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Identification of HLA-A3 and -B7-Restricted CTL Response to Hepatitis C Virus in Patients with Acute and Chronic Hepatitis C

Kyong-Mi Chang,* Norbert H. Gruener,† Scott Southwood,‡ John Sidney,§ Gerd R. Pape,† Francis V. Chisari,2,* and Alessandro Sette‡

The inverse relationship between peripheral blood CTL responsiveness to multiple hepatitis C virus (HCV) epitopes and viral titer in patients with persistent HCV infection suggests that enhancement of the CTL response might result in viral clearance. Since several HLA-A2-restricted HCV CTL epitopes are already known, we aimed to identify CTL epitopes restricted by other HLA types in an effort to expand the epitope repertoire available for T cell-mediated therapeutic vaccine development. Scanning of 14 different HCV genome sequences for the presence of conserved peptides containing the HLA-A3 and -B7 motifs revealed 9- to 10-mer peptides that were synthesized and assayed for binding to HLA-A3, -B7 supertype molecules. Peptides with good HLA-binding affinities and cross-reactivities with at least three of five most common molecules of each supertype were tested for the ability to stimulate a memory CTL response in the peripheral blood from selected HCV-infected patients and normal seronegative donors in vitro. We identified eight HLA-A3 supertype-restricted CTL epitopes and one HLA-B7 supertype-restricted CTL epitope that were recognized by infected patients but not by healthy seronegative donors. HLA class I serotyping of 158 chronically infected patients revealed that 80% expressed one or more of HLA molecules belong to either the A2, A3, or B7 supertypes. In conclusion, the epitopes, herein identified combined with previously defined HLA-A2-restricted CTL epitopes, should be useful for the design of an ethnically unbiased, therapeutic CTL vaccine for the treatment of patients with chronic HCV infection. The Journal of Immunology, 1999, 162: 1156 –1164.

Hepatitis C virus (HCV) is a hepatotropic RNA virus that causes persistent infection in the majority of exposed individuals in the face of a humoral and cellular immune response to its Ags (1–13). The role of virus-specific CTLs in HCV pathogenesis is not well understood. Using a modification of the strategy previously developed to study the HBV-specific peripheral blood CTL response (14–16), we have recently shown that one or more members of a panel of 10 HLA-A2-restricted CTL epitopes are recognized in 97% of chronic HCV patients and in only 2.2% of the anti-HCV-negative controls, suggesting that the response observed in infected patients reflects in vivo priming by HCV (12). Furthermore, we and others have reported an inverse correlation between the vigor of the peripheral blood CTL response to HCV and viral titer (12, 17). In addition, the CTL response in several patients has been shown to be associated with the presence or the emergence of epitope variants that are not recognized by the CTL (18), suggesting that although CTLs may exert some control over the virus, they might also contribute to persistent infection by selecting CTL escape variants. Indeed, early selection of CTL escape variant has been described in an experimentally infected chimpanzee that developed chronic hepatitis (19). These observations support the notion that an effective therapeutic vaccine against HCV must be able to induce a strong multispecific HCV-specific CTL response to eradicate HCV before selection of escape mutants can occur. Furthermore, identification of epitopes restricted by multiple HLA alleles is required for such vaccines to be immunogenic in the general population. Fortunately, it has recently been shown that various HLA alleles share common peptide-binding motifs, thus defining a supertype (20, 21). For example, members of the HLA-A3 supertype (e.g., HLA-A3, -A11, -A31, -A33, and -A6801) bind short peptides that contain residues A, V, I, L, M, S, or T in position 2 and R or K at the C terminus, whereas the HLA-B7 supertype (e.g., HLA-B7, -B35, -B53, -B54, and -B51) recognizes peptides with P at position 2 and A, I, L, M, V, F, W, or Y at the C terminus. By scanning the amino acid sequences of target Ags (e.g., HCV or HBV proteins), virus-derived peptides containing such motifs can be identified, synthesized, and tested for HLA-binding affinity, antigenicity, and immunogenicity in vitro. This motif-search strategy has successfully identified many CTL epitopes (2, 4, 14, 22), including several broadly cross-reactive supermotif epitopes in HBV (22) and Plasmodium falciparum (23). In this study, we describe eight HLA-A3 and one HLA-B7 supertype-restricted HCV CTL epitopes.

