Hepatitis C virus core region: helper T cell epitopes recognized by BALB/c and C57BL/6 mice

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In this study, we characterized the B cell and T cell responses to the hydrophilic portion of hepatitis C virus (HCV) core protein in two strains of mice and identified the respective antigen determinants. BALB/c (H-2d) and C57BL/6 (B6:H-2b) mice were immunized by a subcutaneous injection of recombinant HCV core protein together with Freund’s complete adjuvant. The level of antibody production, as determined by ELISA, was consistently higher in BALB/c than in B6 mice. However, antibodies in sera from each strain bound to the N-terminal region of the core protein within amino acids 1 to 28 (MSTNPKPQRKIKRNTNRRPQDVKFGGG), according to an experiment using non-overlapping peptides that covered the hydrophilic portion of HCV core protein. The T cell responses were also higher in BALB/c than in B6 mice with respect to the proliferative responses of the draining lymph node cells in vitro. By limiting dilution cultures of the draining lymph node cells in vitro repetitively stimulated with recombinant core protein, T cell clones were established from both strains of mice and characterized. The surface markers of these clones were Thy-1.2+, CD3+, TCRβ+, CD4+ and CD8+. The proliferative responses were inhibited in the presence of anti-CD4 or anti-MHC class II monoclonal antibodies. The T cell lines in BALB/c mice recognized an epitope in HCV core at amino acids 72 to 91 (EGRAWAQPGYPWPLYGNEGL). The T cell lines in B6 mice recognized an epitope at amino acids 55 to 74 (RPQPRGRQRPQARQPEG). Thus, mice with different MHC haplotypes recognized different non-overlapping T cell antigenic determinants of HCV core proteins.

Introduction

Hepatitis C virus (HCV) is a major causative agent of post-transfusion hepatitis and parentally transmitted, sporadic non-A, non-B hepatitis throughout the world (Alter et al., 1989; Bortolotti et al., 1991; Choo et al., 1989; Kuo et al., 1989; Miyamura et al., 1990). About half of all patients with acute hepatitis C progress to chronic disease (Alter et al., 1992; Dienstag, 1983, 1990; Farci et al., 1991) and many of them eventually develop hepatocellular carcinoma (Colombo et al., 1989; Kiyosawa et al., 1990; Saito et al., 1990; Seeff et al., 1992). A chronic disease state can be the consequence of incomplete eradication of the virus in the patient (Oldstone, 1989). Therefore, interest has focused upon the function of viral proteins and their interactions with the immune system during the course of infection. Cytotoxic T lymphocytes (CTL) mediate protection in vivo against some viral infections (Earl et al., 1986; Jamieson et al., 1987; Plata et al., 1987; Yap et al., 1978). It is possible that the chronic liver disease associated with HCV infections is mediated by the immune response. However, it is not clear whether CTL specific for HCV are implicated for the pathogenesis of disease or are important for protection or recovery from the infection.
Previous investigations have reported that CD8⁺ CTL recognize epitopes within several HCV proteins (Kita et al., 1993; Koziel et al., 1992, 1993; Shirai et al., 1992, 1994). In contrast to the substantial amino acid sequence variation in the predicted envelope glycoproteins, E1 and E2, the core protein of HCV shows greater sequence conservation among different HCV isolates (Houghton et al., 1991) and appears to be worthy of particular interest as a potential vaccine based on the CTL response. HCV core protein is encoded in the N-terminal region of the HCV genome. The amino acid identity of the HCV core protein region is 97% between Japanese HCV isolates and the original HCV which was serially passaged in chimpanzees (Kato et al., 1990; Okamoto et al., 1990a; Takamizawa et al., 1991; Takeuchi et al., 1990a, b, c). Anti-HCV core antibody has been detected in the majority of patients with chronic hepatitis C (Chiba et al., 1991). HCV core protein contains immunodominant, highly conserved epitopes that elicit antibody production at an early stage of HCV infection (Harada et al., 1991; Hosein et al., 1991; Nasoff et al., 1991).

