Identification of the epitopes on HCV core protein recognized by HLA-A2 restricted cytotoxic T lymphocytes

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Abstract

AIM To identify hepatitis C virus (HCV) core protein epitopes recognized by HLA-A2 restricted cytotoxic T lymphocyte (CTL).

METHODS Utilizing the method of computer prediction followed by a 4h ⁵¹Cr release assay confirmation.

RESULTS The results showed that peripheral blood mononuclear cells (PBMC) obtained from two HLA-A2 positive donors who were infected with HCV could lyse autologous target cells labeled with peptide "ALAHGVRAL (core 150-158)". The rates of specific lysis of the cells from the two donors were 37.5% and 15.8%, respectively. Blocking of the CTL response with anti-CD⁸ mAb caused no significant decrease of the specific lysis. But blocking of CTL response with anti-CD⁴ mAb could abolish the lysis.

CONCLUSION The peptide (core 150-158) is the candidate epitope recognized by HLA-A2 restricted CTL.

Subject headings hepatitis C virus; cytotoxic T lymphocyte; HLA-A2; epitope

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INTRODUCTION

Hepatitis C virus (HCV) is a single-stranded RNA virus responsible for the majority non-A non-B hepatitis[1-2]. More than 50% - 60% of acute infection lead to chronic disease, and once chronicity is established, spontaneous recovery is exceptional. The related mechanism is still unknown[3-5]. Recent studies demonstrate that major histocompatibility complex (MHC) class I-restricted cytotoxic T lymphocytes (CTL) of patients with chronic hepatitis C recognize epitopes from different regions of both structural and nonstructural HCV proteins[6-12]. Some scholars speculate that CTL-mediated cellular immune response probably plays an important role in viral clearance[13,14].

CD⁸* CTL interact through their polymorphic T cell receptor with HLA class I molecules containing endogenously synthesized peptides of 9-11 on the surface of infected cells. The presence of allele-specific amino acid motifs has been demonstrated by sequencing of peptides eluted from MHC molecules. Among the best studied motifs is that of HLA-A2, which is prevalent in a high percentage of population. Several reports[17,18,19,20] described the method of using HCV derived synthetic peptides containing the HLA-A2.1 binding motif to identify and characterize the HLA-A2 restricted CTL in the peripheral blood of patients with chronic HCV infection. We[21] have designed a computer programme to score the reported HCV peptides. Our results revealed that all the reported peptides were with a relative high score of 144 points or higher. Based on the previous study, we attempted to identify the epitopes recognized by the HLA-A2 restricted CTL on HCV core protein utilizing the method of computer prediction followed by 4h ⁵¹Cr release assay.

MATERIALS AND METHODS

Materials

Subjects Six patients with chronic hepatitis C and 2 healthy controls were selected from among those monitored at Xijing blood center. Table 1 summarizes patient characteristics and history of treatment. All subjects had not received any antiviral treatment for at least one year.

Table 1 HLA-A and serology of patients studied for CTL response to HCV epitopes

| Subjects | HLA-A | Anti-HCV | HCV-RNA |
|----------|-------|----------|---------|
| Experiment | Li    | A2A31    | +       | +       |
|           | Zhang | A2A11    | +       | +       |
|           | Tang  | A2A33    | +       | +       |
|           | Zhang | A2A11    | +       | +       |
| Patient control | Li    | A11      | +       | +       |
|             | Zhang | A3A33    | +       | +       |

Note: All subjects received no treatment and had been followed-up for one year.

HLA typing HLA typing of PBMC from patients and from normal donors was determined by microcytotoxicity, using trays (One lamda, Canoga Park, CA). The HLA haplotypes of subjects participating in this study are shown in Table 1.

Methods

Prediction of candidate HLA-A2 restricted CTL epitopes Based on previous study, we use our computer programme to predict HLA-A2 restricted CTL on HCV C protein. In brief, a
computer programme with the function of finding peptides containing HLA-A2 allele-specific peptide motif was written in C language. The HCV cDNA is translated into HCV amino acid sequence from which the peptides was chosen, and the selected peptides include those with a length of 9-11 amino acids, a leucine (L), isoleucine (I) or methionine (M) at position 2 and a leucine (L) or Valine (V) at the last position. According to Nijman’s score system, we scored six points for an anchor residue, four points for a strong and two points for a weak residue. The score for a given peptide is obtained by multiplication of the scores for each amino acid position. Predicted candidate CTL epitopes with scores of 144 or higher.

**Synthetic peptides** Peptides YLLPRRGPRL (core35-44), NLGKVIDTL (core118-126), DLMGYIPLV (core132-140) and ALAHGVRAL (core150-158) were selected from the predicted results and synthesized in automated multiple peptide synthesizer (American Research Genetics, Inc). All peptides were >90% pure and diluted to 1g·L⁻¹ with RPMI1640 medium before use (Gibco, Grand Island, N.Y.).

**CTL generation** PBMC from donors were separated on Ficoll-Hypaque density gradients (Shanghai Huajing, Inc), washed three times in phosphate-buffered saline (PBS), resuspended in RPMI1640 medium (Gibco, BRL) supplemented with L-glutamine (10g·L⁻¹), penicillin (5×10⁴U·L⁻¹), streptomycin (50mg·L⁻¹) and Hepes (5mol·L⁻¹) containing 100mL·L⁻¹ fetal calf serum (FCS) and plated in 24-well plates at 4×10⁶ cells per well. PBMC were stimulated with concanavalin A (ConA, 20µg per well) during the first week. On d3, 1mL of complete medium supplemented with rIL-2 at 2×10³ U·L⁻¹ final concentration was added into each well. On d7, the cultures were restimulated with the peptides plus rIL-2 and irradiated (30Gy) autologous PBMC feeder cells, and the cultured PBMC were restimulated five days later with the original peptides plus rIL-2. On d16, the stimulated cells were used as effectors in CTL assay.