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Materials and Methods

Patients

A total of 154 HCV-infected patients at Scripps Clinic in La Jolla were screened with class I HLA serotyping analysis. All 154 patients were persistently infected by HCV as defined by the following criteria: HCV Ab detected by a second generation Ortho HCV ELISA test system (Ortho Diagnostics, Raritan, NJ), or branched DNA assay (Chiron, Emeryville, CA), elevated serum alanine aminotransferase (sALT) activity (greater than 50 U/l), presence of HCV RNA by RT-PCR (National Institutes of Health, Bethesda, MD), or by the second generation Ortho HCV ELISA test system (Ortho Diagnostics, Raritan, NJ), as previously described (18). The clinical and virological characteristics of the patients are shown in Table I.

As shown in Table II, Patients A1, A2, A5, and A6 spontaneously resolved their viremia, and their sALT activity returned to normal. Patient A7 was viremic for 4 mo but cleared HCV RNA and resolved her hepatitis immediately after IFN therapy. In contrast, patients A3 and A4 became persistently infected. The clinical/virological outcome for Patient A8 has not been determined due to the short follow-up interval at this time (<6 mo).

Peripheral blood mononuclear cells

PBMC from patients and normal donors were separated on Ficoll-Histopaque density gradient (Sigma, St. Louis, MO), washed three times in HBSS (Life Technologies, Grand Island, NY), and used for culture directly or cryopreserved in media containing 80% FCS (Life Technologies), 10% DMSO (Sigma), and 10% RPMI 1640 (Life Technologies).

Synthetic peptides

The amino acid sequences of the peptides (Table III) were derived from the published HCV genome sequences containing known HLA-A3 or -B7 supertype-binding motifs (20, 21). The peptides were synthesized at Cytel (San Diego, CA) and purified to 95% homogeneity by reverse phase HPLC or were purchased as crude material from Chiron Mimotopes (Clayton, Australia) as previously described (20, 21, 24). The peptides were reconstituted at 20 mg/ml in DMSO and further diluted to 1 mg/ml with RPMI 1640.

HLA-binding affinity analysis

The HLA-binding affinity of peptides was determined by measuring their ability to competitively inhibit the binding of a radiolabeled standard probe at least 20 times the upper limit of normal; presence of HCV RNA at least in acute phase serum samples; de novo seroconversion to HCV Abs; and absence of other causes of hepatitis. Resolution of hepatitis was defined as loss of viral RNA and normalization of serum transaminases within 6 mo of onset of acute hepatitis. Chronic infection was defined as persistent viremia and abnormal transaminases beyond 6 mo.

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peptide to purified detergent-solubilized class I MHC molecules, as previously described (20, 21, 24). Briefly, purified class I molecules were incubated for 2 days at room temperature with varying concentrations of competitor peptides, 5–10 nM concentrations of the labeled peptide, 1 μM human β2-microglobulin (Scripps Laboratories, San Diego, CA), and a mixture of protease inhibitors. After incubation, class I peptide complexes were separated from free peptide by size exclusion gel filtration chromatography on a TSK2000 column (7.8 mm × 15 cm) (TosoHaas, Montgomeryville, PA). The concentration of peptide yielding 50% inhibition of the binding of the radiolabeled probe peptide was calculated. The HLA-binding affinities of individual peptides to various HLA subtype molecules are shown in Table III.