Several CTL epitopes recognized in mice and humans have been defined within the HCV core protein. CTL may play an important role in the pathogenesis of HCV infection in humans. A highly conserved antigenic site in the HCV core recognized by both murine and human CTL, was recently identified (Shirai et al., 1994). In BALB/c mice, a single conserved epitope represented by a 16 residue synthetic peptide is presented by D₄ class I MHC molecules to conventional CD4⁺ and CD8⁺ CTL. Although the optimal epitope length was different, the same peptide has been identified using CD8⁺ human CTL from two of eight patients studied. These CTL recognized a nine residue portion of this peptide associated with HLA-A₂, the most common class I HLA molecule. These results suggested that animal model experiments using mice to identify the antigenic epitopes in HCV may be useful for the identification of these epitopes in humans. Although the CTL population seems to play a dominant role in virus-induced diseases, it has been demonstrated that non-cytolytic CD4⁺ T cells of helper phenotypes (T₄) also participate as effector cell populations in immune responses (Fujiwara et al., 1984; Greenberg et al., 1981; Udono et al., 1989). HCV core protein was the most immunogenic antigen for MHC class II-restricted T cells (Ferrari et al., 1994). However, neither the function of T₄ cells nor the viral antigens recognized by T₄ cells generated during the immune responses to HCV infection have been clarified at the clonal level, probably because of the difficulty in establishing T₄ clones in these systems (Bookman et al., 1987; Matis et al., 1985). In this study, the characteristics of T₄ clones specific for HCV in mice were examined prior to human study, and the epitopes were mapped.

### Methods

**Mice.** BALB/c (H-2b) and C57BL/6 (H-2b) mice were obtained from the breeding colony at the Institute for Virus Research, Kyoto University. Female mice, 6 to 8 weeks of age at the initiation of the experiments, were used in all studies.

**Expression and purification of recombinant core protein (C115).** Because we could not express full-size core protein (amino acids 1-191) efficiently in Escherichia coli, only the hydrophilic portion (amino acids 1-115) was expressed. HCV cDNA clones were isolated from the serum of a patient with chronic hepatitis C by the reverse transcription–polymerase chain reaction (RT–PCR) (Seki et al., 1994). The DNA fragments to be expressed were obtained by the PCR using sense primer, 5' GCAAGCTTATGAGCACAAATCCAAACCCCA- and the antisense primer, 5' GCGAATTACAGATCTTCACC-TAGCCGGGGGTCCGTGGG 3'. The oligonucleotides were constructed using an Applied Biosystems model 394 DNA synthesizer. The HCV strain used for cloning was of genotype II/1b (Bukh et al., 1994). The DNA fragments were inserted into the cloning vector pUC19 which was then designated pUC115. The clone was sequenced in both directions by the dideoxynucleotide chain-termination method using a DuPont GENESIS 2000 automated laser fluorescence sequencer. The expression vector was pCZ, which is composed of a strong and controllable tac promoter, the Shine-Dalgamo (SD) sequence, the lacI repressor gene, and the ampicillin resistance gene (Takahashi et al., 1990).

To express the hydrophilic domain of core (C115) protein, pCZ was linearized with HindIII and BglII and ligated to a 36 base pair (bp) HindIII-BglII fragment from pUC115. This plasmid was designated pCZC115. To overproduce C115 protein, pCZC115 was linearized with BglII and ligated to a large amount of the 370 bp BamHI-BglII fragment from pCZC115, which contains one expression unit, to obtain pCZC115tan8, which contained eight expression units from the SD sequence region to the stop codon. Plasmid pCZC115tan8 was transformed into E. coli strain BL21 and expressed as described previously. To purify C115 protein, induced cells were dissolved in 200 ml of buffer C (50 mM-Tris-HCl, pH 8.0, 8 M-urea, 1 mM-DTT, 1 mM-PMSF), and the supernatant was harvested by centrifugation at 10000 g for 20 min at 4 °C. The pellet was washed with buffer C twice and the supernatants were pooled. The pooled extract was applied to a CM52 (Whatman) ion-exchange column, washed with buffer C containing 0.15 M-KC1, and eluted with a linear gradient from 0.15 to 0.4 M-KCl in buffer C. Elution was monitored by the absorbance at 280 nm, and the peak fractions were pooled. Final purification was achieved by Sephacryl S-200 (Pharmacia) column chromatography. The N-terminus amino acid sequence of purified proteins was analysed by automatic Edman degradation using an Applied Biosystems model 470A protein sequencer.

