Indeed, a series of CTL-directed epitopes has been identified from proteins of HCV genotypes 1a and 1b (30). However, few HCV genotype 2a-derived CTL-directed epitopes have been identified to date. In the present study, we attempted to identify HCV2a-derived CTL-directed epitopes presented by the HLA-A2 molecule, which is the most major HLA type in the world population (11).

Materials and Methods

Subjects. The Institutional Ethical Review Board of Kurume University approved this study protocol (Protocol # 2244), and informed written consent was obtained from all the blood donors. Ten HLA-A2$^+$ HCV2a-infected patients and five HLA-A2$^+$ healthy donors were enrolled in this study. HCV2a patients were seropositive for anti-HCV antibodies (Abs) as confirmed by second- or third-generation immunoassay tested by a clinical laboratory company, SRL, Tokyo. The healthy donors were without any symptoms of hepatocellular dysfunction.

Peptide. Nine different kinds of 9- or 10-mer synthetic peptides derived from the protein of HCV genotype 2a, all of which contained an HLA-A2-binding motif, were used in this study (Table 1). Influenza (Flu) A virus matrix protein 1-derived (GILGFVFTL), Epstein-Barr virus (EBV) BMLF1-derived (GLCTLVAML), and HIV-1 gag protein-derived peptides (SLYNTVATL) with the HLA-A2-binding motif were used as controls. All peptides (>90% purity) were purchased from BIO SYNTHESIS (Lewisville, Tex., U.S.A.) or SynPep (Dublin, Calif., U.S.A.), and were dissolved in dimethyl sulfoxide (DMSO) at a concentration of 10 mg/ml and stored at −20 C.

Table 1. HCV2a-derived peptides used in this study

Number	Peptides		
	Region	Sequence	Binding score[a]
1	C 35–44	YLLPRRGPRL	363
2	E1 284–293	VMLAAQMFIV	1,728
3	E1 285–293	MLAAQMFIV	646
4	E2 432–441	SLHTGFLASL	186
5	E2 716–724	YIVRWEWVV	135
6	NS2 888–897	VVFDITKWLL	361
7	NS5 2251–2260	VVLDSLDPMV	156
8	NS5 2850–2858	WLGNIIQYA	289
9	NS5 3012–3021	RLLLLGLLLL	181

[a]The peptide binding scores were calculated based on the predicted half-lives of dissociation from HLA-A0201 molecules, as obtained from a Website (Bioinformatics and Molecular Analysis Section, Computational Bioscience and Engineering Laboratory, Division of Computer Research & Technology, NIH).

Cell lines. The HLA-A2-expressing cell lines, T2 and HEK 293-A2, were cultured in RPMI-1640 medium (Gibco BRL, Grand Island, N.Y., U.S.A.) supplemented with 10% FCS (Gibco BRL).

RNA synthesis. The plasmid pSGR-JFH1 (15), which contained the consensus sequence of NS3–NS5 of HCV2a subgenomic JFH-1, was kindly provided by Dr. Takaji Wakita (Tokyo Metropolitan Institute for Neuroscience, Tokyo). The plasmid was linearized by *Xba*I digestion and further treated with mung bean nuclease (New England Biolabs, Beverly, Mass., U.S.A.) to remove four nucleotides and leave the correct 3' end of the HCV cDNA. Digested plasmid DNAs were purified and used as templates for *in vitro* RNA synthesis using the MEGAscript™ T7 kit (Ambion, Austin, Tex., U.S.A.). Synthesized HCV subgenomic RNA was treated with DNase I (RQ1TM RNase-free DNase; Promega, Madison, Wis., U.S.A.) followed by acid phenol extraction to remove any remaining template DNA.

RNA transfection. Trypsinized HEK 293-A2 cells were washed with PBS and resuspended with ice-chilled Cytomix buffer (29) at a concentration of $0.5–1\times10^7$ cells/ml; Synthesized replicon RNA (5 μg) was mixed with 400 μl of the cell suspensions, transferred to an electroporation cuvette (Bio-Rad, Hercules, Calif., U.S.A.), and pulsed at 260 V and 950 μF with the Gene Pulser II apparatus (Bio-Rad). Transfected cells were then transferred to RPMI-1640 medium with 10% FCS and cultured in a culture flask. G418 (1.2 mg/ml) (Nacalai Tesque, Kyoto, Japan) was added to the culture medium at 18–24 hr after the transfection, and twice a week the culture medium was replaced with fresh medium supplemented with G418. Three weeks after transfection, cells were diluted with selective medium to a concentration of 1 cell per 200 μl. Cells were then plated on a 96-well plate at 100 μl cells per well and cultured for an additional 2–3 weeks. G418-resistant colonies were collected and expanded until they were 80–90% confluent in 10 cm culture dishes for use in nucleic acid and protein analyses.

RT-PCR. The total RNA of the G418-resistant colonies was isolated with RNA-Bee™ (Tel-Test, Friendswood, Tex., U.S.A.) according to the manufacturer's instructions. cDNA was synthesized from 5 μg total RNA. HCV cDNA was detected by PCR amplification using a set of oligonucleotide primers specific to HCV 2a (forward primer at nucleotide position 7244–7263: 5'-AGGAGGCCAGATTACCAACC-3'; reverse primer at nucleotide position 7376–7395: 5'-AAGGTCTTGATGGCCAGTTG-3'). PCR was performed in 30 cycles (1 min at 95 C, 1 min at 60 C, and 1 min at 72 C) using *Taq* DNA polymerase (Promega).

The PCR product was analyzed on 2% agarose gel.

In vitro *induction of CTLs from HCV2a-infected patients with HCV2a-derived peptides.* The method used for the detection of peptide-specific CTLs has been reported elsewhere (18). In brief, PBMCs (1×10^5 cells per well) of HLA-A2$^+$ HCV2a$^+$ patients were incubated with 10 μg/ml of each peptide in a U-bottom 96-well microculture plate (Nunc, Roskilde, Denmark) in a volume of 200 μl of culture medium. The culture medium consisted of 45% RPMI-1640, 45% AIM-V medium (Gibco BRL), 10% FCS, 100 U/ml of interleukin-2 (IL-2), and 40 μg/ml gentamycin. Half of the culture medium was removed and replaced with new medium containing 20 μg/ml of the respective peptide every 3–4 days. On day 15, half of the cultured cells in each well were equally separated into four wells; two wells were further stimulated with the corresponding peptide-pulsed T2 cells, and the other two wells were stimulated with the control HIV peptide-pulsed T2 cells. After 16–18 hr of incubation, the IFN-γ levels of the supernatants were examined by ELISA. The background IFN-γ production in response to the control HIV peptide was subtracted from the value given in the data. The HCV2a peptide-stimulated PBMCs were further cultured with irradiated HLA-A2$^+$ buffy coat cells as feeder cells for approximately 2–3 weeks to obtain a sufficient number of cells for cytotoxicity analysis.

Generation of dendritic cells (DC) from blood monocytes. PBMCs were isolated by Ficoll-Hypaque density gradient centrifugation from HLA-A2$^+$ healthy donors. After 4–5 washes with PBS, PBMCs were suspended in 10% FCS-PRMI-1640 medium at 4×10^6 cells/ml. The cells were placed in culture dishes (10 ml/dish) and incubated for 60–90 min at 37 C under 5% CO_2. The non-adherent cells were removed by gentle washing, and the remaining adherent monocyte-enriched population was cultured for 7 days in medium consisting of 45% RPMI-1640, 45% AIM-V medium, 10% FCS, 100 U/ml of interleukin-2 (IL-2, Shionogi & Co., Osaka, Japan), 10 ng/ml of GM-CSF (Pepro Tech Ec, London, U.K.), 10 ng/ml of IL-4 (Pepro Tech Ec), and 40 μg/ml gentamycin. No medium was added or removed during this culture period. On day 5, 10 ng/ml of TNF-α (Sigma, St. Louis, Mo., U.S.A.) was added to the culture medium to mature DC. The cells were harvested at day 7 and used as DC.

In vitro *induction of CTLs from healthy donors using HCV2a peptide-pulsed DC.* On day 0, DC were washed with PBS, resuspended in the culture medium (45% RPMI-1640, 45% AIM-V, 10% FCS, 100 U/ml IL-2) at 1×10^6 cells/ml, and incubated with 3 μg/ml β2-microglobulin (Sigma) and 10 μg/ml peptide at 37 C for 2 hr. After irradiation (40 Gy), DC were mixed with non-adherent PBMCs at a ratio of 1:20 and cultured in wells of a 96-well culture plate (1×10^5 cells per well). On days 7, 14 and 21, the PBMC cultures were restimulated with irradiated peptide-pulsed DC (2.5×10^3 cells/well). The cytotoxicity assays were performed on day 28.

