Short communication

Three new cytotoxic T cell epitopes identified within the hepatitis C virus nucleoprotein

Takashi Kaneko,1 Ikuo Nakamura,2 Hiroto Kita,2 Kazumasa Hiroishi,1,3 Takashi Moriyama1 and Michio Imawari1

1 Hepatology Division, Jichi Medical School, 3311-1 Yakushiji, Minamikawachi-machi, Kawachi-gun, Tochigi 329-04, 2 Third Department of Internal Medicine, Faculty of Medicine, University of Tokyo, Hongo, Tokyo and 3 Second Department of Internal Medicine, Faculty of Medicine, University of Showa, Hatanodai, Tokyo, Japan

Cytotoxic T lymphocytes (CTLs) may play a role in host defence against hepatitis C virus (HCV) infection, and HCV-specific CTL epitopes may be included in vaccines to induce protective CTLs. We identified three new epitopes within the HCV nucleoprotein recognized by CTLs. HCV nucleoprotein residues 28-37 are the minimal epitope recognized by CTLs in association with the class I human leukocyte antigen B60, and epitopes in HCV nucleoprotein residues 111-130 and 161-180 are both recognized by CTLs in association with the class II human leukocyte antigen DRB1*08032.

Hepatitis C virus (HCV) infection frequently persists and results in chronic hepatitis, cirrhosis, and, eventually, hepatocellular carcinoma. Although post-transfusion acute hepatitis C has substantially decreased since the introduction of an anti-HCV assay for blood screening, sporadic acute hepatitis C still occurs. Interferon therapy is effective for less than 50% of patients with chronic hepatitis C. HCV has been reported to escape recognition by neutralizing antibodies by mutating amino acids in a potential antigenic region within an HCV envelope protein (Shimizu et al., 1994). Thus, it is necessary to develop new strategies to prevent and treat HCV infection.

Cytotoxic T lymphocytes (CTLs) are thought to be a major host defence against viral infection and have also been implicated in the immunopathogenesis of viral infection. HCV-specific CTLs are present in both the peripheral blood (Kita et al., 1993) and among lymphocytes infiltrating the liver (Koziel et al., 1992) in patients with chronic hepatitis C.

Since CTLs restricted by distinct human leukocyte antigens (HLAs) recognize different epitopes, a universally immunogenic HCV CTL vaccine would require multiple epitopes. Epitopes in HCV antigens recognized by CTLs cultured from peripheral blood lymphocytes (PBLs) or lymphocytes infiltrating the liver of patients with HCV infection have been identified in association with HLA B44 (Kita et al., 1993, 1995), B35, A29 (Koziel et al., 1992), B50, A11, B7, B51 (Koziel et al., 1993), B53, A23, A3, B8 (Koziel et al., 1995) and A2 (Shirai et al., 1994; Battegay et al., 1995; Cerny et al., 1995; Koziel et al., 1995). In the present study, we describe three new epitopes within the HCV nucleoprotein that are recognized by CTLs.

Two patients with biopsy-proven chronic hepatitis C were studied. Both patients were infected with HCV of genotype Ib/II according to the classifications of P. Simmonds and colleagues (Chan et al., 1992) and H. Okamoto (Okamoto et al., 1992). Patient 1 had fluctuating serum concentrations of alanine aminotransferase (ALT); the patient’s HLA haplotype was A2, A31, B35, B60, Cw3, DRB1*08032/1502, DQB1*0601/0601 and DPB1*0501/0901. Patient 2 had increased concentrations of serum ALT (> 300 IU/l) during the study; this patient’s HLA haplotype was A24, A33, B44, B46, Cw1, Cw3, DRB1*08032/1302, DQB1*0601/0604 and DPB1*0201/0501. Five other HLA B60-positive patients with chronic hepatitis C were also studied. Informed consent was obtained from all patients studied, and the study was approved by the Ethical Review Committees of the Faculty of Medicine, University of Tokyo and of Jichi Medical School.