**Fragmentation of the C115 protein.** For epitope mapping, the purified C115 protein was digested with Achromobacter protease I (LEP) (a gift from Dr T. Masaki, 1:400, mol/mol) (Masaki et al., 1981), which cleaves peptides at lysine bonds, or with Staphylococcus aureus protease V8 (V8) (ICN ImmunoBiologicals; 1:50 w/w), which cleaves peptides at glutamic acid bonds. After a 15 h incubation at 37 °C, the digested peptides were separated by RP-HPLC on a Bakerbond Octyl column (4.6 x 250 mm). The solvent system was a linear gradient of 0 to 60 % CH₃CN in 0.1% TFA, with a linear gradient for 60 min at a flow rate of 14 ml/min. Elution was monitored by the absorbance at 215 nm.

**Synthetic peptides.** All the peptides were synthesized by Chiron Mimotopes (Victoria, Australia). The amino acid sequence of the C115 protein is shown in Fig. 1. The peptides synthesized and used in this study are also shown in Fig. 1.
Immunization of mice with HCV core protein. Mice were immunized with 10 µg of C115 protein or synthetic peptide in equal volumes of Freund’s complete adjuvant (FCA) (Difco) by subcutaneous injection into the footpads and the dorsal regions. Control mice were immunized with FCA alone.

Measurement of in vivo antibody production and determination of antibody binding epitopes in C115 protein. The sera of immunized mice were collected 14 days after immunization. Preimmune sera were used as controls. Serial dilutions of sera were evaluated for antibody by an ELISA method. Flat-bottomed polystyrene microtitre plates (96 wells; Falcon/Becton Dickinson) were coated overnight at 4 °C with 100 µl of PBS containing 100 ng of C115 protein. To determine the binding epitopes in C115 protein, XENOBIND (covalent binding microwell plates; Xenopore, Saddle Brook, NJ) were coated overnight at 4 °C with 100 µl of PBS containing 1 µg of each synthetic peptide. After blocking non-specific binding with PBS containing 1% BSA, the wells were washed three times with 250 µl of PBS containing 0.05% Tween-20. Sera (50 µl) were plated and incubated for 1 h followed by three washes. Subsequently, 50 µl of peroxidase-conjugated goat anti-mouse IgG (heavy and light chain specific; Cappel, Malvern, PA; diluted at 1:1000) was added. After 1 h incubation, the plates were washed five times. Substrate (100 µl) containing o-phenylenediamine was added for 15 min. The reaction was terminated after 15 min with 25 µl of 2 M-H₂SO₄ and the absorbance at 500 nm was measured with an ELISA plate reader (MTP-12 microplate photometer; Corona, Tokyo, Japan).

Generation of HCV specific Tₘ clones. Draining lymph node cells (5 × 10⁶) from immunized mice were cultured in complete medium (CM) for 7 days at 37 °C in 5% CO₂ and air with 2 µg/ml of C115 protein. CM consisted of RPMI 1640 medium (Nissui Seiyaku, Tokyo, Japan) supplemented with 10% fetal calf serum, 5 × 10⁻⁵ M-2-mercaptoethanol, 100 U of penicillin, and 100 µg of streptomycin per ml. Limiting dilution (LD) cultures (one cell per well) were performed in the presence of 2-5 ng of human recombinant interleukin-2 (rIL-2) (TGP-3; Takeda Pharmaceutical Industries, Osaka, Japan) per ml. Growing colonies were screened by flow cytometry for the surface expression of either CD4 or CD8. All the CD4+ T cells of LD cultures were expanded and tested to determine whether they were specifically reactive to C115 protein. Three CD4+ T cell clones in BALB/c mice and two in C57BL/6 mice were found to be specifically reactive to C115 protein. Each of the five clones was recloned at 0-5 cells per well. Those Tₘ...
clones were passaged every 7 days together with irradiated syngenic spleen cells (20 Gy) and 2 μg per ml of C115 protein in the presence of human recombinant interleukin-2 (rIL-2).

T cell proliferation assay. Draining lymph node cells were harvested at 7 days post-immunization, and 1 × 10⁶ cells were cultured for 72 h in 200 μl of CM containing C115 protein or medium alone. Cloned CD4⁺ T cells (1 × 10⁶) were cultured for 48 h in the presence of irradiated (20 Gy) spleen cells (5 × 10⁶) as antigen-presenting cells (APC) and various concentrations of the C115 protein or a synthetic peptide in 200 μl of CM in each well without rIL-2. The cells were cultured in triplicate wells of flat-bottomed microculture plates at 37 °C in a humidified 5% CO₂ atmosphere, and 18.5 kBq of [³H]thymidine ([³H]TdR; 74 GBq/mmol, NEN) was added to each well 4 h before the termination of the culture. The cells were then harvested onto filter strips to determine the level of [³H]TdR incorporation. The data were expressed as c.p.m., corrected for background proliferation in the absence of antigen (Δ c.p.m.) and the mean c.p.m. ± SD were calculated.

