Table 3. Occurrence of CT Image Patterns

	MTX-P (n=10)	RA-PCP (n=14)	AIDS-PCP (n=11)
type A	7	6	1
type B	2	5	10
type C	1	3	0

type A, type B, type C : see text

such a situation, traditional staining is often not sufficiently sensitive. PCR for *P. jirovecii* is a much more sensitive technique than traditional staining (5) and its usefulness in the diagnosis of PCP, especially with low organism burden, was reported by many investigators (4, 6, 7). On the other hand several studies found incontrovertible incidence of colonization of *P. jirovecii* among immunosuppressed patients, suggesting that a positive PCR result alone may lead to overdiagnosis (8, 9). Meanwhile the measurement of β-D-glucan, a quantitative marker for mycotic diseases, has been reported as a useful and reliable marker in the diagnosis of PCP (18-21). Thus, we considered that, for the diagnosis of RA-PCP, detection of *P. jirovecii* by traditional staining is desirable but cases of positive PCR results with negative smears are also eligible when the plasma β-D-glucan level is significantly elevated.

Radiologic features of MTX-P

The radiologic features of MTX-P have been reported by many authors (10-12). They have been noted only as diffuse infiltrates on radiography and GGO on CT, with no further details described. Through the analysis of CT images of our cases, we found conspicuous features of MTX-P, that is, type A GGO as the predominant pattern on CT, which has never been reported.

RA-PCP compared to AIDS-PCP

Several important differences were found between the two groups clinically and radiologically. RA-PCP developed more rapidly than AIDS-PCP. Respiratory impairment was more severe in RA-PCP, and resulted in two deaths, while there was no fatality in the AIDS-PCP group. The level of plasma β-D-glucan, a quantitative marker for *P. jirovecii*, was significantly lower compared to AIDS-PCP, suggesting a lower organism burden in RA-PCP cases. All these differences have been well documented in many studies as the differences of PCP in patients with and without AIDS (13-17). Limper et al conducted a clinicopathological and comparative study of PCP of both conditions, including quantitative assay of *P. jirovecii* and inflammatory cells in BAL fluid, demonstrating fewer parasite numbers and more intense lung inflammation and also severe clinical symptoms in non-HIV PCP (15).

It is noteworthy that in our RA-PCP patients, the immunological status was not impaired as severely as in AIDS-PCP patients. These facts, i.e., relatively preserved immunity in RA-PCP patients, have been pointed out in several reports (22, 23). Why PCP can occur in patients who are not severely immunosuppressed is a problem to be solved, especially in relation to some particular immunomodifying actions of anti-rheumatic drugs.

The radiologic features of AIDS-PCP have been extensively reported (24-26), but not as thoroughly for non-AIDS-PCP or for the difference between the two, PCP with and without AIDS. Through detailed radiologic analysis, we found differences between these two disorders, which have apparently never been documented previously. In most AIDS-PCP cases, CT presented type B GGO. We consider this finding, which coincides with features previously reported (26-28), to be characteristic of this disease. However, in 6 of the 14 RA-PCP cases, CT showed type A GGO, while 5 presented type B GGO. RA-PCP showed complex radiological findings, intermediate between AIDS-PCP and MTX-P. Since the radiologic features might reflect the pathophysiology of each disease, we conducted a comparative analysis of the CT patterns and the clinical features of each disease, but failed to demonstrate any correlation, either with the clinical features or with patient outcome.

In all 14 cases of RA-PCP, corticosteroid was administered concomitantly with TMP-SMX. Two died, the mortality rate being 14%. High mortality has been reported in PCP of CTD (33% Sekowitz, 32% Godeau et al) (13, 22), to be much higher than AIDS-PCP. It is suggested that the good outcome of the present cases was the result of the use of corticosteroids added to TMP-SMX.

In AIDS-PCP, the National Institutes of Health - University of California Expert Panel recommends use of steroids as early as possible (27). In those cases, the inflammatory response evoked by *P. jirovecii* is assumed to contribute to the lung damage, indicating the need for corticosteroid treatment. However for RA-PCP or PCP of CTD in general, the validity of corticosteroid use has not been discussed in depth. Pareja et al retrospectively analyzed the clinical course of 30 cases of severe PCP without AIDS, among

whom 16 cases were treated with adjunctive corticosteroids (28). They reported good clinical outcome in patients who received high doses of adjunctive corticosteroids. In RA-PCP, the host inflammatory response is assumed to be more intense, in spite of lower organism burden, contributing to severe lung injury. It is therefore reasonable that corticosteroids may play a beneficial role in treatment of RA-PCP, when used concomitantly with antipneumocystic drugs. This issue should be examined in a prospective study.

Discrimination between RA-PCP and MTX-P

Comparison of clinical features of RA-PCP and MTX-P revealed their close resemblance, in terms of major symptoms, rapid progression, and severe oxygenation impairment. Levels of serum albumin, LDH, CRP, and KL-6 were also similar. Immunological status at presentation was also preserved relatively well in both groups. Thus, in the clinical setting of an acute respiratory event in a patient under MTX treatment for RA, discrimination between RA-PCP and MTX-P is challenging.