Stimulation of PBMC with synthetic peptides

Cryopreserved PBMC (rapidly thawed at 37°C and washed three times in cold HBSS) or freshly isolated PBMC were resuspended in RPMI 1640 supplemented with l-glutamine (2 mM), penicillin (50 U/ml), streptomycin (50 μg/ml), HEPES (10 mM) (Life Technologies), and 10% heat-inactivated human AB serum at a 4 × 10^6 cells/ml. To expand peripheral blood mononuclear cells, as previously described (12). PBMC were stimulated with the single B7 peptide. Cultures were restimulated on days 7 and 14 with autologous irradiated (3000 rads) PBMC (1 × 10^6 cells/well) and peptide (10 μg/ml), and on days 3, 10, and 18 with 20 U/ml human rIL-2 (Hoffmann-La Roche, Nutley, NJ) in fresh media as described previously (12). The CTL responses of the acute and chronic HCV patients evaluated at day 21 in a standard 4-h 51Cr release assay using round bottom 96-well plates containing 3000 target cells/well. Allogeneic HLA-matched EBV-transformed B cell lines (described above) were pulsed overnight with peptide (10 μg/ml) labeled for 1 h with 51Cr (0.2 mCi), and used as target cells in these assays, as previously described (4, 13, 18). Percent cytotoxicity was calculated using the formula: 100 × ([experimental release – spontaneous release]/maximum release – spontaneous release)). Maximum release was determined with 10% Triton X-100 (Mallinckrodt, Paris, KY). Spontaneous release was always <30%. The cutoff value for a positive response was determined as 12%, which was more than 3 SDs above the mean cytotoxicity detected for each peptide in the uninfected donors. To compare the strength of the CTL response in patients with chronic or resolved hepatitis C, the CTL response index for each peptide (CRI-P) was calculated by totaling % cytotoxicities for all 8 replicate wells using the 96-well microwell cultures, as previously described (12). CRI-P values greater than or equal to the 3 SD + mean CRI-P observed in normal uninfected donors was considered positive response. For the panel of eight A3 peptides, individual CRI-P values were added and expressed as total CTL response index to reflect the overall strength of the CTL response to these peptides, as previously described (12).

Anti-CD4 and anti-CD8 blocking assay

The contribution of CD4 and CD8 T cells to the CTL activity was determined by incubating effector cells with anti-CD8 or anti-CD4 Ab as previously described (12). Briefly, the effectors were preincubated with 10 μg/ml anti-CD4 or anti-CD8 (Becton Dickinson, San Jose, CA) for 1 h at 4°C before addition of target cells, and tested in duplicates against the peptide-pulsed, 51Cr-labeled targets in the standard 4-h CTL assay as described above.

Statistical analysis

Nonparametric Wilcoxon two-sample rank test was used to compare the sALT activities or the number of HLA-A3 epitope peptide recognized in patients receiving IFN and in patients not receiving IFN.

Results

Distribution of HLA alleles in chronic HCV patients

As shown in Table IV, the frequency of HLA-A2, -A3, and -B7 supertype alleles present in the 158 chronic HCV patients (154 from La Jolla, 4 from Munich) is comparable with the known

<table>
<thead>
<tr>
<th>Patient</th>
<th>Acute vs Chronic</th>
<th>Outcome of Acute Hepatitis</th>
<th>HLA Subgroup</th>
<th>HLA</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>HCV Genotype</th>
<th>No. of mo After Onset of Acute Hepatitis</th>
<th>T-CRI</th>
<th>No. of Peptides Recognized</th>
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<td>Clearance</td>
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<td>M</td>
<td>1b</td>
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<td>5, 0′</td>
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</tr>
<tr>
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<td>Chronic</td>
<td>A3, A24, B7</td>
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<td>F</td>
<td>ND</td>
<td>29</td>
<td>121</td>
<td>3</td>
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<td>1a</td>
<td>Not known</td>
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<td>Chronic</td>
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<td>A3, A1, B55</td>
<td>39</td>
<td>M</td>
<td>3</td>
<td>Not known</td>
<td>116</td>
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Table II. Acute/chronic HCV patients from Munich analyzed for CTL response to A3/B7 HCV peptides

<table>
<thead>
<tr>
<th>Patient</th>
<th>Acute</th>
<th>Chronic</th>
<th>Outcome of Acute Hepatitis</th>
<th>HLA Subgroup</th>
<th>HLA</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>HCV Genotype</th>
<th>No. of mo After Onset of Acute Hepatitis</th>
<th>T-CRI</th>
<th>No. of Peptides Recognized</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>Acute</td>
<td>Clearance</td>
<td>A3, A24, B7, B55</td>
<td>37</td>
<td>F</td>
<td>1b</td>
<td>2, 4, 9′</td>
<td>21, 41, 94′</td>
<td>0, 1, 1′</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A2</td>
<td>Acute</td>
<td>Clearance</td>
<td>A3, A24, B7, B35</td>
<td>46</td>
<td>M</td>
<td>1b</td>
<td>12, 15′</td>
<td>60, 169′</td>
<td>1, 1′</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A3</td>
<td>Acute</td>
<td>Chronic</td>
<td>A3, A24, B7</td>
<td>45</td>
<td>F</td>
<td>ND</td>
<td>29</td>
<td>48</td>
<td>12</td>
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<td></td>
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<tr>
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<td>Acute</td>
<td>Not yet known</td>
<td>A3, A24, B7, B8</td>
<td>18</td>
<td>F</td>
<td>4</td>
<td>5</td>
<td>0</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>C28</td>
<td>Chronic</td>
<td>A3, A24, B7, B51</td>
<td>44</td>
<td>M</td>
<td>1a</td>
<td>Not known</td>
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* T-CRI, total CTL response index.