Assay of cytotoxicity. The cell-mediated cytotoxicity assays were performed using a standard 6-hr ^{51}Cr release assay. A total of 1×10^3 ^{51}Cr-labeled target cells were incubated with various effector cell-to-target cell (E/T) ratios in wells of a U-bottom 96-well plate, and the ^{51}Cr release into the supernatants was examined in triplicate. To eliminate nonspecific lysis, the cytotoxic activity was tested in the presence of 4×10^4 cells/well of unlabeled K562 cells. In antibody blocking experiments, either anti-HLA class I (W6/32: mouse IgG2a), anti-HLA-DR (L243: mouse IgG2a), or anti-CD14 (H14: mouse IgG2a) mAb was added into the wells at a concentration of 20 μg/ml at the initiation of the assay. The CD8$^+$ cells were purified using a CD8 Isolation Kit (DYNAL, Oslo, Norway) in some experiments. L243 mAb is capable of blocking HLA-DRB1- or DRB4-restricted responses, but not HLA-DQ- or -DP-restricted responses, and the effects on DRB3- or DRB5-restricted responses have not yet been clarified.

Statistics. The statistical analyses were performed by a two-tailed Student's *t*-test. *P*-values of less than 0.05 were considered statistically significant.

Results

Determination of T-Cell Epitope Peptides

Putative HLA-A2-binding peptides encoded by HCV2a were analyzed by a BIMAS, U.S.A. software (Bioinformatics and Molecular Analysis Section, NIH). A total of nine peptides were selected for further analyses (Table 1). The BIMAS binding scores used for predicting the half-lives of the peptide-MHC dissociations of the nine peptides were also listed. The peptides were tested for their ability to induce CTLs in the PBMC cultures of ten HLA-A2$^+$ HCV2a-infected patients and five HLA-A2$^+$ healthy donors. The results of IFN-γ production in response to peptide-loaded T2 cells are shown in Table 2. PBMCs of all patients showed a response to at least one of the epitope peptides tested, with the exception of patients 2 and 7. All of the peptides were able to induce peptide-specific IFN-γ production in the PBMC culture from at least one of the patients. These nine HCV2a-derived HLA-A2-binding peptides also induced peptide-specific CTLs from at least one of the five healthy donors. Among these nine peptides, three HCV2a-derived peptides (HCV2a 432–441, HCV2a 716–724, and HCV2a 2251–2260) induced peptide-spe-

Table 2. Induction of peptide-reactive CTLs from PBMCs of HLA-A2$^+$ HCV2a-infected patients and HCV-negative healthy donors

Subject	Peptides											
	C 35–44	E1 284–293	E1 285–293	E2 432–441	E2 716–724	NS2 888–897	NS5 2251–2260	NS5 2850–2858	NS5 3012–3021	HIV	EBV	Flu
	IFN-γ production (pg/ml)											
Pt.1	0	3	39	890	5	3	341	9	0	0	2,000	N.D.
Pt.2	35	N.D.	19	39	N.D.	18	0	N.D.	N.D.	0	28	N.D.
Pt.3	N.D.	39	13	76	26	78	N.D.	0	N.D.	0	N.D.	316
Pt.4	233	0	163	296	N.D.	0	1,255	60	N.D.	N.D.	115	N.D.
Pt.5	0	300	5	429	24	64	118	5	34	N.D.	N.D.	N.D.
Pt.6	107	38	384	N.D.	209	1	47	225	75	0	11	73
Pt.7	5	0	27	7	34	16	2	1	17	N.D.	N.D.	N.D.
Pt.8	0	36	0	21	212	39	9	24	0	N.D.	N.D.	N.D.
Pt.9	206	N.D.	368	127	1,737	36	108	53	246	N.D.	N.D.	N.D.
Pt.10	19	19	52	80	586	0	135	0	164	13	496	2,384
Positive/total	3/9	1/8	3/10	6/9	4/8	2/10	5/9	3/9	3/7			
HD1	4	40	19	52	4	93	0	3	20	0	18	92
HD2	8	0	25	68	10	0	5	34	22	0	15	38
HD3	28	34	7	0	44	5	74	49	36	17	0	70
HD4	90	130	44	45	68	21	0	6	83	4	40	638
HD5	3	0	23	3	45	8	53	23	12	6	0	15
Positive/total	1/5	1/5	1/5	3/5	3/5	1/5	2/5	1/5	1/5			

The PBMCs from patients and HCV-negative healthy donors were tested for their reactivity to peptides after *in vitro* stimulation with each peptide for 2 weeks. Values represent the IFN-γ concentration produced by the effector PBMCs in response to T2 cells prepulsed with each corresponding peptide. Background IFN-γ response to T2 cells prepulsed with the HIV peptide was subtracted. Significant values ($P<0.05$ by the Student's *t*-test) are underlined. N.D., not determined, Pt., patient; HD, healthy donor.

cific CTLs efficiently, and these peptides could generate peptide-specific CTLs from 67%, 50%, and 56% of the patients tested, respectively. The other six HCV2a-derived epitopes could induce the positive CTL responses at percentages lower than 50%. Meanwhile, the HCV2a 432–441, HCV2a 716–724, and HCV2a 2251–2260 peptides induced peptide-specific CTLs in 3, 3 and 2 of 5 healthy donors, respectively. Thus, these results indicated that the HCV2a 432–441, HCV2a 716–724, and HCV2a 2251–2260 peptides were promising, and the subsequent studies were focused on these peptides.

Cytotoxicity of the HCV2a Peptide-Specific CTLs from the PBMCs of Patients

Then we determined whether these HCV2a peptide-stimulated PBMCs would show any cytotoxicity against corresponding peptide-pulsed T2 cells. The cytotoxicity of the peptide-induced CTLs was further confirmed by a 6-hr ^{51}Cr-release assay. The CTLs that were induced by each of the three peptides (HCV2a 432–441, HCV2a 716–724, and HCV2a 2251–2260) exhibited significantly higher levels of cytotoxicity against the corresponding peptide-pulsed T2 cells than against the control HIV peptide-pulsed T2 cells. Representative results (patients 1, 4, 5, 6, 9, and 10) are shown in Fig. 1.

Cytotoxicity of the HCV2a Peptide-Specific CTLs Induced from the PBMCs of Healthy Donors

Because of the limited availability of PBMCs of HCV2a-infected patients, we attempted to induce peptide-CTLs from the PBMCs of HLA-A2$^+$ healthy donors using the HCV2a peptide-loaded DC for further analysis. Representative results are shown in Fig. 2. Peptide-specific CTLs were successfully induced from the PBMCs of healthy donors by the stimulation of HCV2a 432–441, HCV2a 716–724, or HCV2a 2251–2260 peptide-loaded DC (Fig. 2A). Purified CD8$^+$ cells exhibited the cytotoxicity against corresponding peptide-pulsed T2 cells. The cytotoxic activity was blocked by the addition of anti-HLA class I (HLA-A, B, C) antibody, but not anti-HLA class II (HLA-DR) or anti-CD14 antibody (Fig. 2B), which indicates that the cytotoxicity of peptide-specific CTLs was dependent on MHC class I-restricted CD8$^+$ T cells.

Because the CTLs were generated by *in vitro* stimulation with synthetic peptides, we wanted to make sure that the CTLs recognized the endogenously processed peptides of HCV2a protein. We prepared *HCV2a NS3–NS5*-expressing HEK293-A2 cells as targets in the ^{51}Cr-release assay. The presence of the *HCV2a NS3–NS5* subgenomic mRNA in transfected HEK293-A2 cells was confirmed by RT-PCR analysis (Fig. 3A). The HCV2a NS5 2251–2260 peptide-induced CTLs

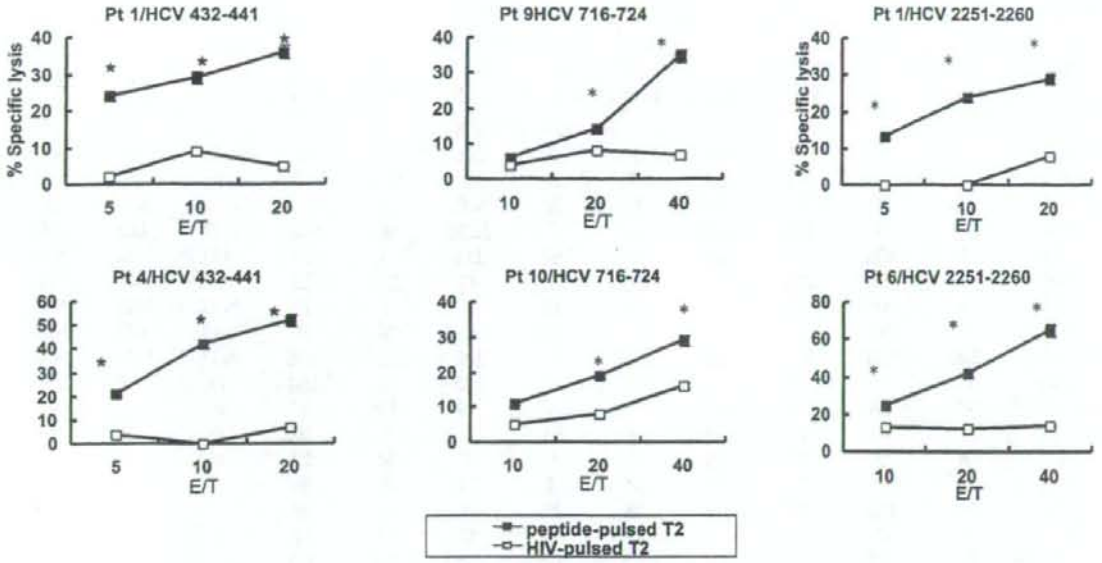

Fig. 1. Cytotoxicity of HCV2a peptide-specific CTLs generated *in vitro* from HCV2a-infected patients. The cytotoxicity against T2 cells pulsed with the corresponding HCV2a peptide or the control HIV-peptide was tested by a standard 6-hr ^{51}Cr-release assay. Representative results in ten experiments using different patient PBMCs are shown. Values represent the mean of triplicate determinations, and statistical analyses were performed by a two-tailed Student's *t*-test (*$P<0.05$).

lysed the *HCV2a NS3–NS5*-transfected HEK293-A2 cells significantly, but not the non-transfected HEK293-A2 cells (Fig. 3B). Collectively, these results indicate that three HCV2a-derived epitope peptides (HCV2a 432–441, HCV2a 716–724, and HCV2a 2251–2260) have the potential to induce HLA-A2-restricted CTLs recognizing HCV2a-infected cells, and that the cytotoxicity is mainly mediated by peptide-reactive and $CD8^+$ T cells.