CT features have limited usefulness. When CT shows GGO of type A pattern or Type B pattern, MTX-P as well as RA-PCP are equally likely, because these patterns are seen in both diseases. Distinction is impossible by CT imaging alone. Thus the discrimination of RA-PCP from MTX-P should be based on detection of *P. jirovecii*, combined with serology.

In RA patients under MTX treatment with acute onset lung injury, we should treat them as MTX-P with corticosteroids, if *P. jirovecii* is not detected. If traditional staining or PCR reveals *P. jirovecii*, along with elevated plasma β-D-glucan level, we should treat it as RA-PCP, with antipneumocystic drugs. Use of adjunctive steroids is a matter to be examined in future.

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Original article

HLA-A*2402-restricted HIV-1-specific cytotoxic T lymphocytes and escape mutation after ART with structured treatment interruptions

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Abstract

Although a limited duration of immune activation of structured treatment interruptions (STIs) has been reported, the immune escape mechanism during STIs remains obscure. We therefore investigated the role of three immunodominant cytotoxic T lymphocyte (epitopes) in 12 HLA-A*2402-positive patients participating longitudinally during the clinical study of early antiretroviral treatment (ART) with five series of structured treatment interruptions (STIs). The frequency of HLA-A*2402-restricted CTLs varied widely and a sustained CTL response was rarely noted. However, a Y-to-F substitution at the second position in an immunodominant CTL epitope Nef138-10 (Nef138-2F), which was previously demonstrated as escape mutation, was frequently detected in seven patients primarily and emerged in the remaining five patients thereafter, and the existence of escape mutations was correlated with high pVL levels early in the clinical course. These findings suggest that escape mutation in the immunodominant CTL epitope may be one of the mechanisms to limit HIV-1-specific immune control in STIs.

Keywords: Structured treatment interruptions; Cytotoxic T lymphocyte; HLA-A*2402; Escape variant

1. Introduction

Structured treatment interruption (STI) is considered one of the immune stimulatory interventions for HIV-1 infection, based on the hypothesis that viral rebound during treatment interruption might induce HIV-specific immune responses [1–3]. Since the 1999 case report of the early-treated patient who achieved sustained viral suppression without highly antiretroviral therapy (HAART) after two occasional treatment interruptions [1], the STI strategy has been studied in various clinical settings [4–7]. Because cytotoxic T lymphocytes (CTLs) play a critical role in the control of HIV-1 replication and HIV-specific CD4+ T-cell response is important to maintain effective HIV-1-specific CTLs [8-11], early treatment that can preserve HIV-1-specific-CD4+ T cells is considered to have the greater impact on STI in early infection than in chronic infection [11–13]. However, the majority of previous STI trials revealed the limitation of immune activation with risk of viral resistance [4,14,15] and the mechanisms of viral control failure in STI strategy have remained unclear.

Viral mutation in immunodominant epitopes is one of the obstacles to HIV-1 vaccine development [16–21]. Since HIV-1-specific T-cell responses are restricted by HLA alleles, its escape variant can be transmitted and adopted in populations sharing some dominant HLA alleles [19–21]. In Japan where HLA-A*2402 is the most frequent HLA class I allele with 70% prevalence, HLA-A*2402-restricted CTLs and its immunodominant epitopes have been extensively assessed [22]. Nef138-10, which has been proved previously as an HLA-A*2402-restricted CTL epitope provoking strong cytolytic activity [22], is one of the immunodominant CTL epitopes in HLA-A*2402-positive Japanese patients [21,22].

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Our previous study showed that a Y-to-F substitution at the second position in Nef138-10 epitope (Nef138-2F) impairs the ability of the Nef138-10-specific CTLs to suppress HIV-1 replication, indicating that Nef138-2F is an escape mutation from CTLs [23]. Since Nef138-2F is observed in both HLA-A*2402-positive and -negative patients, Nef138-2F variant may be stable and adopted at a population level [21].

In the present study of early antiretroviral treatment with five series of STIs for HLA-A *2402 positive Japanese patients, we investigated the longitudinal magnitudes of HIV-1-specific HLA-A*2402-restricted CTLs by using HLA-epitope tetramer binding assay and sequenced the most immunodominant epitopes Nef138-10 to evaluate whether escape mutation might negatively influence viral control in an STI study.

2. Methods

2.1. Study design and patient population

This trial was designed as a prospective study at the AIDS Clinical Center, International Medical Center of Japan. Between November 2000 and December 2001, patients with early HIV infection, with or without acute retroviral symptoms, were recruited. Early HIV infection was confirmed within 6 months before recruitment by a documented history of seroconversion in enzyme-linked immunosorbent assay (ELISA) or longitudinal increase of bands in Western blot test. Patients with active opportunistic infections or psychological disorders, or those treated with immunomodulatory agents were excluded. Antiretroviral therapy was initiated after obtaining a signed informed consent. The first-choice regimen for this study consisted of stavudine, lamivudine and indinavir boosted with ritonavir, but the patient was allowed to use other antiretroviral drugs when the first regimen could not be tolerated. To avoid emergence of drug resistance to indinavir, ritonavir-boosting was stopped more than 1 week before treatment interruption. The duration of treatment interruption was fixed for 3 weeks. The first treatment was interrupted after more than 3 months of HAART, when CD4+ cell count was $>500/mm^3$ and plasma viral load (pVL) had been <50 copies/ml for at least 1 month. Other interruptions were also carried out when pVL became <50 copies/ml and CD4+ cell count was $>300/mm^3$. Five series of STIs were scheduled during the treatment.