† Eight peptides total studied for A3 subgroup, 1 peptide for B7 subgroup.

Assays were performed serially and corresponding T-CRI and number of peptides recognized are listed in the same order as the months.

†† Clearance following IFN therapy within 6 mo of acute hepatitis.

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frequencies of these alleles in the general population (26, 27). The variations from the expected frequency (e.g., twofold increase in the HLA-A3 and -B7 subtypes or twofold reduction in HLA-A11, -A31, and -B51 among the patients) is consistent with the frequency observed among Caucasians and most likely reflects the prevalent ethnicity of the local patient population. These results suggest that the presence of these class I alleles is not related to persistent HCV infection. The relative frequency of A3 and B7 superfamily alleles in these patients as well as the general population suggests that identification of HCV CTL epitopes restricted by these supertypes will expand the pool of potential epitope vaccine recipients to most of the world population.

Identification of candidate HLA-A3 and -B7 supertype-restricted HCV CTL epitope peptides

Potential HLA-A3 supertype-restricted CTL epitopes were identified by scanning the predicted amino acid sequence of complete polyproteins from 14 different HCV isolates for the presence of 9- and 10-mer sequences containing the HLA-A3 supermotif main anchor specificity (AILMVST in position 2 and R or K at the carboxy terminus). The candidate sequences identified were also evaluated using a customized algorithm, which takes into account the presence of positive or deleterious secondary anchor residues (21). Algorithm-identified sequences were then assessed for conservancy, and 27 sequences in which 100% of the residues were conserved in 75% or more of the 14 isolates scanned were synthesized. When the 27 corresponding peptides were tested for binding to HLA-A3 and -A11, the 2 most prevalent A3 supertype alleles, 15 peptides were identified that bound A3 and/or A11 with affinities of 500 nM or less. These 15 binders were next tested for cross-reactivity to the other common A3 supertype alleles (A3101, A3301, and A6801). Seven of the 15 peptides were found to bind at least 3 of the 5 A3 supertype alleles tested with 50% inhibitory concentration (IC50) ≤ 500 nM. In a separate analysis, it was noted that an additional peptide (NS3 1267) carrying a G2-K9 motif was also capable of binding three A3 supertype alleles. Thus, this additional peptide was included in the study described herein.

Potential HLA-B7 supertype-restricted CTL epitopes were identified by scanning the same 14 HCV isolates for the presence of 9- and 10-mer sequences containing the broad B7 supertype motif (Pin position 2 and AILMVFW or Y at the carboxy terminus) (20). After evaluation for conservancy, as described above, 35 peptides were synthesized and tested for binding to HLA-B0702, the most common B7 supertype allele. Thirteen peptides bound B0702 with IC50 ≤ 500 nM. These peptides were then tested for binding to other common B7 supertype alleles (B3501, B51, B5301, and B5401). One peptide, Core 169, was capable of binding to three or more of the five B7 supertype alleles tested. In summary, eight A3-supertype and one B7 supertype candidate CTL epitopes were identified (Table III). Each of these peptides are degenerate superfamily binders and are derived from conserved regions of the HCV genome.

