping errors and population stratification. All genotype frequencies in the present investigation were in Hardy–Weinberg equilibrium.

None of the GM or HLA-C genotypes by themselves were associated with the resolution or persistence of HCV infection (Table 1). Also, none of the KM genotypes alone, or when combined with GM genotypes, were associated with the outcome of HCV infection (data not shown). Particular combinations of HLA and GM genotypes, however, were associated significantly with the outcome of HCV infection. Subjects with the HLA C1C1 genotype, in the absence of GM ff, were more than seven times [OR 7·15; 95% confidence interval (CI) 1·54–33·20] as likely to have persistent infection as the subjects who lacked both these genotypes (Table 2). The presence of GM ff, in the absence of HLA C1C2, was associated with the resolution of infection (OR 0·27; 95% CI

Table 2. Distribution of combined human leucocyte antigen (HLA) C1C1 and immunoglobulin gamma (GM) ff genotypes in relation to persistence and resolution of hepatitis C virus (HCV) infection.

	Subjects with HCV infection				
	Persistent	Resolved			
Genotypes	No. (%)	No. (%)	OR	95% CI	*P*-value
C1C1 (+)/ff (+)	13 (11·1)	10 (20·0)	0·81	0·30–2·14	0·668
C1C1 (+)/ff (−)	23 (19·7)	2 (4·0)	7·15	1·54–33·20	0·012
C1C1 (−)/ff (+)	44 (37·6)	15 (30·0)	1·82	0·83–3·99	0·133
C1C1 (−)/ff (−)	37 (31·6)	23 (46·0)	1·00		

0·10–0·78) (Table 3). The absence of GM fz, in the presence of HLA C2C2, was also associated with the resolution of infection (OR 0·27; 95% CI 0·08–0·93) (Table 4). Simultaneous presence of heterozygosity at one locus and its absence at the other locus was associated with the persistence of infection. Thus, compared to the subjects who lacked both these genotypes, subjects with GM fz, in the absence of HLA C1C2, were almost four times as likely to have persistent infection (OR 3·91; 95% CI 1·32–11·60); similarly, subjects with HLA C1C2, in the absence of GM fz were almost three times as likely to have persistent infection (OR 2·80; 95% CI 1·15–6·81) (Table 5). No other significant associations were found.

Discussion

We have shown complex non-additive interactive effects of two separate genetic regions – GM genes located on chromosome 14, and HLA genes located on chromosome 6 – on the outcome of HCV infection. There are at least two possible explanations for the associations. The GM and HLA genes could themselves affect the outcome of HCV infection.

Table 3. Distribution of combined human leucocyte antigen (HLA) C1C2 and immunoglobulin gamma (GM) ff genotypes in relation to persistence and resolution of hepatitis C virus (HCV) infection.

	Subjects with HCV infection				
	Persistent	Resolved			
Genotypes	No. (%)	No. (%)	OR	95% CI	*P*-value
C1C2 (+)/ff (+)	40 (34·2)	10 (20·0)	0·97	0·34–2·74	0·953
C1C2 (+)/ff (−)	27 (23·1)	17 (34·0)	0·38	0·14–1·03	0·057
C1C2 (−)/ff (+)	17 (14·5)	15 (30·0)	0·27	0·10–0·78	0·014
C1C2 (−)/ff (−)	33 (28·2)	8 (16·0)	1·00		

Table 4. Distribution of combined human leucocyte antigen (HLA) C2C2 and immunoglobulin gamma (GM) fz genotypes in relation to persistence and resolution of hepatitis C virus (HCV) infection.

	Subjects with HCV infection				
	Persistent	Resolved			
Genotypes	No. (%)	No. (%)	OR	95% CI	*P*-value
C2C2 (+)/fz (+)	9 (7·7)	4 (8·0)	0·85	0·24–3·03	0·800
C2C2 (+)/fz (−)	5 (4·3)	7 (14·0)	0·27	0·08–0·93	0·039
C2C2 (−)/fz (+)	42 (35·9)	16 (32·0)	0·99	0·47–2·09	0·978
C2C2 (−)/fz (−)	61 (52·1)	23 (46·0)	1·00		

Table 5. Distribution of combined human leucocyte antigen (HLA) C1C2 and immunoglobulin gamma (GM) fz genotypes in relation to persistence and resolution of hepatitis C virus (HCV) infection.

	Subjects with HCV infection				
	Persistent	Resolved			
Genotypes	No. (%)	No. (%)	OR	95% CI	*P*-value
C1C2 (+)/fz (+)	22 (18·8)	14 (28·0)	1·27	0·50–3·21	0·611
C1C2 (+)/fz (−)	45 (38·5)	13 (26·0)	2·80	1·15–6·81	0·023
C1C2 (−)/fz (+)	29 (24·8)	6 (12·0)	3·91	1·32–11·60	0·014
C1C2 (−)/fz (−)	21 (17·9)	17 (34·0)	1·00		

Alternatively, linkage disequilibrium between particular GM or HLA alleles and the alleles of another putative risk-conferring locus for HCV pathogenesis may give rise to the associations observed. Both HLA and GM genes are endowed with certain immunological properties that make their direct involvement in the outcome of HCV infection likely. The HLA-C genes investigated in this report could influence the outcome of HCV infection by regulating the activity of natural killer (NK) cells, key players in anti-viral immunity. The killer immunoglobulin-like receptors (KIR) on NK cells bind their HLA-C ligands on target cells with differential affinity. Particular allelic combinations of KIR and HLA-C genes have been reported to be associated with the resolution of HCV infection in one study [11], but not confirmed in our study population reported earlier [13], perhaps owing to the differences in KIR and HLA-C gene frequencies in the two populations.

