Reduced Antibody Reactivity to Hepatitis C Virus Antigens in Hemodialysis Patients Coinfected with Hepatitis B Virus

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Antibody reactivities to hepatitis C virus (HCV) antigens and to synthetic peptides derived from different parts of the HCV genome (core, NS4, and NS5) were evaluated in HCV-infected hemodialysis patients. In the RIBA 3 assay, NS5 was significantly less recognizable by sera of hemodialysis patients compared to other HCV-infected subjects. Among hemodialysis patients, those coinfected with hepatitis B virus (HBV) (positive for hepatitis B surface antigen [HBsAg]) showed a reduction in reactivity to C33 and C100. Sera of only 23% of the hemodialysis patients (37 of 161) reacted with more than three of eight peptides tested, significantly fewer than the 60% (12 of 20) of the sera of other HCV-infected patients tested (P = 0.001). This immunosuppression was also manifested by a reduced frequency of recognition of additional peptides on follow-up. An even more reduced reactivity was observed among the HBV-coinfected patients (HBsAg+). The low-responder hemodialysis patients were not infected with any particular genotype of HCV, and the same HCV genotypes observed in the whole group of hemodialysis patients (1a, 1b, 2a, and 3a) were found circulating in the low-responder group. Even in this low-responder population, the good performance of two peptides (peptide 716, corresponding to a portion of the core, and peptide 59, corresponding to a portion of NS4) corroborates the immunodominance of the conserved epitopes within these peptides.

Dialysis patients have an increased risk of exposure to the parenterally transmitted hepatitis virus. Hepatitis C virus (HCV) prevalence in hemodialysis patients is highly variable between different countries and between different centers in the same locality. Previously, we studied four groups in Caracas, Venezuela, with a high prevalence (71% in 1994) and incidence (38% from 1994 to 1995) of HCV infection (16). Seronegative and/or seroconverting specimens are frequently found in this population because of the high incidence of infection and due to the intrinsic immunosuppression of these patients (6, 16). Recently, we reported the performance of overlapping synthetic peptides corresponding to portions of the core, NS4, and NS5 regions for detection of antibodies in HCV-infected patients (15). Hemodialysis patients represent a particular group for whom sensitivity of antibody-screening assays, especially when synthetic peptides are used, may be critical. The aim of this study was to evaluate the antibody response in hemodialysis patients to selected antigenic synthetic peptides derived from different parts of the HCV genome and the evolution of this response over time. A restricted antibody reactivity was in fact observed, particularly in hepatitis B virus (HBV)-coinfected patients, although some immunodominant epitopes in the core and NS4 regions were still consistently recognized by the sera of these patients.

MATERIALS AND METHODS

Population. Hemodialysis patient sera (n = 225) were collected in April 1994 from four different units in Caracas. Sera from some of these patients were obtained 1 year later (n = 61). A high prevalence of HCV antibodies (71%) was determined by testing sera with three different second-generation kits (Abbott [North Chicago, Ill.] HCV enzyme immunoassay 2 [EIA 2], Ortho [Raritan, N.J.] EIA 2.0, and UBI HCV EIA) and confirmed by RIBA 3 (Ortho). RIBA 3 includes recombinant proteins derived from the nonstructural regions NS3 (C33) and NS5 and peptides from the core (C22) and NS4 (C100-3 or C100) regions. A specimen was considered seropositive for HCV if it was reactive in all EIAs and/or confirmed by RIBA 3. Hepatitis B surface antigen (HBsAg) was detected by HBSAg Uni-Form II (Organon Teknika) and AUS/ SYME (Abbott). A sample was considered HBsAg positive if it was reactive by both immunoassays. Anti-hepatitis B core (anti-HBc) and hepatitis B e antigen (HBeAg) were detected by Corezyme (Abbott) and Hepanostika HBeAg/anti-HBe (Organon) (16).

For comparison, HCV-positive blood donors (n = 8) and other HCV-infected patients (n = 12), without any evident cause of immunosuppression and without any HBV serological marker, were also tested. As no difference in antibody reactivity was observed between the two groups, these 20 sera were used as positive controls for comparison with the sera of the hemodialysis patients. The genotypes of HCV circulating in 38 hemodialysis patients and in 10 other HCV patients were determined by restriction fragment length polymorphism as described previously (15).

