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We examined the role that immunoglobulin GM and KM allotypes— genetic markers of γ and κ chains, respectively—play in the outcome of hepatitis C virus (HCV) infection in white Americans. A total of 119 persons who had cleared HCV and 111 with persistent HCV infection were genotyped for the presence of several GM and KM determinants. Persistent HCV infection was more than three times as likely (odds ratio, 3.50; $P = .01$) in subjects who were carriers of the GM3 allele than in those who were noncarriers. These results show that particular GM alleles may be important determinants of the outcome of HCV infection.

Hepatitis C virus (HCV) is a common infection and a major cause of hepatitis. Worldwide, >170 million people are affected. Of persons acutely infected with HCV, ∼20% spontaneously clear the virus. Both viral and host genetic factors play important roles in the clearance of HCV. Among the host genetic factors, genes of the major histocompatibility complex (HLA) have been the primary focus of studies. Associations with several HLA alleles have been reported, but the results are conflicting [1]. Polymorphic determinants of interleukin-10 and interferon-γ have also been implicated in spontaneous recovery from HCV [2, 3]. Allelic variations at these loci, however, do not account for the total interindividual variation in the outcome of HCV infection, suggesting that additional genetic factors are involved in the clearance of HCV and the persistence of HCV infection.

We have previously reported that particular combinations of GM and KM allotypes are associated with spontaneous clearance of HCV or persistent HCV infection in African Americans [4]. To our knowledge, the role that these genes play in the outcome of HCV infection in white Americans—whose GM and KM gene frequencies differ significantly from those in African Americans—has not been examined [5]. There are significant racial differences in rates of spontaneous and treatment-induced clearance of HCV, which may be due, at least in part, to the host genetic differences [3]. Therefore, it is important to examine the role that GM, KM, and other candidate genes play in various ethnic groups. In the present report, we have examined the contribution of particular GM and KM alleles to the outcome of HCV infection in a white American population.

Patients, materials, and methods. The study subjects were enrolled from the Multicenter Hemophilia Cohort Study and from the Division of Gastroenterology and Hepatology at the University of Colorado in Denver. The study was approved by the local institutional review board for human research. A total of 111 patients in whom HCV RNA was detected in 2 plasma specimens collected at least 1 year apart constituted the group with persistent HCV infection, and 119 who were positive for anti-HCV and negative for HCV RNA constituted the group with spontaneous clearance of HCV. All subjects were negative for HIV and were white Americans. Most (97%) of the subjects were male. The median age at initial evaluation was 35 years (range, 1–83 years). Both cohorts were predominantly HCV genotype/serotype 1.

For the determination of IgG1 allelic markers GM3 and GM17 (arginine-to-lysine substitution, a G→A transition in the CH1 region of the $\gamma 1$ gene), a direct DNA-sequencing method was used. The DNA segment encoding the CH1 region of the $\gamma 1$ chain was amplified by polymerase chain reaction (PCR) performed according to the procedure described by Balbín et al. [6] and using primers 5'-CCCCTGGCACCCTCCTCCAA-3' and 5'-GCCCTGGACTGGGGCTGCAT-3'. The purified double-stranded PCR product (364 bp) was subjected to automated DNA sequencing on an ABI PRISM 377.

GM23 (valine-to-methionine substitution, a G→A transition in the CH2 region of the $\gamma 2$ gene) was determined by use of a nested-PCR–restriction fragment length polymorphism (PCR-

Table 1. Distribution of GM and KM genotypes, in relation to persistent hepatitis C virus (HCV) infection and clearance of HCV.

Genotypes	Persistent HCV infection	Cleared HCV
GM		
3/3	57 (54.2)	56 (49.1)
3/17	43 (41.0)	41 (36.0)
17/17	5 (4.8)	17 (14.9)
23+/23+	22 (20.2)	20 (16.8)
23+/23−	56 (51.4)	57 (47.9)
23−/23−	31 (28.4)	42 (35.3)
KM		
1/1	3 (2.8)	1 (0.9)
1/3	17 (16.2)	18 (15.7)
3/3	85 (81.0)	96 (83.5)

NOTE. Data are no. (%) of subjects. Because of typing failures, totals do not sum to 111, in the case of persistent HCV infection, or to 119, in the case of cleared HCV.

RFLP) method. In brief, a 915-bp region of the $\gamma 2$ gene that incorporates the site for the allelic substitution was amplified as described by Brusco et al. [7], by use of primers 5'-AAATGTTGTGTCGAGTGCCC-3' and 5'-GGCTTGCCGG-CCGTGGCAC-3'. A 197-bp segment was further amplified from this 915-bp fragment, by use of primers 5'-GCACCACCTGTGGCAGGACC-3'and 5'-TTGAACTGCTCC-TCCCGTGG-3'. Digestion of the amplified product by the restriction enzyme *Nla*III resulted in the following products corresponding to the following 3 genotypes: GM23+, 90 bp, 63 bp, and 44 bp; GM23−, 134 bp and 63 bp; and GM23+,23−, 134 bp, 90 bp, 63 bp, and 44 bp.

κ-Chain determinants KM1 and KM3 were characterized by a PCR-RFLP technique [8] using primers 5'-ACTGTGGC-TGCACCATCTGTCT-3' and 5'-TCAGGCTGGAACTGAGG-AGCAG-3'. Digestion of the amplified product (360 bp) by the restriction enzyme *Acc*I resulted in the following products corresponding to the following 3 genotypes: KM1, 360 bp; KM3, 247 bp and 113 bp; and KM1,3, 360 bp, 247 bp, and 113 bp. Three alleles—KM1, KM1,2, and KM3—segregate at the KM locus. The KM1 allele is extremely rare, and >98% of subjects positive for KM1 are also positive for KM2; thus, positivity for KM1 includes positivity for both KM1 and KM1,2 alleles.