GM genes could contribute to the outcome of HCV infection through allotype-restricted antibody responses to the HCV epitopes. Although GM markers are located on the constant region, there is a growing body of evidence for the involvement of these regions in antibody specificity usually associated with the variable (V) region of the Ig molecule. Possible mechanisms include direct contribution to the formation of idiotypic determinants, modulation of antibody binding affinity and linkage disequilibrium with alleles coding for the V-region epitopes [14–16]. Recent evidence has challenged the current central tenet of immunology that the V region is the sole determinant of antigen specificity [17]. GM genes could also contribute to the outcome of HCV infection by influencing the strategies employed by this virus to evade host immunosurveillance. The HCV core protein and HCV nucleocapsids circulating in HCV-infected individuals [18] have functional properties of Fcγ receptor (FcγR) that may allow them to interfere with certain host defence mechanisms – antibody-dependent cellular cytotoxicity (ADCC), cytokine release and activation of the classical complement pathway – mediated by the Fcγ part of the anti-HCV IgG antibodies [19]. In support of this mechanism of the involvement of GM allotypes in the outcome of HCV infection, we have recently reported a highly significant difference ($P = 0{\cdot}0003$) in the binding affinity of the HCV core protein to the IgG1 molecules expressing two main GM haplotypes, which include the f/3 and z/17 alleles investigated here [20].

The interaction between HLA-C and GM f,z genotypes observed here could be explained by possible modulation of NK-dependent ADCC against HCV-infected cells by the HLA-C molecules. Evidence for the modulation of NK-dependent ADCC by HLA-C molecules has been presented for the human immunodeficiency virus, which uses a sophisticated immune evasion strategy: to avoid recognition and subsequent elimination by cytotoxic T lymphocytes, it selectively down-regulates HLA-A and B molecules but not HLA-C molecules, which can inhibit NK cell killing by interacting with KIR [21]. The HCV may have evolved similar strategies. The HCV core protein up-regulates HLA class I molecules on liver cells [22]. Antibodies against HCV protein E2 have been shown to mediate ADCC [23]. NK cells express predominantly FcγRIIIa, which is genetically polymorphic. Thus, for instance, anti-HCV-E2 IgG antibodies with Fc of a particular GM genotype could associate preferentially with the FcγRIIIa of a particular genotype [24] and influence the destruction of HCV-infected liver cells through ADCC, and this host defence mechanism could be modulated by the immune evasion strategies of the virus, such as up-regulation of NK-inhibiting HLA-C molecules [22].

We did not find the interactive effect of GM and KM genes on the outcome of HCV infection in Caucasians, as reported for African Americans [4]. The reason for this ethnic difference in genetic association is not clear. Linkage disequilibrium between GM alleles in African Americans is different from that in Caucasians, resulting in distinct arrays of GM haplotypes in the two groups [6]. It follows that linkage disequilibrium between any putative risk-conferring genes for HCV pathogenesis and GM alleles might also be different in the two groups, contributing to the ethnic differences in genetic associations. Differences in allele frequencies and linkage disequilibrium among populations originating from different continents are well documented [25]. A very recent study involving over 4000 genes has shown significant racial differences in the level of gene expression, which could contribute to the differences in genetic associations among various groups [26]. It is also likely that multiple genes interact to influence the outcome of HCV infection, and racial differences in gene frequencies at these loci may contribute to the differences in the observed associations. The results presented here show that GM genes do contribute to the outcome of HCV infection in Caucasians, but in this group their influence is expressed only in the presence of certain HLA genes.

Evidence for gene–gene interaction in infectious diseases is growing. As the documentation of such interaction requires a larger sample size than that needed for individual gene effects, multi-centre studies, employing recently implicated candidate genes, are needed to understand the immunogenetic mechanisms underlying the resolution or persistence of HCV infection. To our knowledge, this is the first report of interaction between HLA and GM genotypes in the outcome of HCV infection. The *P*-values for the associations reported here were not adjusted for multiple comparisons, for there is strong biological rationale – based on their immunological properties and previously published reports – for the involvement of HLA and GM systems in the outcome of HCV infection. Moreover, standard corrections, such as Bonferroni's, do more harm than good to sound statistical inference in biomedical research [27]. Rather than relying on the validity of statistical corrections, the best way to determine whether our observations have a biological

basis (and are not a result of chance occurrences) would be to repeat these studies using an independent study population.

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