Antibodies. The anti-CD3(145-2C11), anti-CD4(GK1.5), anti-TCRμβ(H57-597), anti-Thy-1.2, and anti-CD8 monoclonal antibodies (MAbs) have been described (Iwashiro et al., 1993). Non-polymorphic rat anti-murine class II (anti-I-Aᵇ⁺,α⁺ and I-Eᵇ⁺,k⁺)(M5/114.15.2) and Anti-I-Eᵇ⁺,α⁺,k⁺(14-4-4S) MAbs were obtained from the ATCC (Rockville, MD). Anti-I-Eᵇ⁺,α⁺,k⁺(14-4-4S) MAbs were used in ascitic form. M5/114.15 was used as a hybridoma culture supernatant fluid for blocking T cell proliferation.

Results

In vivo antibodies raised against the hydrophilic portion of HCV core (C115) protein in BALB/c and C57BL/6 mice

BALB/c and C57BL/6 mice were immunized with 10 μg of recombinant core (C115) protein emulsified in FCA. Two weeks thereafter, serum was collected and evaluated for anti-HCV core antibody production by an ELISA. Though anti-HCV core antibody was efficiently produced in both strains of mice, the titre of the antibody from sera of BALB/c mice was 10-fold higher than that of C57BL/6 mice. BALB/c mice were higher responders than C57BL/6 mice to HCV core protein in terms of antibody production (Fig. 2a).

To localize relevant antibody epitopes within C115 protein, immunized murine sera were analysed for antibody reactivity with six sequential synthetic peptides, CP-0 to CP-5, derived from C115 (Fig. 1). Because the peptides were not overlapping, some potential epitopes could have been missed. However, anti-core sera from both BALB/c and C57BL/6 mice bound only to the
HCV core region T\textsubscript{h} epitopes in mice

Fig. 3. Difference of T cell responses to C115 protein in two strains of mice. BALB/c (○) and C57BL/6 (△) mice were immunized with 10 μg of C115 in FCA in the foot pads, and 7 days later the draining lymph node cells were harvested and cultured \textit{in vitro} with varying concentrations of C115 protein or medium alone. Control mice (□) were immunized with FCA alone. Cells (1 × 10\textsuperscript{5}) were cultured in 200 μl of medium containing C115 protein or medium alone for 72 h. The \textsuperscript{3}H\textsuperscript{-}thymidine was added for the last 4 h of culture. The data are expressed as c.p.m. corrected for background proliferation in the absence of antigen. The data are mean ± SD of triplicate samples and are representative of at least two independent experiments. Background counts ranged from 100 to 1000 c.p.m.

CP-0(1–28), representing the N-terminal amino acids 1–28 (MSTNPKQKIRNTNRPQDVFPGGG), where the (major B-type) epitopes for humans are located (Hosein \textit{et al.}, 1991; Nasoff \textit{et al.}, 1991; Sällberg \textit{et al.}, 1992) (Fig. 2b and c).

The C115-specific T cell response in BALB/c and C57BL/6 mice

To determine the difference in the T cell immune response in BALB/c and C57BL/6 mice to C115 protein, BALB/c and C57BL/6 mice were immunized with 10 μg of C115 protein emulsified in FCA. Seven days later, the proliferation of the draining lymph node cells was compared \textit{in vitro} using different C115 protein concentrations. As illustrated in Fig. 3, C115 protein induced significant T cell proliferation in both strains of mice tested. The proliferative responses were blocked when responding lymph node cells were either exposed to anti-Thy-1.2 plus complement before culture or cultured in the presence of non-polymorphic anti-murine class II monoclonal antibody (M5/114.15.2) (data not shown).

Although the C115-primed T cells of BALB/c mice showed a strong response to C115 protein, T cells of C57BL/6 mice responded weakly (Fig. 3). The difference in T cell responsiveness in BALB/c and C57BL/6 mice correlated with their different ability to produce anti-C115 antibody \textit{in vivo} (Fig. 2).