The study protocol was approved by the institutional ethical review boards (IMCJ-H13-10).

2.2. Monitoring and sample collection

Patients were monitored monthly during HAART and at approximately a 4-month interval after treatment discontinuation. Unscheduled visits were permitted according to clinical needs. At each visit, clinical assessment and routine laboratory tests were performed. Blood specimens were collected in ethylenediaminetetraacetic acid (EDTA)-containing tubes, separated into peripheral blood mononuclear cells (PBMCs) and plasma, and stored at −80 °C for assessment of HIV-1-specific CTLs and sequence of the dominant epitope region. pVL was quantified by using the Amplicor HIV-1 Monitor test 1.5 (Roche Diagnostics, Indianapolis, IN) with a detection limit of 50 copies/ml. Antiretroviral drug resistance-associated mutations were examined at baseline and after HAART including STIs in all 26 participants. Each mutation was identified according to the revised August 2006 International AIDS Society Resistance-USA Panel [24].

2.3. HLA typing and epitope-HLA-A*2402 tetramer binding assays

High-resolution HLA class I typing was performed by a PCR-sequence-specific primer method. If HLA-A*2404 was positive, HIV-1 specific CTLs were investigated by using peptide-HLA-A*2402 tetrameric complex synthesized as described previously [21,22,25]. Purified complexes were enzymatically biotinylated at a BirA recognition sequence located at the C-terminus of the heavy chain, and then mixed with phycoerythrin (PE)-conjugated avidin (extravidin-PE; Sigma–Aldrich, St. Louis, MO) at a molar ratio of 4:1. Cryopreserved PBMCs ($0.5–1 \times 10^6$ cells) were stained by the tetramer at 37 °C for 30 min. After double washing with washing buffer (10% fetal calf serum in RPMI 1640), the cells were stained by fluorescein isothiocyanate (FITC)-conjugated anti-human CD8 mAb (BD Biosciences, San Jose, CA) at 4 °C for 30 min. The cells were then washed twice and analyzed using a FACS Calibur with Cell Quest software (Becton Dickinson, San Jose, CA). Based on our previous study [22], three immunodominant epitopes of HLA-A*2402 restricted CTLs; Nef138-10, Gag28-9 and Env584-9, were chosen for this assay. Since we found a high frequency of Y-to-F substitution at the second position in Nef138-10 gene (Nef138-2F) which has been suspected as an escape variant in previous studies [21], Nef138-2F-specific CTLs (Nef138-2F-CTLs) were also measured by tetramers using Nef138-2F variant alone and by competitive double staining using two types of tetramers of both wild type and Nef138-2F variant to compare the frequencies of the two types of HIV-1-specific CTLs.

2.4. Sequence analyses of Nef138-10 gene

For evaluation of escape variants from CTLs, we sequenced the region coding Nef138-10, which is the immunodominant HLA-A*2402-restricted epitope, while Nef138-2F has been suspected as escape mutation in this epitope, using the method described here. Total RNA was extracted from plasma with a High Pure viral RNA kit (Boehringer Mannheim, Mannheim, Germany), followed by RT–PCR with a One Step RNA PCR kit (TaKaRa Shuzo, Otsu, Japan) to amplify the HIV-1 Nef DNA segment (2341 bp) as described previously [21]. The PCR products were purified with SUPREC-02 (TaKaRa Shuzo) and subjected to direct sequencing with an ABI PRISM 3730 automated DNA sequencer (Applied Biosystems, Foster City, CA). Amino acid sequences were deduced with the Genetyx-Win program version 5.1 (Software Development, Tokyo).

2.5. Statistical analysis

Data from patients who completed the treatment protocol including five series of STIs were analyzed. Before analysis, pVL data were log-transformed and undetectable pVL (<50 copies/ml) was considered equivalent to 50 copies/ml. The Mann–Whitney *U*-test was used to compare the pVLs determined every 3 months after treatment cessation to the pVLs of 279 untreated chronic HIV-1 patients in order to assess the durability of viral suppression. The correlation between pVL and percentage of CTLs was assessed by simple regression analysis. Statistical analyses were performed using SPSSII software package for Windows, version 11.0J.

3. Results

3.1. Characteristics of participants

During the enrollment period, 432 new patients were referred to our clinic. Of these, 32 met the criteria of early HIV-1 infection and 6 were excluded due to psychological problems or taking systemic steroid therapy for symptoms associated with acute retroviral syndrome. All 26 recruits were Japanese infected with HIV-1 by sexual intercourse, and 24 were men (92%). The mean age of patients was 35.0 years (range, 21–56 years). The mean pVL at baseline was 5.21 $\log_{10}$ copies/ml (range, 3.28–6.91 $\log_{10}$ copies/ml) and the mean CD4+ cell count at baseline was 413/mm^3 (range, 49–1156/mm^3). Twenty-five patients presented with wide-range clinical symptoms of acute retroviral syndrome. Fifteen out of 26 participants completed the treatment protocol including five series of STI. HAART had to be continued in four patients because CD4+ cell counts had never stabilized above 300/mm^3 despite more than 6 months of treatment. The other seven patients discontinued the treatment protocol after less than five STIs due to adverse events, adherence problems, or no specific problems.

