Restricted Isotypic Antibody Reactivity to Hepatitis C Virus Synthetic Peptides in Immunocompromised Patients

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Received 18 September 1998/Returned for modification 23 November 1998/Accepted 28 December 1998

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Synthetic peptides have proven to be valuable tools for immunodiagnosis of hepatitis C virus (HCV) infection (8, 10, 18). Most of the available serological tests for HCV are based on the combined use of synthetic peptides and recombinant proteins. In a previous study, restricted antibody reactivity to HCV peptides was observed in HCV-infected hemodialysis patients, although some immunodominant epitopes in the core and NS4 regions were still consistently recognized (5). The aim of this study was to design an immunodiagnostic system for HCV based exclusively on synthetic peptides and suitable for epidemiological studies. In addition, isotypic antibody reactivity to these peptides was evaluated and compared between immunocompromised and immunocompetent HCV-infected patients.

A total of 105 sera from HCV-infected hemodialysis patients previously tested for viral hepatitis serological markers (16) and 4 (14, 15, 8, 6, 1, and 1 patients, respectively) and six mixed nucleic acid and protein markers, were also tested. Immune impairment seemed to restrict the spectrum of antibody isotypes reacting to the core peptide.

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| Parameter                  | Sera nonreacting with peptides | Sera reacting only with 286 | Higher-OD recognition of 286* | Similar recognition of both peptides |
|----------------------------|-------------------------------|-----------------------------|-------------------------------|-------------------------------------|
| No. (%) of patients        | 4 (11)                        | 7 (20)                      | 7 (20)                        | 17 (49)                             |
| Mean ODa                   | 0.155                         | 0.505                       | 0.905                         | 1.044                               |
| Peptide 716                |                               |                             |                               |                                     |
| Peptide 286                |                               |                             |                               |                                     |

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a Means of triplicate OD values for each sample were compared with Student’s t test.

b Mean OD of serum samples.
nized in this region (5). Of 35 hemodialysis patient sera tested, 31 (89%) recognized peptide 286 while only 24 (69%) reacted with peptide 716, and a significantly higher optical density (OD) was observed for 14 patients with peptide 286 (Table 1). The use of longer peptides in the core region has previously been suggested by others to be more effective (21). In the region shared by the two peptides, an amino acid change was introduced in the 40-mer peptide, 286, since it was more frequently found in the sequences of the different HCV isolates; this may have contributed to the increased reactivity observed with peptide 286. The length and conservation of peptide 286 ensured appropriate reactivity of sera of patients infected with all of the genotypes tested in this study (data not shown).

No significant increase in reactivity was detected by using polymericic peptides instead of monomers (data not shown). When a mixture of peptides 286, 59, and 290, derived from the core, NS4, and NS5, respectively, was evaluated as a potential diagnostic antigen for HCV infection, high sensitivity and specificity were obtained with immunocompetent patients (66 of 66 patients recognized [100%] and 1 of 96 reactive among negative patient sera [99% specificity]). The mixture, however, was recognized by only 91% of the immunocompromised HCV-infected patients (118 of 129). The presence of an NS3 antigen in the cocktail used may be critical for adequate recognition of this particular group of patients (3–5). Nevertheless, the peptide cocktail analyzed in this study seems to be useful for epidemiological studies in the general population.

The NS5 region was included, as it has been suggested that the use of NS5 antigens may add sensitivity to diagnostic tests (20). In fact, some HCV-infected hemodialysis patient sera from the group studied by Devesa et al. (5) reacted only with peptide 232 from the NS5 region, which is comprised in peptide 290 used in this study (data not shown). A small portion of the 290 peptide is well conserved among different HCV isolates (aa 2288 to 2297). This might explain the frequent recognition of peptide 290 by the sera of patients tested in this study, irrespective of the infecting genotype (data not shown).

The 20-mer peptide derived from NS4 (peptide 59) and included in the cocktail contains a sequence highly conserved among different HCV isolates, which might be one of the reasons for its high frequency of recognition (5, 10, 11). Antibodies against peptide 59 seem to be raised at early stages of infection, with some degree of antigenic cross-reactivity with proteins from different origins (24). In our study, the use of peptide 59 in the cocktail did not produce nonspecific reactions. The only false-positive result was due to recognition of the core peptide, where some nonspecific reaction has also been described (23).

The antibody subclass reactivity was assayed for each of the three peptides from the core, NS4, and NS5. For the latter two peptides, the isotypic reactivity was mostly of the IgG1 subtype, a reactivity similar to that observed in region NS4a (20, 25). No significant differences were found in the isotypic reactivities between immunocompetent and immunocompromised patients (data not shown). Only in two hemodialysis patient sera was the highest reactivity, to both NS4 and NS5 peptides, of the IgG3 subclass (data not shown).

For the core peptide, IgG1 reactivity was observed in all patients. Antibodies of IgG3, IgG4, and/or IgA were found in 44% of immunocompetent HCV-infected patients but in significantly fewer immunocompromised patients, particularly in HBV-infected patients (Table 2). Reactivity to the core region has previously been shown to be predominantly by IgG1 antibodies, although all other isotypes, particularly IgG3, have been detected (6, 19, 22). A similar pattern was found for the 40-mer peptide 286, although IgG3 and IgG4 were present at similar frequencies (Table 2).

The intrinsic immune deficit in hemodialysis patients has already been described (7), and acquired immune impairment has been reported for hemophiliac patients (1, 12). The restricted isotypic response to peptide 286 in both hemodialysis and hemophiliac patients may be due to some degree of impairment of the immune function. Among hemodialysis patients, this effect was more pronounced in HBV-coinfected patients, who were indeed the group with more-reduced reactivity to HCV peptides (5). These results stress the need for highly sensitive diagnostic tools for immunocompromised patients.

This work was supported by grant S1-96000064 from CONICIT, Venezuela, and by Proyecto PCEE.PNUD.VEN/96/002.

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