Isolation and characterization of C115 protein specific T\textsubscript{h} cell clones

To further analyse the T cell responses to C115 protein, we established T cell clones specific to C115 protein in both BALB/c and C57BL/6 mice. The cells showing growing colonies in LD cultures were examined for CD4 expression. Among those CD4\textsuperscript{+} colonies, only three CD4\textsuperscript{+} T cell clones in BALB/c mice and two clones in C57BL/6 mice were specifically reactive to C115 protein and were recloned for further study. AC-1.7, AC-1.10 and AC-1.11 originated from BALB/c mice. BC-1.6 and BC-2.5 originated from C57BL/6 mice (Fig. 4a). These were independent clones derived from a single mouse. The surface phenotypes of these clones were Thy-1.2\textsuperscript{+}, CD4\textsuperscript{+}, CD8\textsuperscript{-}, TCR\textsuperscript{αβ}+, and CD3\textsuperscript{+} (data not shown). Fig. 4(b) shows that the proliferative responses were completely inhibited by anti-CD4 MAb. In addition, non-polymorphic anti-MHC class II MAb (anti-I\textsubscript{A}\textsuperscript{β\(\beta\)} and I\textsubscript{E}\textsuperscript{d\(\beta\)}), and CD3\textsuperscript{+} (data not shown). Fig. 4(b) shows that the proliferative responses were completely inhibited by anti-CD4 MAb. In addition, non-polymorphic anti-MHC class II MAb (anti-I\textsubscript{A}\textsuperscript{β\(\beta\)} and I\textsubscript{E}\textsuperscript{d\(\beta\)}) blocked the proliferative responses as was seen for the bulk cultures. On the other hand, anti-I\textsubscript{E}\textsubscript{α} MAb, 14-4-4S, did not inhibit the responses (Fig. 4b). The C57BL/6 mouse does not express MHC class II I-E antigens. Thus, the restriction molecules seemed to be MHC class II I-A, but not I-E in the T cell clones from both strains.

Determination of the T\textsubscript{h} cell epitopes with synthetic peptides

To define the epitopes recognized by these clones, we completely digested C115 protein with endopeptidase LEP or V8, which generated seven or four peptides, respectively (Fig. 1b). Proliferation was assayed using a mixture of these fragmented peptides (data not shown). If the T cell epitope contains internal lysine or glutamic acid residues, T cell responses would be affected by the digestion of C115 protein. All of the T\textsubscript{h} clones proliferated in the presence of C115 protein digested with V8. Although the C115 digested with LEP could stimulate T\textsubscript{h} clones AC-1.7, AC-1.10 and AC-1.11 of BALB/c origin, T\textsubscript{h} clones BC-1.6 and BC-2.5 derived from C57BL/6 mice were not stimulated. Digestion with V8 did not affect the proliferative response of cells from either mouse strain. We synthesized six non-overlapping peptides, CP-0 to CP-5, some containing internal lysine residues (20 to 28 amino acids long) (Fig. 1). T\textsubscript{h} clones were cultured at various concentrations of each peptide in the presence of irradiated spleen cells, and their proliferation was examined. The T\textsubscript{h} clone BC-2.5
responded only to the peptide CP-3 (RPQPRGRRQP-KARQPEGGR; 55-74). On the other hand, only the peptide CP-4 (EGRAWAQPYPWPLGYNEGL; 72-91) stimulated proliferation of T\(_h\) AC-1.10 over a wide range of concentrations (Fig. 5). AC-1.7 and AC-1.11 responded to the peptide CP-4 as AC-1.10. BC-1.6 was stimulated by peptide CP-3 to the same extent as BC-2.5.

**Fig. 4.** (a) The establishment of the C115-specific T cell clones. Draining lymph node cells (5 \times 10^6) of immunized mice were cultured in complete medium with 2 \(\mu\)g/ml of C115 protein. Growing colonies were assayed for their specific responses to C115 protein. AC-1.7, AC-1.10 and AC-1.11 were established from BALB/c mice. BC-1.6 and BC-2.5 were established from C57BL/6 mice. (b) The effect of anti-MHC class II and anti-CD4 MAbs on the proliferation responses of T\(_h\) clones AC-1.11 (■) and BC-1.6 (□). Each T\(_h\) clone (1 \times 10^5 cells) was cultured for 2 days with 5 \times 10^6 irradiated syngenic spleen cells as APC and 20 \(\mu\)g of C115 protein, in the presence or absence of one of the following MAbs: anti-CD4 (GK1.5), anti-CD8 (anti-Lyt-2.2), non-polymorphic anti-MHC class II (anti-I-A\(^{b,d,g}\) and anti-I-E\(^{k}\))(MS/14.15.2) and anti-I-E\(^{k}\) (14.4-4S) at a concentration of 10 \(\mu\)g/ml. The [\(^{3}\)H]thymidine was added for the last 4 h of culture. Data shown are from a representative of three repeated experiments.