CTL response to HCV-derived candidate A3 and B7 CTL epitope peptides in chronic HCV patients

To examine the immunogenicity of the 8 HCV-derived A3 supermotif peptides, PBMC from 12 HLA-A3 positive individuals with chronic HCV infection and 10 HLA-matched healthy uninfected blood donors were cultured for 3 wk in vitro with the 8 A3 supermotif peptides using the 24-macrowell technique. Little to no CTL activity against these peptides was observed among the 10 normal controls, as shown at the bottom of Fig. 1. In contrast, CTL responses to 1 or more of the A3 peptides were observed in 7 of 12 (58%) patients (mean, 1.5 peptides/patient; range, 0–5 peptides/
Table IV. Frequency of HLA subtypes in patients with chronic hepatitis C

<table>
<thead>
<tr>
<th>Supertype</th>
<th>Ag</th>
<th>Total</th>
<th>%</th>
<th>Distribution of HLA Alleles Among Various Ethnic Groups (%) (26, 27)</th>
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<tr>
<td></td>
<td></td>
<td></td>
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* 154 patients at Scripps Clinic + 4 patients at Munich.
* Does not include 11 patients with A68 allele that have not been subtyped.
* Indicates the number and fraction of individuals expressing one or more of the HLA alleles among the 158 chronic HCV patients analyzed.

A positive CTL response (Fig. 1, □) was observed in 18 of 96 possible instances (19%). All peptides were immunogenic in at least 1 patient (mean, 2 patients/peptide; range, 1–3 patients/peptide), and responses were observed in patients infected by HCV subtype 1a as well as 1b, 2b, or 3a. Interestingly, the NS4 1863 peptide was recognized in 4 of 12 (33%) patients, whereas the NS4 1864 peptide was immunogenic in only a single patient despite similar HLA-binding affinity and a single N-terminal amino acid difference. Two HLA-A11-positive chronic HCV patients were also tested but did not show CTL activity to the same panel of eight A3 HCV peptides (data not shown).

The total number of A3 peptides recognized in each patient was compared with clinical parameters such as sALT activity and IFN therapy. Interestingly, the number of immunogenic peptides correlated inversely with sALT activity (Fig. 2). Furthermore, compared with the patients without ongoing IFN therapy (patients C1, 12, 3, 4, 6, 8, 10, and 11), samples obtained from patients with concomitant IFN therapy (patients C2, 5, and 9) displayed CTL responsiveness to more peptides (mean 3.3 peptides vs 0.9 peptides per patient, p = 0.025) and lower sALT activity (43 U/L vs 122 U/L, p = 0.028). Since all patients with concurrent IFN therapy exhibited low to normal sALT activity (Fig. 2, unfilled diamonds), it is possible that IFN therapy may be responsible for the apparent inverse relationship between CTL and sALT activity. Future studies with additional patients are needed to establish the biological and clinical relevance of this observation. Also, since the viremia levels on the dates of CTL activity were not analyzed, we could not determine the relationship between CTL responsiveness to these epitopes and viral titer.

The CTL response to the single B7 peptide determined in 8 HLA-B7-positive patients and 8 HLA-B35-positive patients is shown on Fig. 3. Using a cutoff of 12% as described in Materials and Methods, a CTL response to the B7 peptide was detected in one (13%) of the eight B7-positive patients, and in two (25%) of the eight B35-positive patients. The CTL activity at varying E:T ratios is shown in Fig. 4. The CTL activity could be blocked by anti-CD8 Ab but not anti-CD4 Ab, consistent with the expectation that CTL activity was mediated by CD8-positive T cells. The mean sALT activity was lower among patients exhibiting B7 peptide-specific CTL response than in patients without B7 peptide-specific CTL response (66 vs 101), although this difference was not statistically significant.

**CTL responsiveness to A3 and B7 HCV CTL epitope peptides in acute hepatitis C patients**

Next, we compared the CTL responses of patients with acute and chronic hepatitis C from Munich, using the semiquantitative microwell technique previously described (12). The CTL responses of group of HLA-A3- and -B7-positive patients to the panel of A3 and B7 peptides are shown in Figs. 5–7, expressed as CRI-P described in Materials and Methods. CRI-P values >3 SD above the mean normal donor CTL response for each peptide are indicated by hashed bars. The time points at which the patients were analyzed are shown in Table II as months after onset of acute hepatitis. Both patients with acute self-limited hepatitis C and patients with chronic HCV infection responded to one or more peptides, although the level of CTL activity was generally low. While there was a tendency for the B7-restricted CTL response to be stronger and more frequent in the patients who resolved the infection as shown in Fig. 6 and Table II, these differences were relatively small and of questionable significance.