EIAs. HCV antibodies were tested in follow-up specimens by Abbott HCV EIA 2. Antibody reactivities to eight selected 20-mer antigenic peptides corresponding to portions of the core (peptides 713, 716, 722, and 725 [amino acids 2 to 21, 13 to 32, 43 to 64, and 58 to 77, respectively]), NS4 (peptides 59 and 139 [amino acids 2121 to 1940 and 1927 to 1946, respectively]), and NS5 (peptides 220 and 232 [amino acids 2157 to 2175 and 2279 to 2298, respectively]) proteins were tested by EIA as previously described (15), except that the peptides were adsorbed to the microtiter plates at a 10-µg/ml dilution. These peptides were selected from 26 previously tested peptides as the most widely recognized for each region (15). These peptides were derived from the Kato sequence (8). The percent amino acid conservation of the peptides used in this study was determined as (1 − n/N) × 100, where n is the number of amino acid substitutions in the sequences analyzed and N is the number of sequences analyzed (166 core sequences, 34 NS4 sequences, and 28 NS5 sequences [11]). Statistical differences were evaluated by the chi-square test with Yates’ correction, and by the Fisher exact test when a value was less than 5, using the Epi Info program, version 5.01b (Centers for Disease Control and Prevention, Atlanta, Ga.). Average statistical differences were evaluated by the Student t test.

RESULTS

A total of 225 hemodialysis patients were tested, and 71% were anti-HCV positive at the beginning of the study. Anti-
body reactivities to HCV proteins and synthetic peptides were evaluated for these patients. Reactivities to proteins corresponding to different parts of the genome were evaluated by RIBA 3 for 152 of the 161 HCV-positive hemodialysis patients (for whom detailed reactivity was available) and compared to the reactivities for 20 HCV-infected patients not undergoing hemodialysis. In the hemodialysis patients, C33 was the protein most frequently recognized, followed by C22 and C100. In the other patients, C33, C22, and C100 were recognized with similar frequencies while NS5 was the protein less frequently recognized (Table 1). The difference in reactivity to the NS5 proteins between hemodialysis patients and other HCV-infected patients was statistically significant (Table 1). Among the hemodialysis patients, the ones coinfected with HBV (HBsAg positive) showed significantly lower responses to C33 and to C100, compared to the patients without HBV coinfection (Table 1).

In testing the antibody responses to selected antigenic synthetic peptides corresponding to portions of the core, NS4, and NS5 regions, a restricted pattern of recognition was observed for hemodialysis patients compared to HCV-positive controls. Only 37 (23%) of the 161 hemodialysis patient sera reacted with more than three of the eight peptides tested, a significantly smaller proportion than 12 (60%) of 20 HCV-positive controls (Table 2). The peptides most frequently recognized by hemodialysis patient sera were peptides 716 (70% reacted with this core peptide) and 59 (65% reacted with this NS4 peptide), and 85% of the sera reacted with at least one of the two peptides (data not shown). These two peptides were among the ones with the lowest number of amino acid substitutions between different HCV isolates. However, particularly in the core region, another peptide (peptide 722) was even more conserved than peptide 716 but was recognized with a significantly lower frequency (Table 2). Only 3 of the 161 sera not reacting with peptide 716 reacted with another peptide corresponding to portions of the core region (peptide 722 or 725), and no sera which did not react with peptide 59 reacted with peptide 139. Reactivities to the nonoverlapping peptides from the NS5 region were more variable: 10 of 161 sera reacted with peptide 232 without recognizing peptide 220, while 8 sera not reacting with peptide 232 reacted with peptide 220. Of the 161 HCV-infected hemodialysis patients, 43 were found to be coinfected with HBV (HBsAg positive) (of whom 21 were HBeAg positive) and 49 had past infections with HBV, as suggested by the presence of anti-HBc positivity without HBsAg positivity. Most of the HBV-coinfected hemodialysis patients recognized only one peptide or none, while most of the patients without HBV coinfection recognized two or three peptides (Table 2). No particular peptide, except peptide 59, was recognized with a significantly lower frequency in HBV-coinfected patients. No significant difference was found between the number of peptides recognized and the presence of HBeAg in the serum (data not shown).

Only 1 of the 64 anti-HCV-negative hemodialysis patient sera reacted with two of the peptides tested, peptides 59 and 139. The actual HCV infection of this patient was confirmed by the presence of HCV RNA in his serum, a positive reaction in RIBA, and second-generation test seroconversion 1 year later (data not shown). Of the 161 HCV-positive sera, 45 (28%) reacted with none or one of the synthetic peptides tested (low responders). These sera showed strong reactivity to C33 in RIBA 3 (Table 3). Of the 24 sera reacting with one peptide, only one reacted with another peptide (peptide 722) different from 716 or 59 (data not shown). No significant difference was found in age or time on hemodialysis between the low-responder group (one peptide or none) and the high-responder group (more than one peptide). The only significant difference between the high- and low-responder groups was the higher prevalence of HBsAg among the low responders (Table 3). Among the subset of hemodialysis patients analyzed for HCV genotypes (n = 38), no significant difference was found in the distribution of subtypes circulating in the low-responder patients compared to the high-responder patients, or in the HBV-coinfected patients compared to the patients without HBV coinfection (data not shown).