**Fig. 5.** Localization of T cell sites within the C115 protein sequence using synthetic peptides. Cloned T\(_h\) cells (1 \times 10^6) were cultured for 48 h with 5 \times 10^5 irradiated (20 Gy) syngenic spleen cells as APC and 50 \(\mu\)g/ml of C115 protein or 25 \(\mu\)g/ml of each synthetic peptide in 200 \(\mu\)l of complete medium in each well without rIL-2. [\(^{3}\)H]Thymidine was added for the last 4 h of culture. AC-1.10 (■) established from a BALB/c mouse specifically responded to CP-4. BC-2.5 established from a C57BL/6 mouse (□) specifically responded to CP-3. The results of repeated experiments were consistent with this representative figure. T\(_h\) clones from BALB/c mouse specifically recognized the peptide CP-4 and those from the C57BL/6 mouse recognized peptide CP-3. These results indicated the presence of at least two T\(_h\) cell epitopes in C115 protein which BALB/c and C57BL/6 mice recognized differentially.

**In vivo immunization with synthetic C115 peptide elicited T cells recognizing C115 protein**

BALB/c and C57BL/6 mice were immunized with 10 \(\mu\)g of each T\(_h\) epitope peptide, (CP-4 in BALB/c mice and CP-3 in C57BL/6 mice). Ten days later, draining lymph node cells were harvested, cultured *in vitro* with various concentrations of each peptide or C115 proteins, and the T cell proliferative responses were determined (Fig. 6). The T cells elicited by each T\(_h\) epitope peptide immunization responded to each peptide and the native C115 protein in both strains of mice. A synthetic T cell determinant derived from the HCV core protein can prime T\(_h\) cell activity *in vivo*. Immunization with CP-4 in BALB/c mice and immunization with CP-3 in C57BL/6 mice did not induce antibody production (data not shown). This was probably because the T\(_h\) epitope peptides CP-4 and CP-3 did not contain the B cell epitope, CP-0 (1-28).
alterations of the amino acid sequence of HVR1. Mutations in HVR1 are likely to result in escape from the immnosurveillance system.

In contrast to the substantial amino acid sequence variation in the E1 and E2 proteins, other regions of HCV like C, NS3, NS4, and NS5 show greater sequence conservation among different types of isolates (Cha et al., 1992; Choo et al., 1991; Houghton et al., 1991; Miller & Purcell, 1990; Okamoto et al., 1990a; Takeuchi et al., 1990a, c; Weiner et al., 1991). We analysed the immune response to HCV core protein in two strains of mice. HCV core protein contains B cell, Th and CTL epitopes in chronic HCV patients (Botarelli et al., 1993; Koziel et al., 1992, 1993; Shirai et al., 1994). The N-terminal portion of the HCV core protein contains a major antigenic region for B cells and synthetic peptides covering the core protein contain linear determinants (Ching et al., 1992; Hosein et al., 1991; Ishida et al., 1993; Nasoff et al., 1991; Okamoto et al., 1990b). In this study, murine sera from each strain of mice immunized with C115 protein bound to the N-terminal portion of the core protein at amino acids 1 and 28 (MSTNPKPLRRKIKRNTPQQDVKFPGGG) on examination with non-overlapping peptides which covered the hydrophilic portion of HCV core protein. Thus we could demonstrate that murine and human B cells recognized the same epitope of HCV core protein.