20-mer (NP1–NP17) or 21-mer (NP18) peptides overlapping 10 amino acids and spanning the HCV nucleoprotein were synthesized and grouped into four peptide mixtures (MIX A, B, C and D) as previously
Peptide specificity of MIX A-stimulated (a), MIX C-stimulated (b) and MIX D-stimulated (c) CTLs. MIX A-stimulated CTLs from patient 1 and MIX C-stimulated and MIX D-stimulated CTLs from patient 2 were assayed for cytotoxicity to autologous BCLs, BCLs pulsed with a mixture of peptides, and BCLs pulsed with individual peptides at an effector-to-target cell ratio of 40. Percentage specific cytotoxicity was calculated by subtracting the percentage cytotoxicity of effector cells to non-pulsed BCLs from that to peptide-pulsed BCLs. Peptide MIX A consists of HCV nucleoprotein peptides NP1 (residues 1-20), NP2 (11-30), NP3 (21-40; DVKFPGGGQIVGGVYLLPRR), NP4 (31-50) and NP5 (41-60). MIX C consists of NP11 (101-120), NP12 (111-130; DPRRRSRNLGKVIDTFTCGL), NP13 (121-140) and NP4 (131-150). MIX D consists of NP15 (141-160), NP16 (151-170), NP17 (161-180; SVNYATGNLPGCSFSSLFA) and NP18 (171-191).
New epitopes for HCV-specific CTLs

Peptide NP3 corresponds to HCV nucleoprotein residues 21-40.

Table 1. Recognition of truncated and overlapping HCV nucleoprotein synthetic peptides by NP3-stimulated CTLs

<table>
<thead>
<tr>
<th>Peptide Amino acid sequence</th>
<th>Specific cytotoxicity (%)</th>
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<tbody>
<tr>
<td>21-40 DVKFPGGGQIVGGVYLLPRR</td>
<td>27</td>
</tr>
<tr>
<td>21-38 DVKFPGGGQIVGGVYLLPP</td>
<td>32</td>
</tr>
<tr>
<td>21-36 DVKFPGGGQIVGGVVY</td>
<td>31</td>
</tr>
<tr>
<td>21-34 DVKFPGGGQIVGVY</td>
<td>5</td>
</tr>
<tr>
<td>23-40 KFGGQIVGGVYLLPRR</td>
<td>29</td>
</tr>
<tr>
<td>25-40 PGGQIVGGVYLLPRR</td>
<td>23</td>
</tr>
<tr>
<td>27-40 GQIVGGVYLLPRR</td>
<td>30</td>
</tr>
<tr>
<td>21-40 DVKFPGGGQIVGGVYLLPRR</td>
<td>38</td>
</tr>
<tr>
<td>26-35 GGQIVGGYY</td>
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</tr>
<tr>
<td>27-36 GGQIVGGVYL</td>
<td>46</td>
</tr>
<tr>
<td>28-37 GQIVGGVYLL</td>
<td>38</td>
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<tr>
<td>27-35 GGQIVGGYY</td>
<td>0</td>
</tr>
<tr>
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<td>49</td>
</tr>
<tr>
<td>28-35 GQIVGGYY</td>
<td>3</td>
</tr>
<tr>
<td>29-36 QTIVGGVY</td>
<td>0</td>
</tr>
</tbody>
</table>

* Cytotoxicity assays were performed at an effector to target cell ratio of 40. Target cells were sensitized with peptides at a concentration of 10 μM. Percentage specific cytotoxicity was calculated as described in the legend to Fig. 1.

recognition is restricted by HLA B60, while recognition of both NP12 and NP17 is restricted by HLA DRB1*08032.

NP3-stimulated CTLs recognized not only peptide-sensitized targets but also recombinant vaccinia virus-infected targets that synthesize HCV nucleoprotein endogenously although lysis of recombinant vaccinia virus-infected targets was less efficient than that of peptide-pulsed targets (data not shown). Whether or not NP12-stimulated CTLs and NP17-stimulated CTLs recognize endogenously synthesized antigens remains unclear since CTLs were detectable in the peripheral blood of patient 2 for only 6 months, and recombinant vaccinia virus was not available during that period. The reason why we could detect the CTL responses for only 6 months remained unknown.