In the protocol-completed 15 patients, 14 were men (92%). The mean age was 34.0 years (range, 21–56 years). At baseline, the median pVL was 5.14 $\log_{10}$ copies/ml (range, 3.28–6.91 $\log_{10}$ copies/ml) and the median CD4+ cell count was 475/mm^3 (range, 245–990/mm^3). The demographic, immunological, and virological factors before initiation of HAART of the protocol-completed group were not statistically different from those of the uncompleted group (Mann–Whitney *U*-test) (data not shown), although baseline CD4+ cell counts of four ART-continued patients: 49, 185, 210, and 351/mm^3 respectively seemed lower than those who completed the treatment protocol. Twelve (80%) patients were positive for HLA-A*2402 and its incidence was similar to those reported previously in Japanese population [21,22]. No specific HLA genotypes that are known to influence the clinical course of HIV infection such as HLA-B*27, HLA-B*57 and HLA-B*35 (except B*3501) [26] were detected in participants. The median length of follow-up after treatment cessation was 961 days (range, 462–1255 days).

No resistance-associated mutations were identified among all the 26 participants at study enrollment except one who had M184V, D30N and L90M mutations despite good virologic responses throughout HAART. There was no increase in resistance-associated mutations during and after five STIs in all participants (data not shown).

3.2. Plasma viral load and CD4+ cell count in protocol-completed 15 patients

Fig. 1 shows serial changes in median pVLs and CD4+ cell counts in protocol-completed 15 patients. Peaks of viral

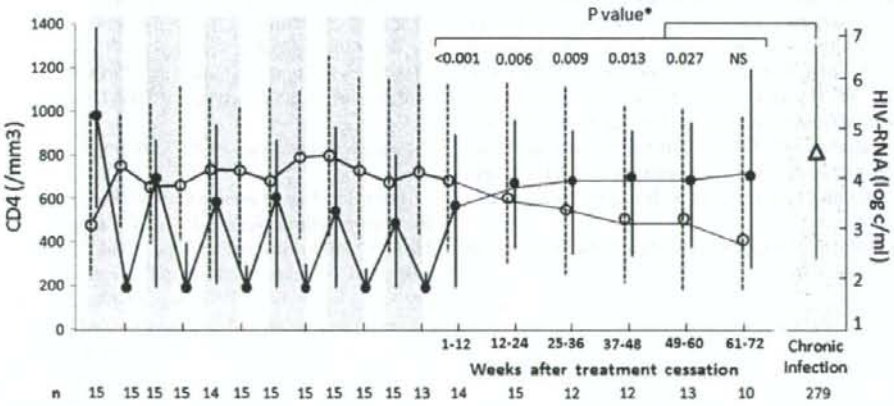

Fig. 1. Serial changes in plasma viral loads and CD4+ cell counts of 15 protocol-completed patients. Plasma viral loads (pVLs) and CD4+ cell counts are expressed as median of 15 protocol-completed patients; at baseline, at the times of treatment interruption, at the peaks of pVL rebound during structured treatment interruption and at every 12 weeks after treatment cessation. Open circles: CD4+ cell counts; solid circles: pVLs; triangle: the median pVL of 279 untreated chronic HIV-1 patients who were referred to our clinic during the study and whose CD4 count was >200/mm^3. Vertical lines provide the ranges with dotted lines in CD4+ cell counts and with light lines in pVLs. Shaded area: time on antiretroviral therapy; unshaded area: time off therapy. Numbers of patients whose data were evaluated at each time point appear at the bottom of the graph. *pVLs of every 12 weeks after treatment cessation were compared to the pVLs of 279 untreated chronic HIV-1 patients by Mann–Whitney *U*-test.

rebounds during treatment interruptions decreased gradually. The pVLs of every 12 weeks after treatment cessation were under 4 $\log_{10}$ copies/ml in most of the patients and they were significantly lower for 60 weeks than the pVLs of 279 untreated chronic HIV-1 patients in our clinic. However, pVLs gradually increased and there was no difference at week 61–72 from pVLs of chronically infected patients. The proportion of patients with a favorable viral control whose median pVL at every 12 weeks after treatment cessation were less than 4.0 $\log_{10}$ copies/ml was 66% in the first 12 weeks but the proportion decreased to 33% in the 61–72 weeks. Along with the increase in pVL, CD4+ cell counts declined after treatment cessation and one patient (KI-134) required restart of HAART because CD4+ cell count decreased below 200/mm^3 at week 52. None of the patients developed episode of opportunistic infections or HIV-related diseases throughout this study.