Discussion

$CD8^+$ T cells have been suggested to play a role not only in chronic hepatitis due to infection by hepatitis B virus (20, 21), but also in the pathogenesis of chronic hepatitis due to HCV infections (13, 17, 25). Rehermann et al. reported that the HCV-specific CTL response in low viral-load patients was stronger than that in high viral-load patients, and suggested that the HCV-specific CTL response might be able to control viral load to some extent in chronically infected patients (23). Moreover, the HCV-specific CTL activity has an impact on the efficacy of interferon therapy, with the patients who show detectable HCV-specific CTL activity developing better or complete responses to IFN treatment (22). Therefore, augmentation of the CTL responses might be useful as therapeutic antiviral strategy, and the identification of T-cell epitopes from HCV protein is a critical step in the development of peptide-based immunotherapy for HCV-infected patients. Actually, a number of CTL-directed epitopes derived from HCV proteins have been identified (5). However, many of the reports have focused on identification of epitopes in HCV1a or 1b proteins, and few epitopes have been identified in HCV2a proteins, although HCV2a is a predominant genotype in various Asian and European countries (12, 31).

In this study, three peptides (HCV2a 432–441, HCV2a 716–724, and HCV2a 2251–2260) were found to be immunogenic in more than half of the patients tested. Meanwhile, all of the peptides failed to induce peptide-specific CTLs in two patients (Pt. 2 and 7). At present, we are unsure why peptide-specific CTLs were not induced in these subjects. This finding may be attributable to one or more of the following causes: the absence of an HCV-specific CTL precursor in these individuals; HCV2a infection with a different strain of HCV2a; or the loss of cells present at a low frequency during the *in vitro* culture. Since HCV is a highly heterogeneous virus and, in some circumstances, even a single amino acid variation within CTL epitopes facilitates viral escape from host immunity (1, 8), amino acid mutation in these individuals may be the most plausible explanation. We previously reported that the HCV1b

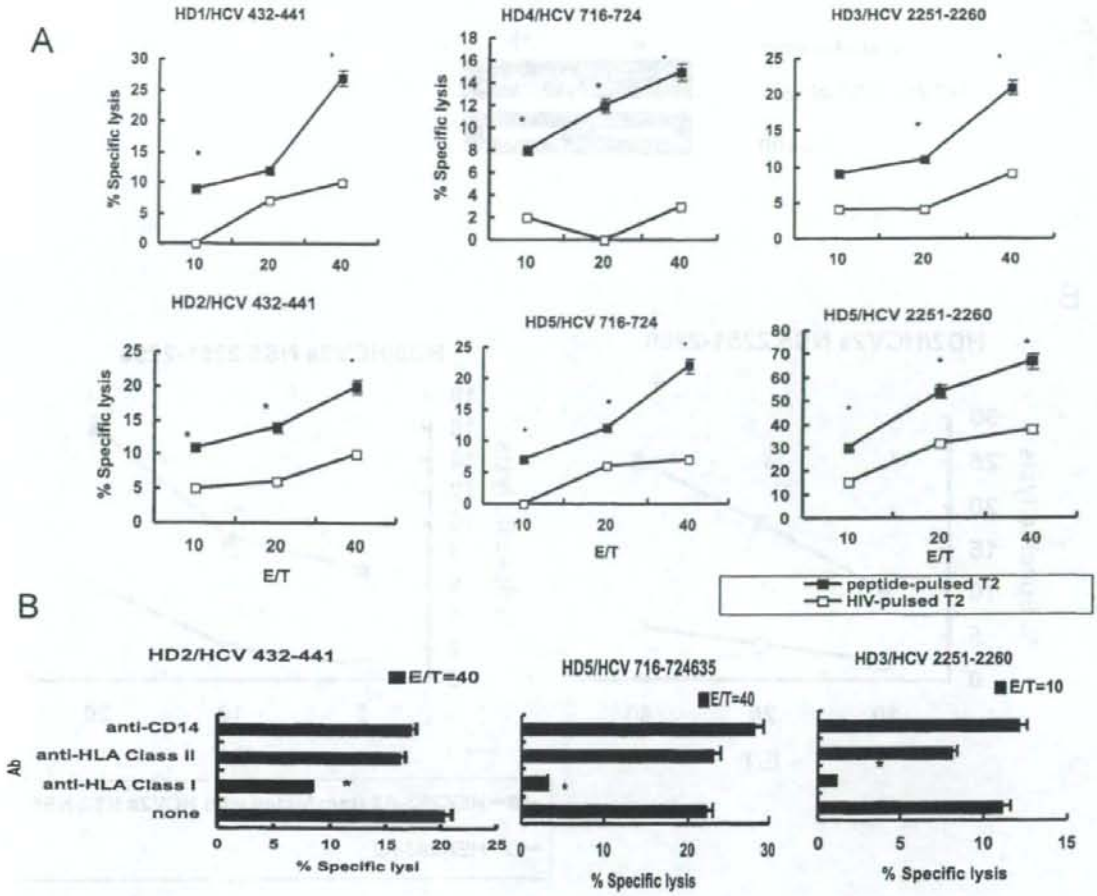

Fig. 2. Cytotoxicity of HCV2a peptide-specific CTLs induced from healthy donors. A: PBMCs of healthy donors were stimulated *in vitro* with HCV2a peptide-loaded autologous DC, and their cytotoxicity was examined by a 6-hr ^{51}Cr-release assay. T2 cells pulsed with the corresponding HCV2a peptide or the control HIV-peptide were used as a target. B: $CD8^+$ cells purified from the HCV2a peptide-stimulated PBMCs of healthy donors were tested for their cytotoxicity against corresponding HCV2a peptide-pulsed T2 cells in the presence of the indicated mAb. Representative results in three experiments are shown. Values represent the mean of triplicate determinations, and statistical analysis was performed by a two-tailed Student's *t*-test (*$P<0.05$).

35–44 peptide induced specific CTLs from the PBMCs of HCV1b-infected patients (26). In this study, the HCV2a 35–44 peptide, which has the same amino acid sequence as HCV1b 35–44, was also found to be immunogenic in some patients, but the rate of successful CTL induction was less than 50%.

We wanted to be sure that the CTLs induced by repetitive stimulation with the synthetic peptides actually recognized the endogenously processed peptides, because there are well-established cases of peptides that stimulate CTL responses *in vitro* but are not generated and presented in cells producing the antigen (4). We confirmed that the NS5 2251–2260 peptide-induced CTLs recognized the endogenously processed peptide expressed on NS3–NS5 gene transfectants. It remains to be determined whether the CTLs specific to the HCV2a 432–441 or the HCV2a 716–724 peptide lysed the HCV2a E2-expressing target cells by using the HCV2a E2 gene. Cerny et al. reported that the HCV1a-derived peptide HCV1a 2252–2260 (ILDSFDPLV), which differs from the present HCV2a 2251–2260 (VVLDSLDPMV) peptide by two amino acids, induced CTLs from PBMCs of HCV1a-infected patients and healthy donors after *in vitro* stimulation (6). In addition, the $CD8^+$ T cell response directed against this epitope in patients with acute hepatitis C