The fine specificity of the HLA B60-restricted CTLs was assessed with panels of truncated and overlapping peptides. Cytotoxic activity was observed exclusively against peptides containing a 9-mer peptide consisting of HCV nucleoprotein residues 28-36, GQIVGGVYLL (Table 1). A recently proposed HLA B60-binding peptide motif has a negatively charged glutamic acid residue at position 2, a hydrophobic amino acid residue, such as isoleucine or valine, at position 7, and a leucine residue at the carboxyl terminus (Falk et al., 1995). However, the minimal epitope for HLA B60-restricted CTLs specific for HCV nucleoprotein had an uncharged polar glu-
unpublished results). This finding suggests that a vaccine
T. Aikawa, N. Tanaka, K. Mitamura & M. Imawari, who express HLA B44 (K. Hiroishi, H. Kita, M. Kojima, T. Moriyama, T. Kaneko, T. Ishikawa, T. Aikawa, N. Tanaka, K. Mitamura & M. Imawari, unpublished results), an HLA B44-restricted CTL response was not demonstrated in patient 2 (data not shown). These findings suggest that the hierarchy of HCV-specific CTL epitopes may differ in each patient even if they have the same HLA antigens.

Although CTLs are generally CD8+ and HLA class I-restricted, the presence of CD4+, HLA class II-restricted CTLs has also been reported (Jacobson et al., 1984; Yasukawa & Zarling, 1984). It has been suggested that CD4+ CTLs terminate the immune response to antigens rather than act as antiviral effector cells (Braakman et al., 1987). However, it has been reported that as well as exogenous antigens, some endogenously synthesized antigens can be presented by MHC class II molecules and be recognized by CD4+ CTLs (Jacobson et al., 1989; Jaraquemada et al., 1990). Such CTLs have been shown to be involved in the pathogenesis of viral infection and the immune clearance of infected cells, although CD4+ CTLs may be less effective for viral clearance than CD8+ CTLs (Muller et al., 1992). Thus, NP12-stimulated CD4+ CTLs and NP17-stimulated CD4+ CTLs may play a role in the host defence against HCV infection, although whether or not these CTLs can recognize endogenously synthesized HCV nucleoprotein remains to be seen. Alternatively, CD4+ HCV nucleoprotein peptide-specific CTLs may be involved in the immunopathogenesis of viral infection rather than viral clearance as postulated for herpes simplex virus infection (Yasukawa et al., 1989).

Recently, we observed that a detectable CTL response to the HCV nucleoprotein is associated with a decreased serum HCV RNA titre in patients with HCV infection who express HLA B44 (K. Hiroishi, H. Kita, M. Kojima, H. Okamoto, T. Moriyama, T. Kaneko, T. Ishikawa, T. Aikawa, N. Tanaka, K. Mitamura & M. Imawari, unpublished results). This finding suggests that a vaccine that induces HCV-specific CTLs may be useful for the prevention and elimination of HCV infection.

A universally immunogenic HCV CTL vaccine would require multiple epitopes, preferably from the conserved regions of HCV. The amino acid sequence of the HCV nucleoprotein between residues 28–36 is highly conserved between HCV isolates from the same group and between the different groups of HCV, while the sequences between residues 111–130 and 161–180 are conserved between isolates from the same genotype (Weiner et al., 1991). The peptides used in the present study were synthesized based on the amino acid sequence of genotype 1b/II HCV. Thus, peptide 28–36 would induce CTLs that would recognize most HCV strains irrespective of the genotype, and peptides 111–130 and 161–180 would induce CTLs that would recognize at least HCV isolates of genotype 1b/II.

In conclusion, the results demonstrate that the 9-mer HCV nucleoprotein peptide 28–36 is the minimal epitope recognized by CTLs in association with HLA B60, and that peptides 111–130 and 161–180 both contain epitopes recognized by CTLs in association with HLA DRB1*08032. These peptides may provide the basis for a vaccine to induce a sufficient anti-viral CTL response to prevent HCV persistence in persons expressing HLA B60 or HLA DRB1*08032 and to eliminate HCV from infected patients expressing HLA B60 or HLA DRB1*08032.

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T. K. and I. N. contributed equally to this study.

References


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