*3.3. Plasma viral loads and frequency of HLA-A*2402-restricted CTLs*

We investigated induction of 3 HLA-A*2402-restricted immunodominant epitope-specific CTLs in 12 patients with HLA-A*2402 by using the corresponding tetramers. Fig. 2 shows the serial changes in HLA-A*2402-restricted HIV-1-specific CTLs. Overall, the frequency of HLA-A*2402-restricted CTLs varied widely among the patients and a sustained CTL response was rarely noted. We investigated the correlation between pVLs at every 12 weeks after treatment cessation and frequency of HLA-A*2402-restricted CTLs according to the epitope. None of Nef138-10-, Gag28-9- or Env584-9-specific CTLs was statistically correlated to pVLs (Fig. 3A).

3.4. Effect of Nef138-10 escape mutation on suppression of HIV replication

A Y-to-F substitution at the second position of Nef138-10 (Nef138-2F) has been suspected as an escape mutation from HLA-A*2402-restricted Nef138-10-specific CTLs in a previous study [21]. In fact, we recently demonstrated that Nef138-10-specific CTLs fail to suppress replication of Nef138-2F mutant [23]. We therefore performed serial sequence analyses of Nef138-10 epitope and investigated whether this 2F mutation is responsible for the limited duration of viral suppression. As shown in Table 1, we found high frequency of this mutation. Seven out of 12 patients had Nef138-2F variant in viral RNA or proviral DNA in the earliest samples (KI-091, KI-126, KI-134, KI-144, KI-150, KI-154 and KI-163). The Nef138-2F variant was not detected in the earliest samples of the other five patients (KI-092, KI-099, KI-102, KI-158 and KI-161) and these patients were considered to have Nef138-10 wild-type infection except a T-to-C substitution at the fifth position (Nef138-5C) in KI-099 which has also been suspected as one of the escape variants from Nef138-10-specific CTLs in a previous study [21], and an L-to-I substitution at the forth position (Nef138-4I) in KI-161. However, Nef138-2F mutation was detected at the latter stage in all the other five patients.

We speculated that Nef138-10-specific CTLs can control replication of HIV-1 in patients who had been infected with Nef138-WT virus. Therefore we compared pVLs according to the existence of escape mutants Nef138-2F or 138-5C at the earliest sample drawn during early phase of infection before treatment initiation. As shown in Fig. 3B, the pVLs between 13 and 36 weeks were significantly lower in the other four patients who were confirmed as Nef138-WT or Nef138-4I infection than in the remaining eight patients who had Nef138-2F or Nef138-5C variant in the earliest samples, which has been suspected as an escape variant from Nef138-10-specific CTLs in a previous study. These indicate that Nef138-10-specific CTLs control replication of wild-type virus but the presence of either Nef138-2F or Nef138-5C negatively influences viral control.

3.5. Nef138-2F variant specific CTLs

We found Nef138-WT-tetramer and Nef138-2F-tetramer bound to both Nef138-WT-specific CTL clones and Nef138-2F-specific CTL clones. In addition, Nef138-WT-tetramer had stronger affinity to Nef138-WT-specific CTL clones than Nef138-2F-specific CTL clones (Fig. 4A) and vice versa (our unpublished work). Therefore, the double-staining assay using both tetramers simultaneously was performed to differentiate the two types of CTLs.

The frequencies of the two types of CTLs are shown in Table 1. In patients negative for Nef138-2F or Nef138-5C initially, Nef138-WT-CTLs were detected early after the treatment cessation (KI-092, KI-102, KI-158 and KI-161) but declined after evolution of Nef138-2F (KI-092, KI-102, and KI-161). Although only a slight elevation of Nef138-2F-CTLs was noted after emergence of Nef138-2F (KI-092 and KI-161), the magnitude was smaller than that of Nef138-WT-specific CTLs before emergence of Nef138-2F.

In patients having Nef138-2F variant initially and suspected as Nef138-2F variant infection, the frequencies of Nef138-2F-CTLs were relatively smaller than those of Nef138-WT-specific CTLs in Nef138-10 wild-type infection, except KI-144 who had marked increase of Nef138-2F-CTLs in week 37.

Fig. 4B and C illustrate the clinical courses of two representative cases; KI-161 was non-Nef138-2F variant infection and KI-144 was suspected as Nef138-2F variant infection. In KI-161 (Fig. 4B), Nef138-WT-CTL response diminished after the emergence of Nef138-2F mutation. Interestingly, the pVL of this patient seemed to increase along with the fall in Nef138-WT-CTLs (Fig. 2). In KI-144 (Fig. 4C), Nef138-2F-CTLs were induced but there was no suppression of pVLs. These results indicate that either infection or emergence of Nef138-2F variant might limit the CTL induction.