The genotype-frequency distribution between the 2 groups of patients was analyzed by a global (3×2) χ^2 test (table 1). The significance of the group genotype-frequency difference between particular allele carriers and noncarriers was determined by use of Fisher's exact test (table 2). An odds ratio (OR) was calculated to measure the strength of the association observed. All tests were 2-tailed, and the statistical significance was defined as $P < .05$.

Results. Table 1 presents the distribution of all GM and KM genotypes examined in the present study, in relation to the clearance of HCV and the persistence of HCV infection. The distribution of κ-chain determinants (KM1 and KM3) did not differ in relation to HCV-infection status ($P = .54$). Small differences in the distributions of the $\gamma 2$ gene (GM23) variants also were not statistically significant ($P = .52$). In contrast, the $\gamma 1$ gene (GM3 and GM17) variants did differ significantly by HCV-infection status ($P = .04$). The GM3 and GM23 alleles are in significant linkage disequilibrium.

Table 2 presents the distribution of GM3-carrier and GM23-carrier genotypes, in relation to the clearance of HCV and the persistence of HCV infection. Compared with GM3 noncarriers, the frequency of GM3 carriers in the group with persistent HCV infection was significantly higher than that in the group who had cleared HCV (95% vs. 85% [OR, 3.50; $P = .01$]). The frequency of GM23 carriers was not significantly different between the 2 groups ($P = .32$). None of the KM genotypes, either alone or when combined with GM genotypes, was associated with persistence or clearance of HCV.

Discussion. We found that carriers of the GM3 variant in the $\gamma 1$ gene were at >3-fold-higher risk of persistent HCV infection. This is plausible, because the IgG molecules carrying the GM3 and GM23 alleles bind the Fcγ receptor (FcγR)–like HCV core protein much more strongly than do those carrying GM17 and GM23 alleles [9, 10]. Presumably, the FcγR-like properties of the HCV core protein aid the virus in evading host immuno-surveillance [11]. Under this hypothesis, anti-HCV IgG antibodies in GM3-carrying subjects are more likely to have their Fc domains scavenged, thereby reducing their immunological competence to eliminate the virus or circulating nucleocapsids via antibody-dependent cellular cytotoxicity and other Fc-mediated effector mechanisms—which, in turn, leads to this allele being more prevalent in subjects with persistent HCV infection than in those who have cleared the virus. Although the prevalence of the GM23-carrying genotype was slightly higher in

Table 2. Distribution of GM 3-carrier and GM 23-carrier genotypes, in relation to persistence of hepatitis C virus (HCV) infection and clearance of HCV.

Genotype, status	Persistent HCV infection	Cleared HCV	OR (95% CI)	P
	Subjects, no. (%)[a]			
GM3				
Carriers	100 (95.2)	97 (85.1)	3.50 (1.24–9.87)	.01
Noncarriers	5 (4.8)	17 (14.9)		
GM23				
Carriers	78 (71.6)	77 (64.7)	1.37 (0.78–2.40)	.32
Noncarriers	31 (28.4)	42 (35.3)		

NOTE. CI, confidence interval; OR, odds ratio.
[a] Because of typing failures, totals do not sum to 111, in the case of persistent HCV infection, or to 119, in the case of cleared HCV.

the group with persistent HCV infection than in the group who had cleared the virus—a finding that is consistent with the observation that GM23-carrying IgG2 has a higher affinity for binding to the core protein than does its allelic counterpart—it did not reach statistical significance. This may be a reflection of the fact that antibodies to HCV proteins are predominantly by the IgG1 and IgG3 subclasses [12]. Linkage disequilibrium, which is very strong in the chromosomal region harboring the IgG loci, may provide an additional explanation for the results presented here. It is possible that, in this region, there is an HCV-pathogenesis gene that is in linkage disequilibrium with the GM3 allele of the γ1 locus but not with the GM23 allele of the γ2 locus. This could explain the finding of a significant association between GM3 and HCV persistence, as well as the lack of association with the GM23 determinant.

In a study of Spanish white subjects [13], the prevalence of GM3 carriers in the group with persistent HCV infection was slightly higher than that in the group who had cleared HCV (92.3% vs. 90.0%), but the increase was not statistically significant. That study and the present study are not comparable, however. In addition to the possible differences between the genetic backgrounds of the 2 study populations, the Spanish study consisted of only 50 subjects (as opposed to 119 in the current study) who had cleared HCV and thus may have had inadequate power to detect a true difference. It is relevant to note that in African Americans [4], the prevalence of GM3-carrying GM phenotypes was found to be higher in the group with persistent HCV than in the group who had cleared HCV (36.7% vs. 25.3%; OR, 1.72 [95% confidence interval, 1.00–2.94]). GM allotypes appear to be involved in the immunobiology of hepatitis C, in several different population groups. In some cases, their contribution requires epistatic interaction with other genes of the immune system, such as KM or HLA. There is growing evidence for such interactions, in a variety of human disorders; for instance, rheumatoid arthritis in a Japanese population was significantly associated with certain genetic determinants of Fc receptor–like protein 3, whereas in a Spanish population this was true only in the presence of particular alleles of the gene encoding the promoter region of nuclear factor κB [14]. These findings further underscore the importance of epistasis, as well as the need to examine different ethnic groups.

It is important to delineate the mechanisms underlying the involvement of GM allotypes in the outcome of HCV infection. Such studies may lead to the identification of novel pathways of host immune response that may be helpful in the designing of vaccines for protection against this infection. Some progress toward this end has been made [9, 10, 15], but more studies are needed.

Acknowledgments

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