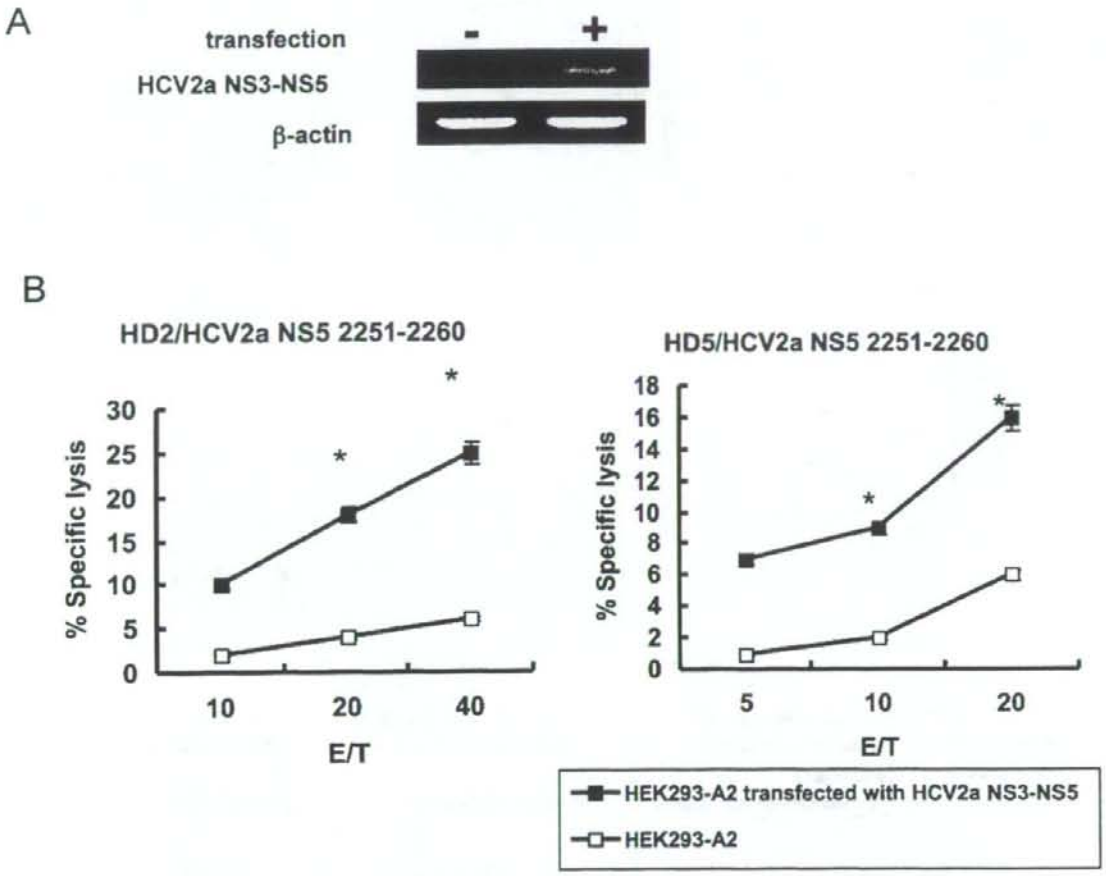

Fig. 3. Recognition of endogenously processed HCV2a peptide by HCV2a peptide-induced CTLs. A: The expression of *HCV2a NS3–NS5* mRNA in the HCV2a NS3–NS5-transfectant and parental HEK293-A2 cells was examined by RT-PCR. β-Actin was used as a control. B: The cytotoxicity of HCV2a NS5 2251–2260 peptide-specific CTLs from two healthy donors against *HCV2a NS3–NS5*-transfected HEK293-A2 cells was examined by a 6-hr ^{51}Cr-release assay. Representative results in three experiments are shown. Values represent the mean of triplicate determinations, and statistical analysis was performed by the two-tailed Student's *t*-test (*$P<0.05$).

correlates with termination of the disease (10). The effect of HCV2a 2251–2260 peptide-specific CTL activity on the viral clearance or antiviral therapy response remains to be investigated in a future study.

In conclusion, our present study indicates that the HCV2a 432–441, HCV2a 716–724, and HCV2a 2251–2260 peptides are new HLA-A2-restricted CTL epitopes capable of inducing peptide-specific CTLs *in vitro*. If the prevalence of HCV2a in the entire genotypes of HCV-infected people is estimated 10%, 18 million people are infected with HCV2a in the world. In addition, the HLA-A2 is the most frequent allele in the world. Therefore, the identification of HCV2a 432–441, HCV2a 716–724, and HCV2a 2251–2260 peptides would contribute substantially to the development of peptide-based vaccines for HCV-infected patients.

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Branched-Chain Amino Acid Supplementation Complements Conventional Treatment for Spontaneous Bacterial Peritonitis

EITARO TANIGUCHI, MD, PhD,† TAKUMI KAWAGUCHI, MD, PhD,* MARI SHIMADA, MD,† REIICHIRO KUWAHARA, MD, PhD,† YUMIKO NAGAO, MD, DDS,* MOMOKA OTSUKA, Dietitian,† SHOKO IWASAKI, Dietitian,† TOKIKO MATSUDA, Nrs,† RYOKO IBI, Nrs,† SATOMI SHIRAISHI, Nrs,† MINORU ITOU, MD,† TETSUHARU ORIISHI, MD,† RYUKICHI KUMASHIRO, MD, PhD,† SHOKO TANAKA, Dietitian,† YUKO SARUWATARI, Nrs,† and MICHIO SATA, MD*

KEY WORDS: nutrition; liver failure; late evening snack; hepatic fibrosis; hepatocyte growth factor.

Spontaneous bacterial peritonitis (SBP) is a life-threatening complication of liver cirrhosis (1). The prevalence of SBP among hospitalized cirrhotic patients with ascites has been estimated to be approximately 15% (2). SBP in cirrhotic patients is thought to occur as a consequence of impaired defensive mechanisms against infection, such as a decrease in the lymphocyte count and an impaired phagocytic function of neutrophils (3).

Mortality rate related to SBP has been improved by the development of new antibiotics (4); however, the rate is still 17–50% (2). Branched-chain amino acids (BCAA) supplementation improves not only the nutritional and metabolic status such as serum albumin concentration and Fisher's ratio, but also defensive mechanisms against infection in cirrhotic patients (5). Strengthening of resistance to infection is believed to be the result of the elevation of the absolute lymphocyte count (6). Moreover, a recent study disclosed that BCAA supplementation improves phagocytic function of neutrophils in cirrhotic patients (7). Although BCAA supplementation seems to be an effective therapy in patients with SBP, the beneficial effects of BCAA supplementation have never been reported in this regard.

In this report, we present the first documented case showing that BCAA supplementation complements conventional treatment for SBP and subsequent liver failure. BCAA supplementation should be considered as a complementary treatment for patients with SBP.

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From the *Department of Digestive Disease Information and Research and †Nutritional Curative Team and Second Department of Medicine, Kurume University School of Medicine, Kurume, Japan.

Address for reprint requests: Eitaro Taniguchi, MD, PhD, Second Department of Medicine, Kurume University School of Medicine, 67 Asahi-machi, Kurume 830-0011, Japan; eitaro@med.kurume-u.ac.jp.

CASE REPORT

A 71-year-old Japanese woman was referred to Kurume University Hospital for abdominal pain. Chronic hepatitis C was diagnosed when the patient was 54 years old. Despite various treatments including interferon, liver cirrhosis developed when the patient was 66 years old. Ascites was evident 2 months before the patient presented with abdominal pain.

Physical examination showed a height of 152 cm and a weight of 51.7 kg. Vital signs were within normal range except for her body temperature (BT) of 37.2 °C. The patient had icteric pigmentation of the sclera and skin, tense ascites, and peripheral edema. Laboratory data on admission are summarized in Table 1. Briefly, liver failure with inflammation was indicated. In the patient's ascitic fluid, the protein concentration was 0.29 g/dL and the polymorphonuclear count was 837/mm^3; few red blood cells and no malignant cells were seen. Abdominal x-ray showed no free air. Abdominal ultrasound examination showed no findings suggesting acute pancreatitis, tumor, or hemorrhage in the abdominal organs. Although no pathogenic organisms could be isolated from the ascites or peripheral blood, the patient exhibited abdominal pain and fever, a polymorphonuclear count >500/mm^3 in the ascitic fluid, and an absence of clinical, laboratory, radiologic, and ultrasound findings suggesting secondary peritonitis. Therefore, a diagnosis of SBP with liver failure was established.

SBP was immediately treated with a third-generation cephalosporin (sulbactam/cefoperaazone; Figure 1). Infusions of albumin were also administered to reduce the risk of renal failure and disseminated intravascular coagulation. At first, the antibiotic treatment seemed to be effective because the serum CRP concentration gradually decreased; however, the patient exhibited persistent fever and abdominal pain. The antibiotic was then

TABLE 1. LABORATORY DATA ON ADMISSION

Hematology		Glucose	84 mg/dL
WBC	5,600/mm^3	BUN	21.7 mg/dL
Neutrocytes	68.5%	Creatinine	0.66 mg/dL
Lymphocytes	23.5%	Na^+	143 mEq/L
RBC	361 × 10^4 mm^3	K^+	4.5 mEq/L
Platelets	3.4 × 10^4 mm^3	Cl^-	107 mEq/L
Biochemical examination		Prothrombin time	55%
AST	142 U/L	Endotoxin	<0.4 pg/mL
ALT	132 U/L	Blood culture	negative
LDH	494 U/L	**Ascitic fluid examination**	
Total protein	5.54 g/dL	Gross appearance	turbid
Albumin	2.7 g/dL	Protein	0.29 g/dL
Total bilirubin	3.74 mg/dL	WBC	1922/ mm^3
Direct bilirubin	1.10 mg/dL	Polymorphonuclear cells	837/ mm^3
CRP	7.15 mg/dL	culture	negative

changed to a carbapenem (imipenem/cilastatin) and infusions of γ-globulin were also administered. To improve the patient's nutritional status and resistance to infection, 50 g of Aminoleban EN containing abundant BCAA with 210 kcal energy (Table 2) was given as a late evening snack, although no findings of hepatic encephalopathy were seen. We also treated a decayed tooth, because it could have been an origin for elevated CRP concentration. With these treatments, abdominal pain disappeared, and BT and serum CRP concentration gradually decreased. However, BT and CRP concentration were not fully normalized, and a subsequent increase in serum total bilirubin concentration and a decrease in prothrombin activity occurred, indicating development of severe liver failure.