4. Discussion

In this study, we could not demonstrate the lowered set-point pVLs in patients who received HAART with five series of STIs in early HIV-1 infection. Previous studies revealed that a vigorous HIV-1-specific CD4 response is associated

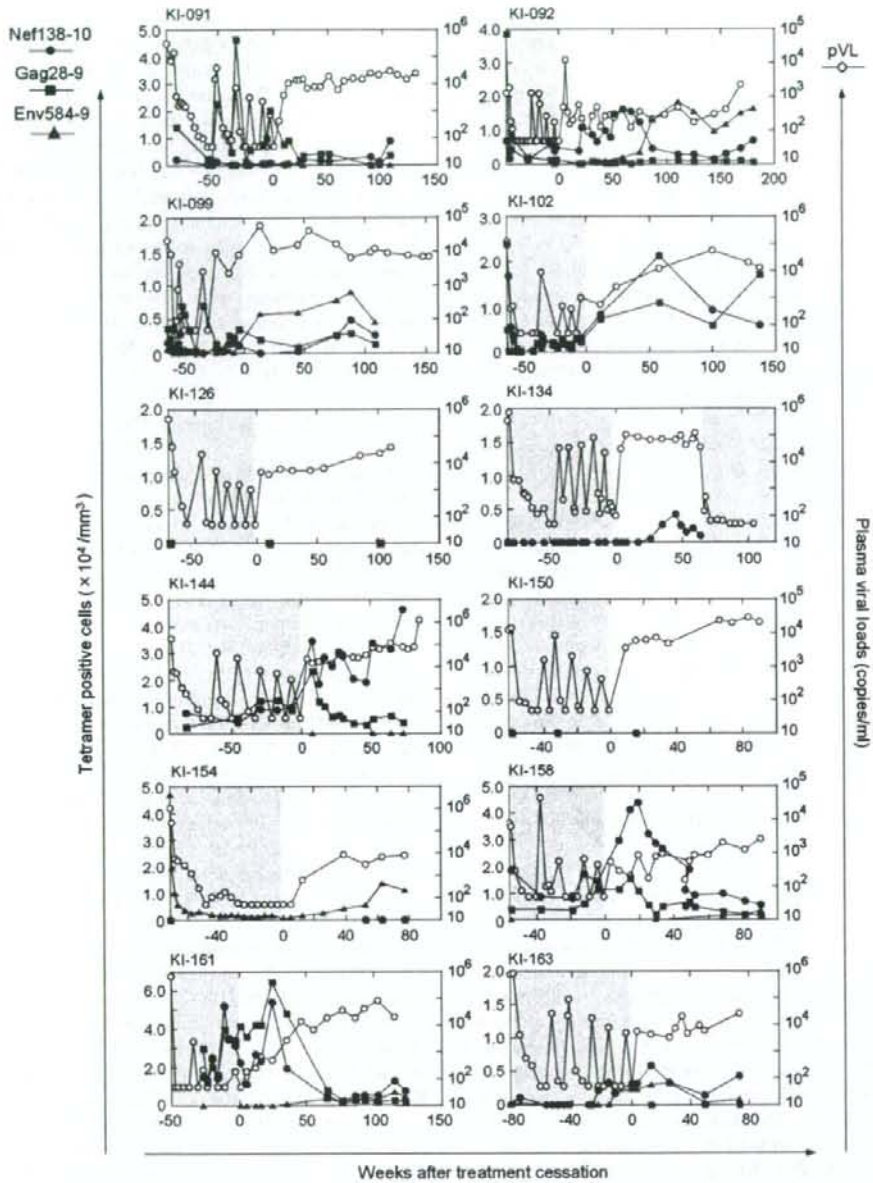

Fig. 2. Frequencies of HLA-A*2402 restricted HIV-1-specific CTLs determined by tetramer binding assay. HLA-A*2402 restricted HIV-1-specific CTLs in PBMCs were determined by using tetrameric complexes of HLA-A*2404 and each of the three types of epitopes. Solid circle: Nef138-10-specific CTL; solid squares: Gag28-9-specific CTL; solid triangles: Env584-9-specific CTL; open circles: plasma viral load. Shaded area: time on antiretroviral therapy; unshaded area: time off therapy.

with a slower disease progression [8–11]; however, despite some reports of boosted immunological responses in acutely treated patients, the evidence of clinical benefits of early treatment has not been established [12,13]. In line with these trials of early initiation of HAART with or without STI, the CTL responses in our study were mostly transient and did not correlate with pVL levels.

We adopted HLA-epitope tetramer analysis for evaluating CTL responses, which provides specific information on HLA class I allele and HLA-restricted epitopes, because CTL