A high incidence of seroconversion (38% from 1994 to 1995) was previously documented for this group of patients (16). A total of 15 of 40 initially HCV-negative patients were found positive for HCV antibodies 1 year later. Of the sera from these 15 patients, 13 (87%) recognized at least one of the peptides tested. The peptides most frequently recognized were

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**TABLE 2. Antibody reactivities of hemodialysis patients to synthetic peptides derived from different parts of the HCV genome**

<table>
<thead>
<tr>
<th>Patients (no. of sera)</th>
<th>Core</th>
<th>NS4</th>
<th>NS5</th>
<th>No. (%) recognizing the following no. of peptides:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>713</td>
<td>716</td>
<td>722</td>
<td>725</td>
</tr>
<tr>
<td>Hemodialysis (161)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>37 (23)</td>
<td>112 (70)</td>
<td>27 (17)</td>
<td>42 (26)</td>
</tr>
<tr>
<td>HCV+c controls (20)</td>
<td>4 (20)</td>
<td>20 (100)</td>
<td>8 (40)</td>
<td>9 (45)</td>
</tr>
<tr>
<td></td>
<td>NSb</td>
<td>0.01</td>
<td>0.03</td>
<td>NS</td>
</tr>
<tr>
<td>Hemodialysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HBsAg+ (43)</td>
<td>9 (21)</td>
<td>27 (63)</td>
<td>6 (14)</td>
<td>12 (28)</td>
</tr>
<tr>
<td>HBsAg+ (118)</td>
<td>27 (23)</td>
<td>85 (72)</td>
<td>23 (19)</td>
<td>30 (25)</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

*a* Amino acid conservation (as defined in Materials and Methods): 713, 92%; 716, 94%; 722, 99%; 725, 86%; 59, 98%; 139, 91%; 220, 98%; 232, 91%.

*b* NS, not significant (*P* > 0.05).
TABLE 3. Characterization of low-responder patients compared to high responders

<table>
<thead>
<tr>
<th>Patients* (no. of sera)</th>
<th>C100 (no. (%) reactive)</th>
<th>C33 (no. (%) reactive)</th>
<th>C22 (no. (%) reactive)</th>
<th>NS5 (no. (%) reactive)</th>
<th>Avg age (yr)</th>
<th>Mo on dialysis</th>
<th>No. coinfected with HBVa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low responders (44)</td>
<td>31 (70)</td>
<td>40 (91)</td>
<td>37 (84)</td>
<td>10 (23)</td>
<td>48</td>
<td>50</td>
<td>19 (43)</td>
</tr>
<tr>
<td>High responders (108)</td>
<td>102 (94)</td>
<td>106 (98)</td>
<td>99 (92)</td>
<td>55 (51)</td>
<td>43</td>
<td>49</td>
<td>24 (22)</td>
</tr>
<tr>
<td>P</td>
<td>0.001</td>
<td>NS</td>
<td>NS</td>
<td>0.003</td>
<td></td>
<td></td>
<td>0.02</td>
</tr>
</tbody>
</table>

a Sera of low responders recognized one peptide or none; those of high responders recognized more than one peptide.
b RIBA 3 assay.
c Positive for HBsAg.
d NS, not significant (P > 0.05).

The intrinsic immunosuppression in hemodialysis patients (6) was shown by a restricted antibody reactivity to recombinant proteins and synthetic peptides corresponding to different parts of the HCV genome. These results are in agreement with those of previous studies comparing reactivities to HCV proteins between competent and immunocompromised hosts (10).

The wide recognition of NS3 was also shown with hemodialysis patients. This result is in agreement with previously reported data for seroconverting follow-up specimens from hemodialysis patients, where C33 was the protein most frequently recognized (2). Additionally, the sensitivity of a screening test for detection of antibodies to HCV seems to be greatly influenced by the optimal presentation of the NS3 antigen (3).