The patient's required energy expenditure was estimated to be 1700–1842 kcal/d based on the Harris-Benedict formula (8). Although the patient seemed to have an adequate dietary caloric intake with the BCAA supplementation, the serum concentration of free fatty acids showed 1531 μEq/L (normal range,

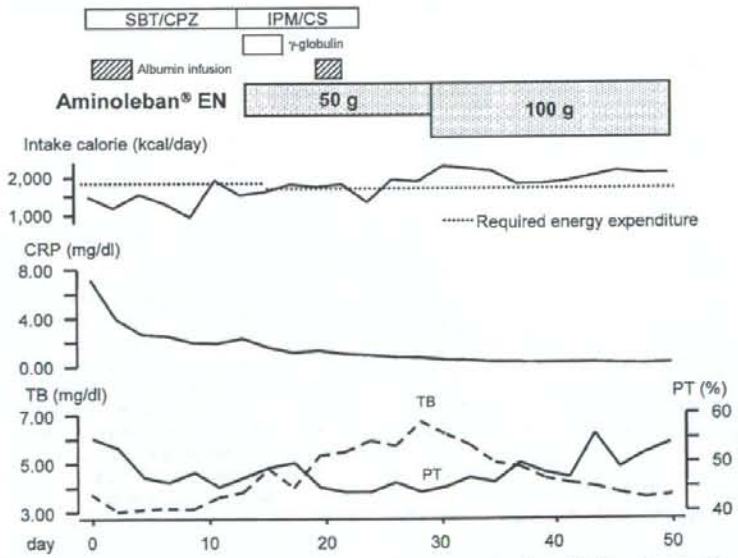

Fig 1. Laboratory indices over the days of admission. By treatment with antibiotics and γ-globulin infusions, CRP concentration gradually decreased. However, CRP concentration was not fully normalized. Total bilirubin reachd peak value and prothrombin activity reached its nadir on day 29. There was a dramatic response to additional administration of BCAA supplementation (total 100 g), with decrease in total bilirubin concentration and increase in prothrombin activity. CRP concentration returned to normal. *Abbreviations:* SBT/CPZ, sulbactam/cefoperazone; IPM/CS, imipenem/cilastatin; TB, total bilirubin; PT, prothrombin activity.

TABLE 2. COMPOSITION OF AMINOLEBAN EN (PER 50 G)

Total energy	210 kcal	Histidine	0.27 g
Protein	13.5 g	Proline	0.98 g
Amino acids		Serine	0.24 g
(Fischer's ratio = 38)			
Valine	1.79 g	Tyrosine	0.05 g
Leucine	2.25 g	Lysine	0.60 g
Isoleucine	2.04 g	Aspartic acid	0.45 g
Threonine	0.29 g	Glutamic acid	0.85 g
Tryptophan	0.08 g	Glycine	1.74 g
Methionine	0.06 g	Fat (rice oil)	3.50 g
Phenylalanine	0.17 g	Carbohydrates (Dextrin)	31.05 g
Alanine	0.75 g	Vitamins	
Arginine	0.88 g	Minerals	

Note. Vitamins include trace amounts of magnesium sulphate, calcium glycerophosphate, potassium iodide, potassium chloride, sodium dihydrogen phosphate dihydrate, sodium ferrous citrate, cupric sulphate, zinc sulphate, and manganese sulphate. Minerals include retinol palmitate, ergocalciferol, bisbentiamine, riboflavin, pyridoxine HCl, cyanocobalamin, folic acid, sodium l-ascorbate, tocopherol acetate, phytonadione, calcium pantothenate, nicotinamide, and biotin.

100–540 μEq/L), suggesting starvation. Moreover prothrombin activity was only 44% of normal, indicating impaired protein synthesis. We therefore administered additional BCAA supplementation after breakfast to improve the protein–energy malnutrition condition.

This action resulted in a dramatic decrease of total bilirubin concentration and increased prothrombin activity as well as the disappearance of ascites and peripheral edema (see Figure 1). Moreover, BT and CRP concentration were normalized with BCAA supplementation without the use of antibiotics. Simultaneously, increases in total lymphocyte count and serum hepatocyte growth factor (HGF) concentration were seen (Figure 2). Thus, SBP and subsequent liver failure were successfully managed.

We continued the BCAA supplementation. At 3 months' follow-up, the patient did not show any evidence of SBP or liver failure, and the serum concentrations of hyaluronic acid and type IV collagen were decreased, suggesting improvement of hepatic fibrosis (Figure 3).

DISCUSSION

This represent the first description of a case showing BCAA supplementation complementing conventional treatment of SBP. Thus, the use of BCAA supplementation as add-on therapy to antibiotics appears to reduce the risk of mortality in patients with SBP.

Although recent advances in the diagnosis and treatment of bacterial infections have improved the prognosis in patients with SBP (9), SBP accompanying severe liver failure continues to have a poor prognosis. Tito *et al.* reported that predictive factors for poor prognosis of SBP patients were serum bilirubin >4 mg/dL, prothrombin activity ≤45%, and protein concentration in ascitic fluid ≤1 g/dL (1). The patient in the current report demonstrated even poorer values for all of the predictive factors; therefore, poor prognosis was anticipated.

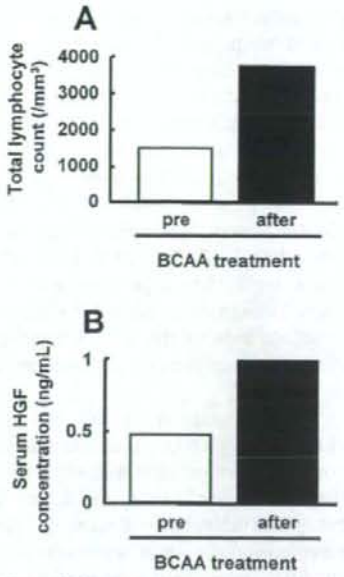

Fig 2. The effects of BCAA supplementation on total lymphocyte count (A) and serum HGF concentration (B). Total lymphocyte count and serum HGF concentration were increased by administration of BCAA supplementation.

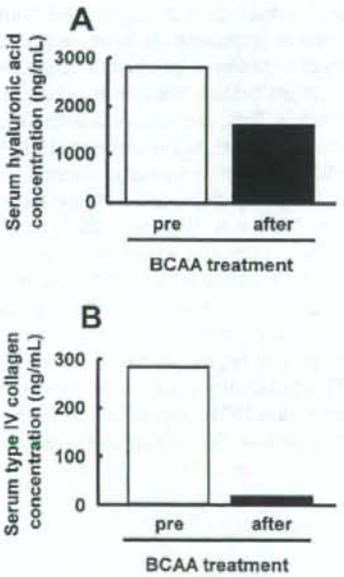

Fig 3. The effects of BCAA supplementation on serum concentrations of hyaluronic acid (A) and type IV collagen (B). Serum concentrations of hyaluronic acid and type IV collagen were decreased by administration of BCAA over 3 months.

After the administration of antibiotics and BCAA supplementation in our patient, abdominal pain disappeared and BT and CRP concentration normalized. Carbapenem and γ-globulin played a significant role in suppression of the bacterial infection; however, BT and CRP concentration did not revert to the normal range. BCAA administration has been shown to improve immune function parameters, for example, the elevation of total lymphocyte count (6). In our patient, total lymphocyte count was increased from 1450–3740/μL. Furthermore, BCAA supplementation is reported to improve phagocytic function of neutrophils in cirrhotic patients (7). Taken together, these observations indicate that BCAA supplementation the the potential to complement conventional treatment for patients with SBP.

Given a similar situation as that of our patient, one would most likely choose total parenteral nutrition rather than enteral nutrition. However, additional bacterial translocation may occurr because the defensive mechanisms of gut against infection are weakened. This suggests that total parenteral nutrition may worsen the prognosis for patients with SBP. Therefore, it is recommended that these patients be treated via enteral nutrition.

Because our patient appeared to suffer from both of starvation and protein synthesis, additional BCAA supplementation was given to improve protein–energy malnutrition. The administration of additional BCAA supplementation caused a dramatic decrease of total bilirubin concentration and an increase in prothrombin activity. BCAA supplementation has been reported to have various pharmacologic effects besides being an essential substrate for protein synthesis. Tomiya *et al.* reported that BCAA, especially leucine, stimulate the production of HGF by hepatic stellate cells (10). HGF stimulates proliferation of hepatocytes and bile duct epithelial cells (10) and facilitates liver regeneration. Moreover, HGF prevents hepatic fibrogenesis. Therefore, a possible explanation for the recovery from severe liver failure is that BCAA supplementation leads to an increase in HGF production by hepatic stellate cells and subsequently improves liver failure through promotion of liver regeneration and inhibition of fibrogenesis. This possibility is supported by observations in our patient: serum HGF concentration was increased after administration of BCAA supplementation and serum hyaluronic acid and type IV collagen concentrations, both indicators of hepatic fibrosis, were decreased.

In conclusion, we report a case herein, where BCAA supplementation complemented conventional treatment for SBP and subsequent liver failure. BCAA supplementation should be considered as complementary therapy for patients with SBP.