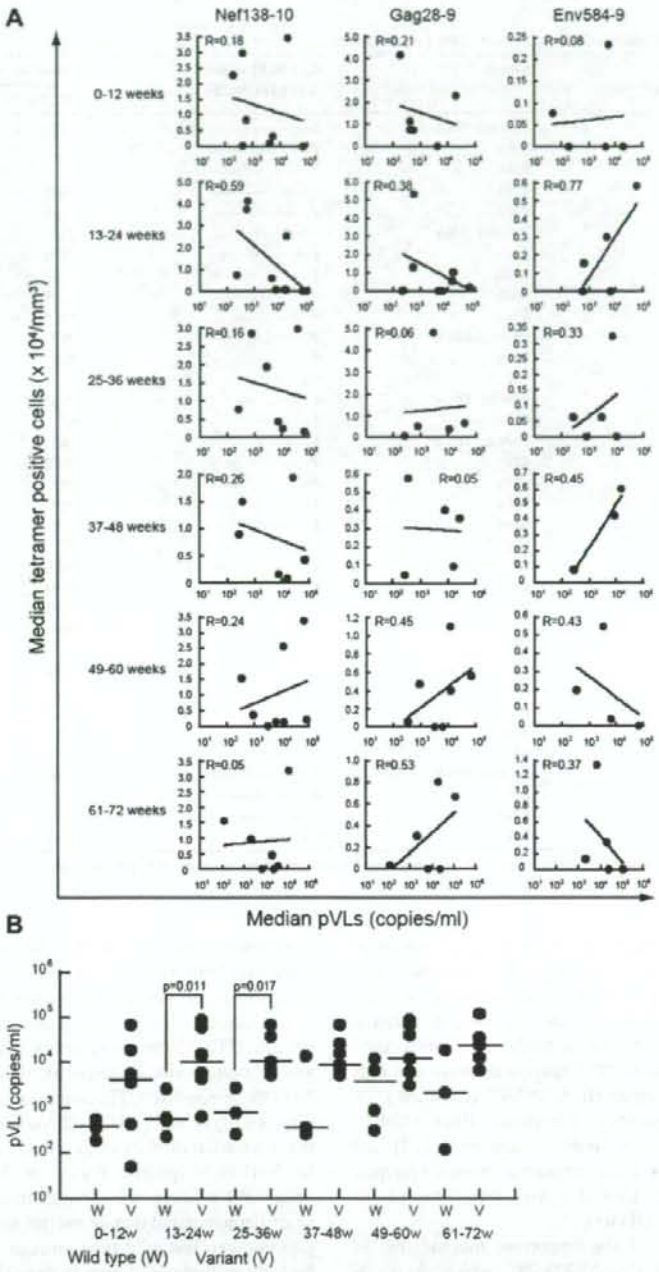

Fig. 3. (A) Plasma viral loads and frequency of HLA-A*2402-restricted HIV-1-specific CTLs. The correlation between the pVL values of every 12 weeks after treatment cessation and frequency of HLA-A*2402-restricted CTLs was assessed by simple regression analysis according to the epitope in 12 HLA*A2402-positive patients. None of Nef138-10-, Gag28-9- or Env584-9-specific CTLs was statistically correlated to pVLs at any time point. R: correlation coefficient. (B) Plasma viral loads and initial type of virus. pVL was compared according to the existence of escape variant in the earliest sample drawn during early phase of infection. Wild type group (W) includes four patients: KI-092, KI-102, KI-158 and KI-161. Variant type group (V) includes eight patients: KI-091, KI-099, KI-126, KI-134, KI-144, KI-150, KI-154 and KI-163, having Nef138-2F or Nef138-5C, which were previously reported as escape variants, in viral RNA or proviral DNA in the earliest samples. The pVLs between 12 and 36 weeks were significantly higher in Variant type group than in Wild-type group. Horizontal lines: median values.

Table 1
Nef138-10 sequence and Nef138-specific CTLs in HLA-A*2402 positive patients

Patient ID	Time (weeks)[a]	Sample	Nef138-10 sequence (RYPLTFGWCF)	Tetramer positive cell (% in CD8+ cells)	
				Wild type	2F
KI-091	−55	Proviral DNA	-F————	NA	NA
	21	RNA	-F————	0	0.46
	89	RNA	-F————	0	0.83
KI-092	39	RNA	————	1.48	0.05
	86	RNA	-F————	0.41	0.13
KI-099	−44	Proviral DNA	——C———	NA	NA
	−4	RNA	-F–C————	0.04	0.06
	44	RNA	-F————	0.02	0.12
KI-102	58	RNA	————	2.11	0.45
	137	RNA	-F————	0.45	0.10
KI-126	−68	Proviral DNA	-F————	NA	NA
	19	RNA	-F————	0.01	0.06
	101	NA	NA	0	0.11
KI-134	9	Proviral DNA	-F————	NA	NA
	49	RNA	-F————	0	0.22
KI-144	−46	Proviral DNA	-F————	NA	NA
	37	RNA	-F————	0.02	2.45
	71	RNA	-F————	NA	NA
KI-150	−43	RNA	-F————	NA	NA
	21	RNA	-F————	0	0.03
	63	NA	NA	0	0.02
KI-154	−70	Proviral DNA	-F————	0.06	0.13
	77	RNA	-F————	0.01	0.35
KI-158	14	Proviral DNA	————	2.91	0.41
KI-161	−26	Proviral DNA	—I————	NA	NA
		RNA	-F-I————		
	24	Proviral DNA	-F-I————	3.94	0.05
		RNA	-F-I————		
	86	Proviral DNA	-F-I————	0.29	0.79
		RNA	-F-I————		
	52	RNA	-F————	0.71	0.66
KI-163	−81	Proviral DNA	-F————	NA	NA
		RNA	-F————		
	26	RNA	-F————	0.09	0.57
	73	NA	NA	0.02	0.59

[a] Time: Time in weeks after treatment cessation. Negative time numbers: before treatment cessation. NA, not available.

responses are different between HLA class I alleles and influenced by viral mutations in epitope regions as described elsewhere [16–22]. HLA-A*2402 is the most frequent HLA class I allele with 70% prevalence in the Japanese population [21,22]. Therefore, the majority of the study participants could be assessed by using HLA-A*2402-epitope tetramer and thus it is most beneficial to evaluate HLA-A*2402 restricted CTL responses for Japanese patients. Moreover, HLA-A*2402-restricted epitopes have been studied extensively [22] and we were able to focus on three immunodominant epitopes. This approach allowed us to find a high frequency of the escape variant Nef138-2F efficiently.