It has been reported that acute-phase sera can be differentiated from chronic-phase sera by a reduction in the number of recognized peptides corresponding to portions of the core region (24). In the hemodialysis patients under study, it is difficult to assess the exact time of infection, and this population is indeed heterogeneous, including (i) recently infected patients, as evidenced by a high incidence rate reported for 1994 to 1995 (16), (ii) chronically infected patients, as evidenced by the presence of HCV infection reported for these populations since 1990 (12), and (iii) patients who might have resolved their HCV infection. In fact, around 28% of the HCV-positive patients tested were found to be negative for HCV RNA (16). No significant difference in antibody reactivity to the HCV antigens was observed between HCV RNA-positive and -negative patients (data not shown). Thus, the restricted antibody response observed could be due, at least in some cases, to the presence of acute-phase specimens. However, we have shown that the higher immunosuppression found in a subgroup of patients was also manifested by a reduced frequency of recognition of additional peptides on follow-up, suggesting that not all the low-responder patients represented recent infections. On the other hand, HCV RNA was found in some of these patients (data not shown), eliminating the possibility that these patients had resolved HCV infections. This delayed response is in agreement with previous reports about delayed seroconversion in hemodialysis patients (20). Thus, it appears that the intrinsic immunosuppression of these patients leads to a more prolonged period of acute-phase-like antibody response. The restricted antibody response to synthetic peptides also has been documented as an impairment for serotyping HCV-infected strains in hemodialysis patients (22). On the other hand, this study cannot exclude the possibility that the absence of reactivity to some HCV antigens in hemodialysis patients is due to a quantitative reduction in the titer of antibodies and not to an absence of reactivity.

Among the hemodialysis patient cohort, the low-responder group (i.e., recognition of one peptide or none) had a higher prevalence of HBsAg. Moreover, HBV coinfection was associated with a reduced antibody reactivity to C33 and C100 in RIBA 3 and a lower number of peptides recognized. Lower optical density values have been previously observed for anti-HCV tests among HBeAg-positive HBV-coinfected patients compared to an HBeAg-negative group (14). These results suggest that coinfection with HBV might provoke an increasing immunosuppressive effect on the patients. Cellular immunosuppression in patients chronically infected with HBV has been well documented (1). In addition, a better renal-allograft actuarial survival has recently been described for HBsAg-positive patients compared to negative ones, suggesting that in HBV infection, a more efficient immunosuppression was occurring after the transplantation (4). Although infection with hepatitis viruses does not provoke a global immunosuppressive effect (1), the evaluation of an immune humoral suppression in response to other infectious agents, caused by HBV infection, deserves further study. An alternative explanation could be the occurrence of interference between HCV and HBV, which has been previously described (9), although not in this particular population (16), and does not seem to be totally understood in immunocompromised patients (5). It is interesting to speculate that if HBV exerts any inhibitory effect on HCV replication, this could somehow modulate the immune recognition of HCV antigens. On the other hand, the possibility that the HBV chronicity state observed for most of these patients (data not shown) is simply another consequence of a more severe immunosuppression suffered by this group of patients cannot be excluded.

An alternative explanation is that some of the low-responder patients may be infected with HCV strains with mutations in the specific regions modeled by the tested peptides. A lower recognition of NS4 antigen has been reported for patients infected with HCV genotype 2 compared to patients infected with genotype 1 (21). It is interesting that C100 and NS5 antigens were significantly less recognized by the low-responder sera in this study (Table 3). However, other studies have shown that the absence of reactivity to the core and other regions is not always attributable to variability in the infecting HCV strain (7, 13), suggesting that the genetic constitution of the host may also play a role in restricting recognition of these viral antigens, especially when synthetic peptides are used. In
this particular cohort of hemodialysis patients, all the common genotypes circulating among different Venezuelan groups (1a, 1b, 2a, and 3a), except genotype 2b, were found (18), and no difference was found in genotype distribution among the low-responder patients compared to the high responders. Moreover, no association could be found between any particular genotype and a low responsiveness to any particular peptide (data not shown). Furthermore, due to the high incidence of HCV seroconversion previously reported for these populations (16), these patients are, in fact, subjected to exposure to multiple strains of HCV, with a high turnover of genotypes circulating in a patient (17). These observations suggest that the reduced antibody reactivity to any HCV antigen may be explained only partially by antigenic variation in the infecting HCV strain and more probably by the intrinsic immunosuppression in this patients and a still-unexplained reduction in reactivity induced by HBV coinfection in hemodialysis patients.

Even in this population, two peptides—peptide 716, corresponding to a portion of the core region (amino acids 13 to 32), and peptide 59, corresponding to a portion of the NS4 region (amino acids 1921 to 1940)—were widely recognized, suggesting that these peptides model immunodominant epitopes, as previously described (15, 19, 23). These two peptides correspond as expected to regions conserved among different isolates of HCV, and this explains, in part, their optimal performance.

In conclusion, intrinsic immunosuppression in hemodialysis patients was reflected by a restricted reactivity to HCV peptides corresponding to different parts of the HCV genome, which resembles the response of an acute-phase serum. Coinfection with HBV exacerbates this phenomenon. The presence of NS3 antigens in screening assays seems to be even more critical for hemodialysis patients. Even in this low-responder population, antibody reactivity to peptides 716 (core) and 59 (NS4) corrobartes the presence of immunodominant epitopes in these regions.

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