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Case Report

Kurume Medical Journal, 53, 41-46, 2006

Appearance-specific Satiety Increases Appetite and Quality of Life in Patients with Metastatic Liver Tumor: A Case Report

TAKUMI KAWAGUCHI*,**, EITARO TANIGUCHI**, MINORU ITOU**, JYUNJI AKIYOSHI**, SATOSHI ITANO**, MOMOKA OTSUKA†, SHOKO IWASAKI†, TOKIKO MATSUDA‡, RYOKO IBI‡, SATOMI SHIRAISHI‡, TETSUHARU ORIISHI**, SHOKO TANAKA†, YUKO SARUWATARI‡ AND MICHIO SATA*,**

Departments of Digestive Disease Information & Research and Medicine**, Kurume University School of Medicine, Departments of Nutrition†, Nursing‡, Kurume University Hospital, Kurume 830-0011, Japan*

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Summary: Loss of appetite is frequently seen and is a main factor affecting quality of life (QOL) in patients with advanced cancer. The etiology for loss of appetite in patients with cancer is multifactorial. The sensory properties of food are factors regulating appetite. Changes in taste, smell and texture of foods influence food intake. The appearance of the food is also a notable factor in sensory-specific satiety. We described a 46-year-old Japanese woman with multiple metastatic liver tumors. Although there was no obvious factor for loss of appetite, she suffered from a loss of appetite and subsequent malnutrition. In order to improve the appearance of food, we reduced the diet to 1000 kcal/day from 1500 kcal/day. On the new diet, the patient's appetite significantly increased and patient's nutritional status was improved. Eating whole diet was an important achievement and increased in mental aspects of QOL. Arrangement for the appearance of food may be a simple and nontoxic therapeutic strategy for patients with cancer suffering a loss of appetite.

Key words advanced cancer, malnutrition, sensory-specific satiety, anorexia, nutritional status, SF-36

INTRODUCTION

Patients with advanced cancer often suffer from a loss of appetite, leading to malnutrition [1]. As many as 20% of patients with cancer die of the effects of malnutrition rather than of the malignancy [2]. Loss of appetite is also a main factor affecting quality of life (QOL). Thus, maintaining appetite is a valuable part of managing patients with advanced cancer.

The etiology for loss of appetite in patients with cancer is multifactorial [3]. Loss of appetite can result from systemic effects of cachexia and altered metabolism by tumor. Local effects of tumor, such as obstruction and pain, can also interfere with dietary intake. Furthermore, treatment for cancer may cause fatigue, taste changes, diarrhea, and nausea, which lead to a subsequent loss of appetite [4,5].

Currently prescribed appetite stimulatory drugs are corticosteroids, megestrol acetate, and metoclopramide. Although corticosteroids may increase appetite, their metabolic, infectious, and psychiatric side effects usually limit their use to the short term. Megestrol acetate has been found to improve appetite [6], but is associated with water retention and an increase in the risk of venous thromboembolism. Metoclopramide has been shown to improve chronic nausea [7], but it has no proven effect on appetite. Clearly, many patients are not helped by or cannot tolerate currently available treatments to stimulate appetite. A simple and nontoxic therapeutic strategy

Corresponding author: Takumi Kawaguchi, MD., PhD, Department of Digestive Disease Information & Research, Kurume University School of Medicine, 67 Asahi-machi, Kurume 830-0011, Japan. Tel: +81-942-31-7902 Fax: +81-942-31-7747 E-mail: takumi@med.kurume-u.ac.jp

Abbreviations: MH, mental health; QOL, quality of life; RE, role-emotional; SCC, squamous cell carcinoma; SF, social functioning; SF-36v2, medical outcomes study 36-item short-form health survey version 2; VT, vitality.

for increasing appetite is needed.

The sensory properties of food regulate appetite. Several studies have been carried out to investigate the impact of food varying in sensory properties. Changes in taste, smell, and texture of foods can influence food intake [8,9]. An increase in the amount of pasta offered affects its' appearance, resulting in a decrease in the pleasure of eating [8]. The appearance of the food seems to be a notable factor in sensory-specific satiety. In other words, the appearance of food may influence appetite through visual information.

Patients with cancer are usually prescribed a high-calorie diet because cancer increases energy expenditure. High-calorie diet is accompanied by heavy appearance, which can provide a visual stress leading to a decrease in appetite. It is possible that the appearance of the food in a high-calorie diet spoils appetite in patients with advanced cancer.

In this report, we describe a case in which the appearance-specific satiety of food increased appetite and improved nutritional status and QOL as well. Arrangement for appearance of food may be a simple and nontoxic therapeutic strategy for patients with cancer suffering a loss of appetite.

CASE REPORT

A 46-year-old Japanese woman was referred to Kurume University Hospital for treatment of multiple metastatic liver tumors and obstructive jaundice. The patient was diagnosed at age 44 with cervical cancer and was treated with radiation and chemotherapy following a radical hysterectomy. Two years later, computed tomography revealed multiple hepatic metastases due to cervical cancer and a rapid growth of metastatic liver tumors. These tumors were treated with a continuous intrahepatic arterial injection of 5-fluorouracil (375 mg/day) and intermittent cisplatin (30 mg/day) via a reservoir system for 5 days [10]. With 2 periods of this regimen, the serum squamous cell carcinoma (SCC) antigen level (normal range $<$ 1.5 ng/ml) decreased from 119.4 ng/ml to 30.1 ng/ml. Metastatic liver tumors were responsive to the chemotherapy.

The patient did not show any troublesome adverse effects of the anticancer drugs administered, such as vomiting and the patient's liver function was preserved, however, she suffered from loss of appetite 2 weeks before admission, which resulted in 4 kg loss of body weight. The patient's body mass index, arm muscle circumference, and triceps skin-fold thickness decreased to 14.6 kg/m^2, 16.3 cm, and 8 mm, respectively. The percentage of the age-adjusted standard value for Japanese women was 65.3%, 80.5%, and 47%, respectively. Laboratory data showed decrease in total lymphocyte count and levels of hemoglobin and albumin (Table 1). Thus, physical examination and laboratory data indicated a state of severe malnutrition.

TABLE 1.
Characteristics of the patient on admission

	Value	% of standard value
Physiological measurements		
Height	158.5 cm	101.9%
Weight	36.5 kg	67.9%
Body mass index	14.5	65.3%
Arm muscle circumference	16.3 cm	80.5%
Triceps skin-fold thickness	8 mm	47.0%
Loss of body weight in 2 weeks	4 kg	
Laboratory data		(Normal range)
Hemoglobin	7.0 g/100ml	(11-15)
Total lymphocyte count	435/μl	(1200-3870)
Albumin	3.2 g/dl	(4.0-5.0)
Total cholesterol	246 mg/dl	(128-256)
Glucose	86 mg/dl	(80-112)
Aspartate aminotransferase (U/l)	22 U/l	(13-33)
Alanine aminotransaminase (U/l)	34 U/l	(6-27)
Total bilirubin (mg/dl)	2.2 mg/dl	(0.3-1.5)
Prothrombin time	96%	(60-130)

Note. Standard values of physiological measurements were referred to Japanese anthropometric reference data

Acute bowel toxicity is one of the common complications of chemotherapy that leads to malnutrition. Oral administration of glutamine reduces this anticancer drug-induced bowel toxicity. The patient was treated with glutamine via oral administration, however, her appetite did not increase. Granisetron is effective against nausea induced by anticancer drugs. Nevertheless, loss of appetite persisted after the patient received an infusion of 3 mg of granisetron. Corticosteroids and megestrol acetate are also known to increase appetite in patients with advanced cancer. On the other hand, the long term use of these agents is associated with decreased efficacy and increasingly unacceptable adverse effects. Therefore, corticosteroids and megestrol acetate were not used in our case.

The patient's energy requirement was estimated to be 1400 to 1600 kcal/day, based on the Harris-Benedict equation [11]. Accordingly, the patient was prescribed a diet of 2000 or 1500 kcal/day. She understood the importance of nutritional therapy, however, her actual intake was only about 600 kcal/day. The sensory properties of the food play important roles in controlling the patient's food intake. Appearance is one of the important sensory properties and heavy appearance of food spoils appetite. In order to improve the appearance of food, we reduced the diet to 1000 kcal/day (Fig. 1). On the new diet, the patient's appetite significantly increased and she ate not only the prescribed diet but also snack. She maintained an intake of more than 1000 kcal/day (Figs 2A and B). Then, we followed her nutritional status until Day 76. Although total lymphocyte count showed no change on the new diet, there was a significant increase in serum albumin level (Figs 3A and B).

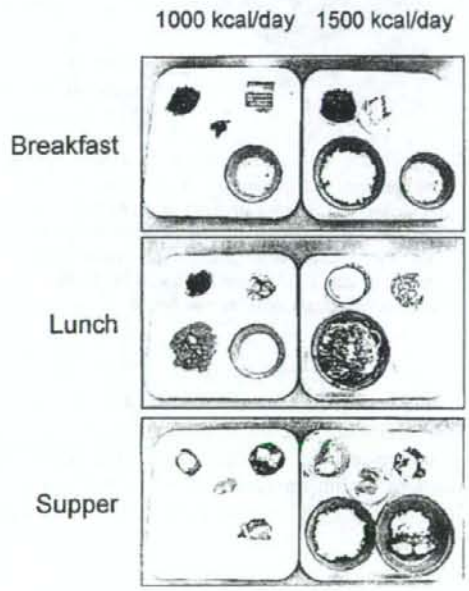


Fig. 1. Appearance of food in the 1000 kcal diet (left) and the 1500 kcal diet (right). A nutrient balance of 1000 kcal diet is similar to that of 1500 kcal diet.