HCV core protein induced not only a B cell response but also a T cell response specific to HCV core protein in mice. The HCV core-primed T cells of BALB/c mice responded more vigorously to HCV core protein, especially in bulk cultures, than those of C57BL/6 mice. These findings might help to explain why the outcomes of HCV infection differ from one person to another. Some people are able to control the infection, but in 60% of HCV-infected individuals, the disease progresses and becomes chronic (Dienstag et al., 1983, 1990). The difference in T cell responsiveness as seen in BALB/c and C57BL/6 mice might influence the severity of the infection and the different manifestations of the disease in man. We established helper T cell clones and determined the Th epitope at the peptide level. To estimate the location of the T cell epitope, we digested C115 protein with endopeptidase. Two endopeptidases, LEP or V8, were expected to cleave the native C115 protein at several sites. LEP abolished the stimulating capacity for only Th clones of B6 origin, whereas no change was seen for that with V8. Thus, it was suggested that the epitope(s) recognized by the C57BL/6 mouse contains lysine, whereas that recognized by BALB/c mice lay between glutamic acid residues and was not affected by V8 digestion. We synthesized six non-overlapping peptides to determine the T cell recognition site more precisely. T cell clones derived from the two

**Discussion**

The mechanism responsible for the lack of protective immunity against chronic infection with HCV is at present unknown. The virus fails to induce an effective neutralizing antibody response, or genetic variation leads to the rapid development of escape mutants that circumvent the immune response (Kato et al., 1990; Martell et al., 1992; Ogata et al., 1991). Alternatively, the inability of the host to mount a protective immune response could lead to reactivation of the same virus (Abe et al., 1992; Oldstone, 1989). Studying the immune response to HCV is one of the most important approaches to understanding the pathogenesis of chronic hepatitis and for the development of an effective vaccine.

In some antiviral immune responses, such as to human immunodeficiency virus, influenza A virus and HCV, the same epitopes are recognized by both human and murine T cells, suggesting the validity of epitope mapping in man. We established helper T cell clones and determined the Th epitope at the peptide level. To estimate the location of the T cell epitope, we digested C115 protein with endopeptidase. Two endopeptidases, LEP or V8, were expected to cleave the native C115 protein at several sites. LEP abolished the stimulating capacity for only Th clones of B6 origin, whereas no change was seen for that with V8. Thus, it was suggested that the epitope(s) recognized by the C57BL/6 mouse contains lysine, whereas that recognized by BALB/c mice lay between glutamic acid residues and was not affected by V8 digestion. We synthesized six non-overlapping peptides to determine the T cell recognition site more precisely. T cell clones derived from the two
strains of mice, BALB/c and C57BL/6, recognized the different epitopes in the HCV core protein. T cell clones from BALB/c mice recognized CP-4 (EGRAYQPGYWPWCYLGENEG; 72-91) and those from C57BL/6 mice recognized CP-3 (RPQPRGQRQPKARQPEGR; 55-74). However, it is possible that other T<sub>h</sub> epitopes than those reported here do exist, since we do not know at present whether the T<sub>h</sub> clones used in the experiment are really representative of T<sub>h</sub> repertoire involved in the anti-C115 responses. To identify less immunodominant T<sub>h</sub> epitope(s), use of a greater number of T<sub>h</sub> clones and a panel of overlapping peptides would be required. Although the reason for the lower response in C57BL/6 mice to HCV core protein remains unclear, it is possible that non-MHC genetic background as well as MHC molecules presenting the HCV epitope to T cells influence the magnitude and quality of the immune responses by some regulatory mechanism (Milich et al., 1987; Morioka et al., 1994; Moskophidis et al., 1994; Oldstone, 1989; Peters et al., 1991; Plata et al., 1987).

It is believed that antibodies to HCV core protein would have no prophylactic value, because they are detectable in the blood of persistently infected hosts. On the other hand, it was reported that peripheral blood mononuclear cells from HCV-infected individuals proliferated in response to HCV recombinant proteins and that peripheral responses to the core protein correlated with a benign course of infection (Botarelli et al., 1993; Ferrari et al., 1994). We demonstrated that T<sub>h</sub> epitope peptides could induce a T cell proliferative response to HCV core protein in both strains of mice. We would like to know whether a T<sub>h</sub> vaccine could modulate the immune response to HCV in chronically infected individuals. Although the significance of CD4<sup>+</sup> T cell responses to HCV was not assessed in vivo, it may be critical to keep the infection under control by providing continuous help for B and CD8<sup>+</sup> T cells, both of which may be required to maintain humoral and cellular responses against the disease. There are no appropriate animals except chimpanzees which are susceptible to HCV infection. Transgenic mice expressing whole or various portions of HCV antigens would provide a useful model with which to analyse the roles of HCV antigen-specific T cell clones both in protection against and pathogenesis by HCV infection (Ando et al., 1993; Moriyama et al., 1990).

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