Influence of Hepatitis C Virus (HCV) Genotypes on HCV Recombinant Immunoblot Assay Patterns

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Ninety-six patients with chronic hepatitis C were studied. A second-generation recombinant immunoblot assay detected anti-NS4 antibodies significantly more often in patients infected by hepatitis C virus genotype 1 than in patients infected by other types. By a third-generation recombinant immunoblot assay, the prevalences of the four antibodies measured did not differ according to the hepatitis C virus genotype.

Hepatitis C virus (HCV) strains classified into different genotypes or subtypes differ in their nucleotide sequence and, as a consequence, in the amino acid composition of the epitopes present on HCV proteins (14, 16). Therefore, the question of whether HCV genotypes might influence antibody response in patients with chronic hepatitis C can be raised.

Ninety-six patients with chronic hepatitis C (64 males and 32 females; mean age, 47 ± 14 years) were studied. All were positive for anti-HCV antibody by a second-generation enzyme-linked immunosorbent assay (ELISA2.0; Ortho Diagnostic Systems, Raritan, N.J.) and positive for HCV RNA PCR. The same serum as used for ELISA2.0 was tested by (i) a third-generation ELISA (ELISA3.0; Ortho Diagnostic Systems) that includes, in addition to the core (c22-3r) and the NS3-NS4-encoded (c200r) recombinant proteins already present in the ELISA2.0 kit, an NS5-encoded recombinant protein (NS5r) (Fig. 1) and (ii) second- and third-generation recombinant immunoblot assays (RIBA2.0 and RIBA3.0; Ortho Diagnostic Systems). RIBA2.0 detects antibodies directed to four recombinant HCV proteins bearing structural (core, c22-3r) and nonstructural (NS4, 5.1.1r and c100-3r; NS3, c33cr) epitopes (Fig. 1). In the RIBA3.0 kit, (i) 5.1.1r and c100-3r were replaced by a mixture of synthetic peptides bearing the 5.1.1 (5.1.1p) and the c100 (c100p) epitopes, (ii) a conformationally modified recombinant c33c protein was added to the initial c33cr, (iii) c22-3r was replaced by a four-epitope core synthetic peptide (c22p), and (iv) a recombinant NS5 protein (NS5r) was added (Fig. 1). The RIBA2.0 and RIBA3.0 tests were performed according to the manufacturer's instructions.

The HCV genotype was determined, by using the same serum as used for anti-HCV assays, by slight modifications of the Inno-LiPA HCV technique (Innogenetics S.A., Gent, Belgium) (10, 15). Amplification of the highly conserved 5' noncoding region of the HCV genome was performed by a reverse transcriptase nested PCR with two sets of universal biotinylated HCV primers. The amplified products were then hybridized to immobilized oligonucleotide probes designed to be specific for the different HCV types and subtypes. For statistical analysis, comparisons of categorical parameters were per-

formed by using the chi-square test. For comparisons of quantitative parameters, the Kruskal-Wallis test was used. Correlations between the RIBA2.0 and RIBA3.0 reactivities were studied by means of the Spearman rank correlation coefficient.

According to the consensus classification (12), the distribution of HCV genotypes was as follows: 1a, 20 of 96 patients (21%); 1b, 42 of 96 (44%); 3a, 19 of 96 (20%); others, 15 of 96 (16%), including type 2a in 9 cases and type 4a in 6 cases. The results obtained with RIBA2.0 and RIBA3.0, according to HCV genotype, are presented in Table 1. With RIBA2.0, anti-5.1.1r and anti-c100-3r antibodies were significantly more prevalent in patients infected by HCV genotype 1 than in patients infected by other genotypes ($P < 0.0001$ and $P < 0.003$, respectively). No difference among HCV genotypes was found for other antibodies. All the patients were positive by ELISA3.0. In contrast with results obtained with RIBA2.0, no significant difference among HCV genotypes was found for the prevalences of any of the four antibodies sought by RIBA3.0. However, weaker c100 reactivity, as assessed by optical interpretation, was found to occur in patients infected by types 2a and 4a than in those infected with other types ($P < 0.02$). As shown in Table 1, compared with those of RIBA2.0, mean RIBA3.0 reactivities were increased for all antibodies, suggesting greater overall sensitivity of RIBA3.0. The RIBA2.0 and RIBA3.0 c100 and c33c reactivities were significantly correlated, whereas the c22 reactivities were not.

In the present study, antibodies directed to NS4-encoded antigens were found by RIBA2.0 in most of the patients infected by HCV type 1 (including subtypes 1a and 1b), whereas they were present in only 13 to 47% of the patients infected by other HCV genotypes. These results are in accordance with those reported for HCV-infected Scottish blood donors (13), for blood donors from different regions of the world determined to be HCV positive by ELISA (7), and for a short series of patients with chronic hepatitis C from The Netherlands (17). These results are likely due to genotype-dependent sequence variability in the NS4 region of the genome. Indeed, only 75 to 77% sequence similarity in the region encoding the c100 antigen has been observed between HCV types 1 and 2 (13). Furthermore, two major sequential antigenic determinants were identified at similar positions within the NS4 sequences of HCV types 1, 2, and 3. These epitopes are partially or fully included in 5.1.1r and c100-3r of RIBA2.0 and were shown to

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FIG. 1. HCV antigens present in second- and third-generation ELISA kits and in second- and third-generation RIBA kits (Ortho Diagnostic Systems). a.a., amino acids; SOD, superoxide dismutase.

differ substantially in sequence according to the HCV type (13). The fact that the 5.1.1 and c100-3 antigens included in serological assays were initially derived from the American prototype HCV strain (type 1a) (5) explains the lower prevalence of antibodies directed to these proteins in patients infected by HCV types 2 to 5. In contrast, antibodies directed to epitopes encoded by the core region (c22-3r) or the NS3 region (c33cr) of the genome, more highly conserved among HCV

genotypes, were detected at similar frequencies for all HCV genotypes.