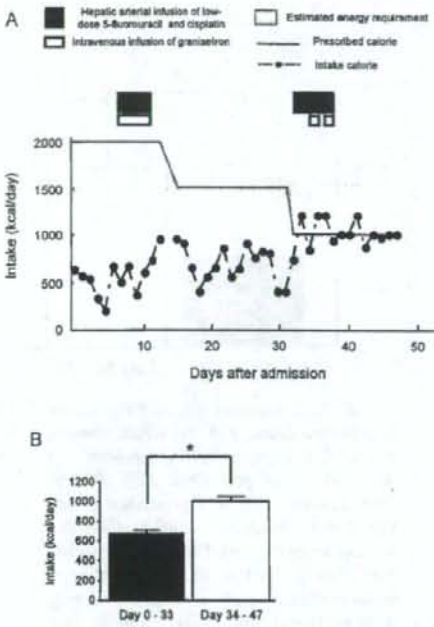


Fig. 2. (A) Time course of daily caloric intake. Metastatic liver tumors were treated with a continuous intrahepatic arterial injection of 5-fluorouracil (375 mg/day) and intermittent cisplatin (30 mg/day) via a reservoir system (■). Granisetron (3 mg) was administrated by intravenous infusion at 30 min before chemotherapy (□). The patient's energy requirement was estimated to be 1400 to 1600 kcal/day, based on the Harris-Benedict equation. (B) Caloric intake after improvement of the appearance of food in the prescribed diet. 2000 or 1500 kcal/day diet was prescribed during Day 0 to 33 (n=34; black bar). 1000 kcal/day diet was prescribed during Day 34 to 47 (n=14; white bar). Caloric intake was evaluated everyday and changes in caloric intake were statistically analyzed by the Mann-Whitney *U* test. *$P<0.01$.

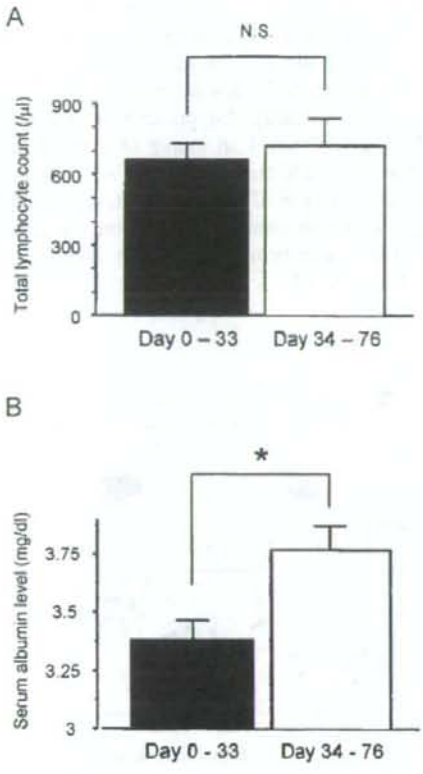
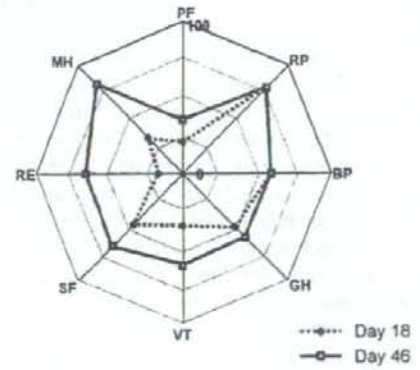


Fig. 3. Long-term effects on (A) total lymphocyte count, and (B) serum albumin levels after improvement of appearance of the food in the prescribed diet. 2000 or 1500 kcal/day diet was prescribed during Day 0 to 33 (black bar). 1000 kcal/day diet was prescribed during Day 34 to 76 (white bar). During Day 0 to 33, total lymphocyte count (n=5) and serum albumin levels (n=5) were measured. During Day 34 to 76, total lymphocyte count (n=4) and serum albumin levels (n=5) were measured. Changes in total lymphocyte count and serum albumin were statistically analyzed by the Mann-Whitney *U* test. N.S.: not significant. *P<0.05.



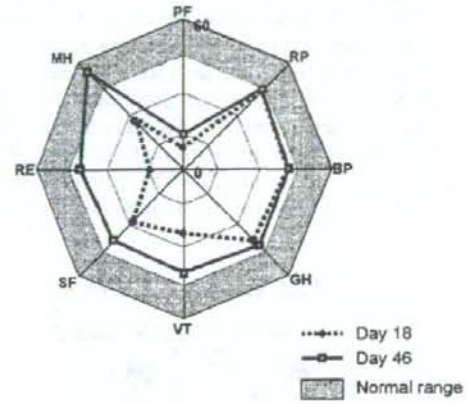


Fig. 4. Changes in SF-36v2 scores after improvement of appearance of the food in the prescribed diet. (A) actual score on a scale of 0 to 100. (B) Norm-based score. PF: physical functioning; RP: role-physical; BP: bodily pain; GH: general health; VT: vitality; SF: social functioning; RE: role-emotional; MH: mental health.

We also evaluated the effects of appearance-specific satiety on the patient's QOL by using the Medical Outcomes Study 36-Item Short-Form Health Survey version 2 (SF-36v2) on Day 18 and 46. By improvement in appearance of food, her scores for vitality (VT), mental health (MH), role-emotional (RE), and social functioning (SF) were markedly increased on a scale of 0 to 100 (Fig. 4A). In particular, the MH score reached to normal range (Fig. 4B). Appearance-specific satiety stimulated appetite and resulted in the improvement of nutritional status as well as in the QOL in this patient with metastatic liver tumors.

DISCUSSION

In this study, we describe a case in which the appearance-specific satiety of food increased appetite

and improved both nutritional status and QOL. Improvement in the sensory properties of food, including appearance, is a simple and nontoxic strategy to increase appetite and QOL in patients with advanced cancer suffering from a loss of appetite.

Loss of appetite is frequently associated with advanced cancer and its treatment. Multiple, interactive factors that are the probable cause of loss of appetite include direct interference of tumors with food intake, malabsorption and poor digestion, and treatment-related complications such as changes in smell and taste. No effective therapy has been established previously for improvement of anorexia in patients with advanced cancer. A simple nontoxic means to improve anorexia would be most benefical in the supportive management of the cancer patient and also could possibly enhance the effectiveness of other therapeutic measures.

Sensory-specific satiety has an important influence on the amount of food eaten [12]. Invariable foods decrease the pleasure of eating even though the food are savory [13-16]. The same phenomenon occurs when eating a sweet food to satiety [13-15] or when drinking a weak-smelling tea compared to a strong-smelling tea [17]. On the other hand, varying the texture of the yogurt in the diet caused a 12.6% elevation of intake compared to the amount of the previously preferred yogurt eaten [14]. Taste, smell, and texture-sepcific satieties are important for regulation of appetite.

Appearance of food is also an important factor involved in sensory-specific satiety. Cancer increases energy expenditure and patients with cancer are normally prescribed a high-calorie diet with heavy appearance of food. In fact, our patient was prescribed a diet of 2000 or 1500 kcal/day, but achieved a caloric intake of only about 600 kcal/day. Since there was no obvious reason for loss of appetite, we assumed that heavy appearance of food spoiled her appetite. Although she was in a state of malnutrition, we reduced her diet to 1000 kcal/day in order to improve appearance of food. In results, the patient's appetite was significantly increased. One would think that chemotherapy itself increased appetite through improvement in cachexia, however, she complained of persistent appetite loss after tumors showed partial remission. On the other hand, Marcelino et al. [18] reported that the desire to eat pizza depended on the visual quality of the pizza. Appearance-specific satiety is also related to areas of the brain that control motivation and the reward value of foods [19-21]. These reports suggests appearance-specific satiety stimulated appetite in our case.

Long-term effects of appearance-specific satiety on the patient's nutritional status was evaluated by measureing total lymphocyte count, an indicator of visceral proteins, and serum albumin levels. We must be cautious in the interpretation of these results because there was no time-couse study in lymphocyte count and albumin levels. Alhough total lymphocyte count did not change, serum albumin levels were significantly increased. The discrepancy between total lymphocyte count and serum albumin levels may be due to adverse effects of chemotherapy. Bone marrow suppression is observed during the chemotherapy which is same regimen used in this case [22] and total lymphocyte count might not reflect the nutritional status adequately in our case. Serum albumin level is a standard marker for evaluation of nutritional status and is not influenced by anti-cancer drug itself. Since serum albumin levels were significantly increased, it is possible that appearance-specific satiety improved long-term status of nutrition in our case.