Interestingly, the pattern of CTL response varied over the course of early infection. For example, as shown in Fig. 7, Patient A1 responded to six A3 peptides at 2 mo during which time she was viremic but displayed normal sALT activity. In contrast, as the virus became undetectable by PCR, the response decreased to only 3 A3 peptides at 4 mo and 2 A3 peptides at 9 mo. In contrast, the response to B7 peptide (Core 169) gradually increased over this
observation period. Patient A2 showed similar fluctuations although he was studied later, at 12 and 15 mo after the onset of acute hepatitis and viral clearance.

**Discussion**

CTLs are thought to play an important role in viral clearance. For example, a strong multispecific CTL response to HBV is characteristic of patients with acute hepatitis B who clear their infection whereas the CTL response is rarely detectable in patients with chronic hepatitis B. In HCV infection, however, virus-specific CTL are detectable despite persistent infection. Although this response appears to be relatively weak (13), its inverse relationship with viral titer (12) suggests that amplification of the CTL response might be a useful therapeutic antiviral strategy. Importantly, since CTL escape variants seem to occur in chronic HCV infection (18, 19), a multispecific CTL response must be induced to avoid selecting for escape variants.

In this study, we identify nine new HLA-restricted CTL epitopes, thereby expanding the known HCV CTL epitope repertoire that can contribute to the development of a broad spectrum CTL epitope vaccine relevant to the general population. Indeed, considering all members of the HLA-A2, -A3, and -B7 superfamilies, the current set of epitopes together with the previously described HLA-A2-restricted epitopes will cover >80% of the world population. Identification of multiple epitopes is of potential importance in development of immunotherapy of HCV infection since multispecific responses have been shown to correlate with a better clinical outcome (12). Similar observations have been made in the case of HIV infection (28–31). Therapeutic augmentation of CTL response has already been reported in human malignancies such as melanoma (32, 33) and B cell lymphomas (34, 35). Furthermore, a synthetic peptide vaccine derived from the HLA-A2-restricted HBV core 18–27 CTL epitope was found to be immunogenic in HLA-A2-positive donors (36), and a Phase II trial of the same vaccine in HLA-A2-positive patients with chronic hepatitis B is currently underway. Our study also supports the usefulness of the HLA-binding supermotif and HLA-binding affinities to predict
potential CTL epitopes, as has already been reported for HBV (22) and other conditions (2, 4, 37).

The HLA-A3-restricted CTL epitopes are derived from both structural and nonstructural regions of HCV and are relatively conserved (75–100% conservation). Focusing the immune response against highly conserved epitopes might be of particular importance in the case of the highly variable hepatitis C virus.

Each peptide was immunogenic in one or more patients studied (range, 8–33%; mean, 19%). The most frequently immunogenic epitope was NS4 1863 (GVAGALVAFK) which was recognized in 4 of 12 patients (33%). Interestingly, the NS4 1864 peptide (VAGALVAFK), which lacks the N-terminal glycine residue of NS4 1863, was recognized by only one patient (patient C9), who also showed response to NS4 1863. It is possible that the additional N-terminal G may influence the processing and transport of the peptide since the HLA-binding affinity of the two peptides is quite comparable. Alternatively, the N-terminal glycine may interact with the TCR and the MHC-peptide complex. It is important to note that, because they are highly conserved, the CTL responses to these genotype 1a-derived peptides were observed in patients infected with viral strains other than genotype 1a. For example, patient C5 who was infected with HCV subtype 2b responded to Core 51, E1 290, and NS4 1863. This is consistent with high amino acid sequence conservation of the peptides, as shown on Table III, and CTL cross-reactivity between the viral subtypes as we previously reported (12, 18).

There was a correlation between the number of the A3 epitopes recognized and sALT activity among the HLA-A3-positive chronic HCV patients, compatible with a potential curative role of the CTL response as described in hepatitis B (38, 39). A similar inverse relationship between CTL responsiveness and sALT activity has been previously demonstrated in chronic HCV patients using a panel of 10 HLA-A2-restricted HCV CTL epitope peptides (12) but not in another study looking at the intrahepatic CTL (40). However, the patients with low to normal sALT activity and with greater CTL epitope reactivity were also undergoing IFN therapy, which could enhance CTL activity due to increased class I HLA expression. It is also possible that the HCV-specific CTL no longer...
The current results demonstrate the feasibility of developing a broad spectrum therapeutic CTL vaccine for the treatment of chronic HCV infection that should cover most infected patients, irrespective of genotype or ethnicity.

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