**Preparation of autogeous B lymphoblastoid cell line** After Ficoll-Hypaque separation, PBMC were suspended in the RPMI1640 medium containing 200 mL·L⁻¹ FCS and then plated in 24-well culture plate at a concentration of 2×10⁶ cells per well. EBV-transfected B cell lines were established by culturing 2×10⁶ PBMC with 100g·L⁻¹ of cyclosporin A and 1mL B95-8 EBV culture supernatant (provided by Dr. Jin, the Fourth Military Medical University, Xi’an). After transformation, the lymphoblastoid cell lines (B-LCL) were maintained in RPMI1640 medium with 200 mL·L⁻¹ FCS, with media change twice each week. The cell lines were maintained at 37°C in a humidified chamber with 5% CO₂ and used as targets.

**CTL assay** Target cells were incubated overnight with synthetic peptides at 200 mg·L⁻¹, and then were labeled with 3.7MBq 1-Cr for 1h and washed three times with PBS. Cytotoxicity activity was determined in a standard 4h Cr release assay using U-bottom 96 well plates containing 5000 autogenous targets per well. All assays were performed in triplicate with effector target cell (E/T) ratios of 100:1, 50:1, and 1:1. Maximum release was determined on the basis of lysis of labeled target cells with 50g·L⁻¹ Triton X-100. We examined spontaneous release by incubating target cells in the absence of effector cells. It was less than 25% of the maximum release. Percent cytotoxicity was determined by the formula: 100×[(experiment release-spontaneous release)/(maximum release-spontaneous release)].

**Blocking of CTL response by antibodies** CTL responses were tested in the presence of anti-CD8 or anti-CD4 monoclonal antibody added to the 96-well plates at the indicated concentrations used for the CTL assay.

**RESULTS**

**Prediction of HLA-A2 restricted CTL epitopes on HCV protein**

Seven high-scoring peptides (≥144 points) were selected from HCV C protein using our computer programme. Among them, peptide 1 and peptide 4, namely peptide YLLPRRGPRL and peptide DLMGYIPLV, have been reported to be epitopes recognized by HLA-A2.1 restricted CTL. Predicted peptide 7, namely FLALLSCL (core177-185) was almost the same as the reported peptide FLALLSCLT (core178-187). The rest predicted peptides have not been proved to be epitopes recognized by HLA-A2 restricted CTL. Four peptides (peptide 1, 2, 4, 5), were selected randomly from the seven predicted peptides to be used in CTL assay (Table 2).

**Screen of HCV peptide-specific response**

In this experiment PBMC from 8 subjects were stimulated individually with the four peptides from HCV C protein, and cultures were tested after 16d of expansion for peptide-specific CTL activity. Two of the four donors of HLA-A2 and HCV RNA positive responded to peptide 5. After induced by peptide 5, the two donors’ PBMC can lyse autologous target cells pulsed with peptide 5 and the specific lysis was 37.5% and 15.8%, respectively (Table 3). Treatment of the CTL specific for peptide 5 with anti-CD8 mAb, but not anti-CD4 mAb, plus complement markedly reduced cytotoxic activity on target cells (Figure 1).

**Figure 1** Blocking of CTL activity of Mr. Tang with mAb.
Four synthesized peptides of the HCV core protein were tested using CTL assay. Four donors were positive for HLA-A2. Among donors positive for HCV RNA, 2 donors’ PBMC were found to have lysed autologous target cell-labeled with peptide 5. The specific lysis rate was 37.5% and 15.8% respectively. The other 3 peptides didn’t show obvious CTL induction action. CTL response was very weak in two healthy and HLA-A2 positive donors, and also in two HCV RNA+ HLA-A2- donors.

According to the reference[33], the lysis might be considered specific with the lysis rate ≥15%. The specific lysis rate was up to 37.5% in Tang with effector/target cell (E/T) ratio of 50:1, and 15.8% in Zhang with E/T ratio of 100:1. Blocking of the CTL response with anti-CD4 mAb did not decrease the specific lysis significantly. But blocking of the CTL response with anti-CD8 mAb could abolish the lysis. It indicated that[34-40] the lysis was mediated by CD8+ T cells rather than CD4+ T cells, and that the epitope 5 was probably the candidate epitope recognized by HLA-A2 restricted CTL.

Although 3 peptides, including 2 reported in other studies, YLLPRRGPRL (core35 - 44), DLMGYIPLV (core132-140), didn’t demonstrate obvious CTL induced activity, we could not draw a conclusion that they were not HLA-A2 restricted CTL recognized epitopes. The two reported epitopes were recognized by HLA-A2.1 restricted CTL, but in this study, we did not determine the HLA-A2 subtypes. Various subtypes of HLA-A2 restricted CTL probably recognized different epitopes[41-50]. Another possible reason is that HCV protein sequence of HCV-infected patients might not be in complete accordance with the synthesized peptides. To clarify the reasons, further study is still necessary.

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