Replacement of first-generation anti-HCV tests by second-generation tests resulted in a substantial improvement in sensitivity for the detection of HCV antibodies (1, 6, 8). First-generation ELISA and RIBA detected only anti-NS4 antibodies (5) so that, according to our results, they were likely to detect most cases of HCV type 1 infection but a low proportion of other HCV types. Therefore, the increase in sensitivity of second-generation tests, which detected, in addition to anti-NS4 antibodies, antibodies directed to core and NS3 epitopes, reflected the proportion of patients infected by HCV types different from type 1 in the corresponding populations. Indeed, second-generation tests identified 10 to 20% more cases of chronic hepatitis C (1, 2, 4), a finding consistent with the 25 to 40% prevalence of HCV genotypes other than type 1 in patients with chronic hepatitis C (36% in the present series) (3, 10, 11). The enhanced rate of detection of second-generation assays was even greater with high-risk patients, such as hemodialysis patients or intravenous drug users, reaching 40% (1). This last finding is consistent with our recent observation that intravenous drug users are infected by HCV type 3a in more than 60% of cases (10).

Our results suggest that the HCV genotype-dependent differences observed in the rates of detection of anti-HCV antibodies by second-generation assays are no longer observed with the recently developed third-generation confirmatory test. The enhanced sensitivity of the third-generation confirmatory assay for anti-NS4 antibodies can be explained by the replacement of 5.1.1r and c100-3r by synthetic peptides in which a highly conserved epitope of the c100 protein was included (Fig. 1). This epitope, located at the C terminus of the protein, was present in a truncated form, lacking four amino acids (amino acid positions 1932 to 1935), in first- and second-generation tests (Fig. 1), so that they were not able to detect the corresponding antibodies. We recently showed that, for patients with an "indeterminate" c22-3r pattern by RIBA2.0, RIBA3.0 was positive in most cases, mainly because of the latter assay's greater sensitivity for the detection of anti-c33cr and anti-c100p antibodies (9). The ability of third-generation confirmatory tests to detect anti-NS4 antibodies in patients infected by any genotype could therefore be responsible, at least in part, for their improved sensitivity, especially for patients with negative or indeterminate second-generation patterns. These results suggest that including the 4-amino-acid C-terminal region of the c100 epitope into the NS4-encoded antigens of third-

TABLE 1. Prevalences of the four antibodies detected by second- and third-generation RIBAs and mean reactivities, as assessed by optical interpretation, according to HCV genotypes in 96 patients with chronic hepatitis C

Assay and antibody	Result ^a for genotype:				P value
	1a (n = 20)	1b (n = 42)	3a (n = 19)	Other (n = 15)	
RIBA2.0					
Anti-5.1.1r	18 (90); 2.7 ± 1.3	31 (74); 2.2 ± 1.5	8 (42); 1.1 ± 1.5	2 (13); 0.5 ± 1.2	<0.0001
Anti-c100-3r	18 (90); 3.2 ± 1.3	31 (74); 2.1 ± 1.6	9 (47); 1.4 ± 1.7	6 (40); 1.5 ± 1.9	<0.03
Anti-c33cr	20 (100); 3.5 ± 0.6	41 (98); 3.4 ± 0.9	17 (89); 2.6 ± 1.3	15 (100); 3.5 ± 0.8	NS ^b
Anti-c22-3r	18 (90); 3.3 ± 1.3	41 (98); 3.5 ± 0.9	19 (100); 3.6 ± 0.5	15 (100); 3.7 ± 0.6	NS
RIBA3.0					
Anti-c100p	20 (100); 3.8 ± 0.7	40 (95); 3.4 ± 1.1	17 (89); 3.1 ± 1.4	12 (80); 2.5 ± 1.7	NS
Anti-c33cr	20 (100); 4.0 ± 0.0	42 (100); 4.0 ± 0.2	19 (100); 3.4 ± 0.7	15 (100); 3.8 ± 0.5	NS
Anti-c22p	19 (95); 3.8 ± 0.9	41 (98); 3.9 ± 0.6	19 (100); 4.0 ± 0.0	15 (100); 3.9 ± 0.4	NS
Anti-NS5r	18 (90); 3.6 ± 1.2	33 (79); 2.9 ± 1.7	15 (79); 2.7 ± 1.8	12 (80); 3.0 ± 1.6	NS

^a Number (percentage) of positive serum samples; mean reactivity ± 1 standard deviation, as assessed by optical interpretation.

^b NS, not significantly different.

generation screening assays might further enhance their sensitivity.

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