Appearance-specific satiety increased the scores of SF, ME, RE, and VT of the SF-36 score, which is widely used for evaluating QOL [23,24]. Although it was not clear how appearance-specific satiety increased QOL, following possibilities are exist. Increase in appetite improved the nutritional status. Nutritional status is closely related to liveliness, therefore, improvement of nutritional status may increase in lively aspect of QOL, such as VT. Alternatively, the patient could eat prescribed whole diet by changing the appearance of diet. Eating whole diet was an important achievement and it gave her great pleasure that caused an increase in mental aspects of QOL, such as MH, RE, and SF. Thus, appearance-specific satiety may increase QOL by improvement of lively and mental aspects in our case.

In conclusion, we report here a case in which appearance-specific satiety increased appetite and improved both nutritional status and QOL in patient with advanced cancer. Arrangement for the appearance of food in a diet may be a simple and nontoxic therapeutic strategy for patients who suffered from loss of appetite.

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A Decrease in AFP Level Related to Administration of Interferon in Patients with Chronic Hepatitis C and a High Level of AFP

SHIRO MURASHIMA, MD, PhD, MASATOSHI TANAKA, MD, PhD, MAKOTO HARAMAKI, MD, PhD, SHIGERU YUTANI, MD, PhD, YUTAKA NAKASHIMA, MD, PhD, KAZUNORI HARADA, MD, PhD, TATSUYA IDE, MD, PhD, RYUKICHI KUMASHIRO, MD, PhD, and MICHIO SATA, MD, PhD

It is known that there is a very high incidence of hepatocellular carcinoma (HCC) among patients with type C chronic hepatitis and cirrhosis, and α-fetoprotein (AFP) has been widely used as a diagnostic marker for HCC. However, there are some patients showing continuous high AFP values but no evidence of HCC, and some studies have defined such patients as a high-risk group for HCC. In vitro study has shown that interferon (IFN) inhibits cell proliferation and enhances apoptosis as well as specific cytotoxic T lymphocytes against HCC, resulting in direct anticancer actions. In this study, we investigated the effect of IFN on AFP changes in chronic hepatitis C patients. Of 40 patients with chronic hepatitis C in whom diagnostic imaging confirmed the absence of HCC, 24 patients showed high pretreatment AFP values (high AFP group: AFP level > 10 ng/dl; mean ± SD, 46.3 ± 41.5 ng/dl) and 16 showed low pretreatment AFP values (low AFP group: pretreatment AFP level ≤ 10 ng/dl; mean ± SD, 5.3 ± 2.2 ng/dl). Pretreatment clinical parameters were statistically evaluated in relation to the AFP value. In the high AFP group, the platelet count, albumin level, and prothrombin (%) were significantly lower ($P = 0.047$, $P = 0.0002$, and $P = 0.044$, respectively), suggesting that AFP value increases with advancing liver disease. Subsequently 27 patients were administered IFN (IFN group), and the remaining 13 patients were administered Stronger Neo-minophagen C (SNMC), a glycyrrhizin preparation (SNMC group), as a control group receiving liver-protective therapy. Alanine aminotransferase was reduced in both the IFN and the SNMC group (mean, 132.56 to 60.07 mg/ml [$P < 0001$] and 147.85 to 56.23 mg/ml [$P = 0.0240$], respectively). AFP was significantly reduced in the IFN group (mean, 30.03 to 12.65 ng/ml; $P = 0.0034$), but there was no significant change in AFP in the SNMC group (mean, 29.70 to 39.17 ng/ml). AFP is useful for diagnosing HCC; however, some patients show a persistently high AFP level in the absence of HCC, and these patients have been described as a high-risk group for HCC. In this study, we found that IFN therapy but not SNMC universally reduced the AFP baseline. Since AFP is a significant predictor for HCC, therapeutic strategies for hepatitis C, e.g., long-term low-dose IFN treatment, may reduce hepatocarcinogenesis.

KEY WORDS: hepatitis C; interferons; hepatocellular carcinoma; α-fetoprotein.

Recently, combination therapy with pegylated interferon (IFN) and ribavirin for 48 weeks has achieved viral eradication in 54 to 56% of patients, and the occurrence of hepatocellular carcinoma (HCC) was prevented in these responders (1, 2). For nonresponders to IFN therapy, liver-protective therapy, such as oral administration of

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From the Department of Internal Medicine, Kurume Medical Center, Kurume, Fukuoka, Japan.
Address for reprint requests: Shiro Murashima, MD, PhD, Department of Internal Medicine, Kurume Medical Center, 155-1 Kukubu-machi, Kurume, Fukuoka 839-0863, Japan; muracy@h3.dion.ne.jp.

ursodeoxycholic acid or intravenous injection of Stronger Neo-minophagen C (SNMC), is commonly performed in Japan, and it is considered that these treatments may delay the progression of liver disease (3, 4). SNMC is a glycyrrhizin preparation that exhibits potent anti-inflammatory actions and has been used to treat allergic diseases and hepatitis in Japan for centuries. However, this agent is not considered to have any antiviral or anticancer ability (5), while IFN is considered to have antiviral, anti-inflammatory, and anticancer effects, and is employed in clinical practice to treat certain types of cancer, such as germ cell tumor and RCC (6, 7).

α-Fetoprotein (AFP) has been widely used as a diagnostic marker for HCC. However, there are some patients with a high AFP baseline but no evidence of HCC, although some papers have reported that AFP is a significant predictor of HCC in such patients (8, 9). This study investigated the clinical characteristics of such patients with a high AFP baseline and assessed the effect of IFN administration in terms of AFP changes, since AFP is suggested to be an important risk factor for HCC.

METHODS

Forty patients with type C chronic hepatitis and compensatory liver cirrhosis patients who were being followed at Kurume University Medical Center were retrospectively investigated. All patients were confirmed to be positive for serum hepatitis C virus (HCV)-RNA by polymerase chain reaction (PCR). HBs-Ag-positive, autoimmune, alcoholic, and drug-induced hepatitis patients were excluded from the study. Furthermore, the absence of HCC was confirmed by abdominal ultrasonography (US) or dynamic computed tomography (CT) in all subjects.

According to the pretreatment AFP value, the 40 subjects were divided into two groups: the high AFP group (AFP > 10 ng/dl; $n = 24$) and the low AFP group (AFP ≤ 10 ng/dl; $n = 16$). Then the pretreatment clinical background parameters were statistically investigated using the Mann-Whitney U-test and chi-square test to compare the high and low AFP groups.

These 40 subjects were divided into two groups, the IFN group ($n = 27$) and the SNMC group ($n = 13$). Six million units of recombinant IFNα-2b was injected intramuscularly three times a week or more in the IFN group. SNMC was administered intravenously three times a week at a dose of 40 to 100 ml in the SNMC group. Both alanine aminotransferase (ALT) and AFP values after 4 weeks of treatment were compared with the pretreatment values. Paired t-test was used, and $P < 0.05$ was regarded as significant.

RESULTS

Clinical Characteristics in Patients with High AFP Baseline (High AFP) vs. Low AFP Group. There were no significant differences in age, gender, ALT level, HCV genotype, or HCV-RNA level between the high and the low AFP groups; however, in the high AFP group, the platelet count, albumin level, and prothrombin (PT) value were significantly lower ($P = 0.0014$, $P = 0.0026$, and $P = 0.0041$) (Table 1). These results suggest that the AFP level increases with the progression of liver disease.

Pretreatment Backgrounds in IFN and SNMC Treatment Groups. There were no significant differences in the pretreatment background parameters such as AFP value, age, gender, ALT value, platelet count, albumin level, PT (%), and HCV-RNA level between the two groups (Table 2). Fourteen of the 27 IFN-treated patients (52%) showed a high pretreatment AFP value (>10 ng/ml), and 9 of the 13 SNMC-treated patients (69%) showed a high pretreatment AFP value (>10 ng/ml).

ALT Changes in IFN and SNMC Treatment Groups. With respect to changes in the ALT level, the AFP level was significantly decreased in the IFN group (132.6 ± 72.7 to 61.1 ± 43.3 U/L; $n = 27$; $P < 0.0001$). In the SNMC group, ALT levels were also significantly decreased (149.4 ± 17.2 to 83.0 ± 57.7 U/L; $n = 12$; $P = 0.019$) (Figure 1).

AFP Changes in IFN and SNMC Treatment Groups. As for AFP changes, the AFP value was significantly

TABLE 1. PRETREATMENT CLINICAL CHARACTERISTICS ACCORDING TO AFP VALUE

	High AFP (n = 24) (AFP > 10 ng/ml)	*Low AFP (n = 16) (AFP ≤ 10 ng/ml)*	P *value*
AFP (ng/ml)	46.264 ± 41.534	5.348 ± 2.229	—
Age (yr)	55.875 ± 9.252	52.938 ± 12.179	0.3914
Gender (M/F)	14/10	12/4	0.2790
ALT (U/L)	144.333 ± 88.122	125.813 ± 83.818	0.5108
PLT ($\times 10^4/\mu l$)	11.421 ± 4.997	14.550 ± 4.030	0.0467*
Albumin (g/dl)	3.617 ± 0.444	4.138 ± 0.238	0.0002*
PT (%)	72.368 ± 11.923	80.237 ± 10.796	0.0439*
HCV-RNA (KIU/mL)	472.667 ± 286.404	463.067 ± 323.334	0.9257

Note. Mann-Whitney U-test or chi-square test was used. $P < 0.05$ was considered significant.
Values are expressed as mean ± SD.