Viral mutation is one of the important mechanisms of immune escape of HIV-1 [16–23,27–29], which occurs at amino acids responsible for HLA binding, T-cell receptor recognition, or in flanking regions that affect antigen presentation. In our study Nef138-2F, which is a mutation in the immunodominant CTL epitope Nef138-10, had emerged in 5 of 12 HLA-A*2402-positive patients. Although the magnitude of Nef138-10-specific CTLs was not significantly correlated with pVLs as previous trials [15], Nef138-2F variant infection was correlated with high pVL levels in early clinical course and seemed to contribute to lower CTL response. Furthermore, we previously demonstrated the strong and weak ability of Nef138-10-specific CTL clones to suppress replication of the wild-type and 2F mutant viruses respectively [23]. In addition, although Nef138-2F-specific CTL clones suppressed the replication of both wild-type and Nef138-2F variant, their ability to suppress the replication of Nef138-2F virus was much weaker than that of Nef138-10-specific CTLs or Nef138-2F-specific CTLs against the wild-type virus replication. Furthermore, the present study demonstrated that 2F mutant appeared at the late phase in patients who had wild-type virus at the early phase. Together with these findings, frequent detection of Nef138-2F in this study strongly supports the idea that Nef138-2F is one of the escape mutations from HLA-A*2402-restricted CTLs and that Nef138-2F virus was selected by CTL pressure.

Nef138-2F mutation could occur not only by positive selection by CTLs but also by Nef138-2F-variant transmission [19–21]. Furutsuki et al. [21] reported frequent detection of

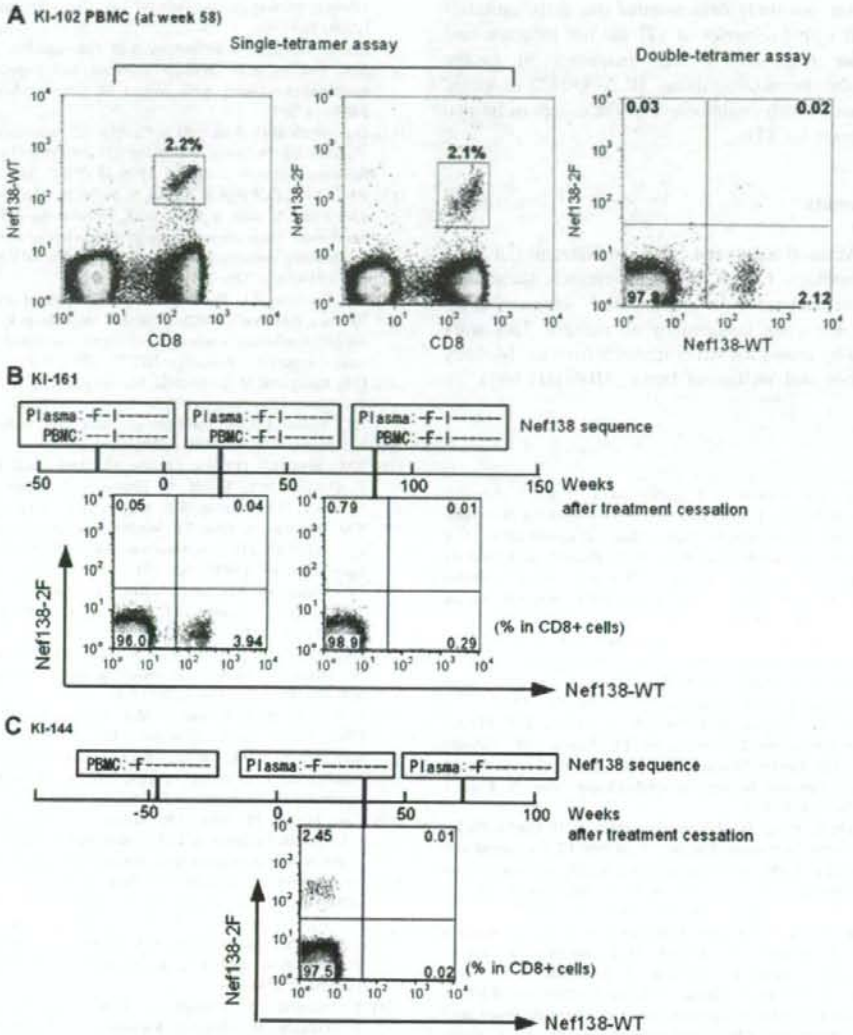

Fig. 4. Nef138-2F variant and CTL specificity. (A) PBMC of KI-102 at week 58, known to coincide with Nef138-10 wild-type infection, were assayed for wild-type Nef138-10-specific CTL (Nef138-WT-CTL) by tetramer-staining with Nef138-WT-tetramer and Nef138-2F-tetramer. The left two charts depict the results of single-tetramer-staining, showing the two tetramers stained for Nef138-WT-CTL equally (2.2% by Nef138-WT-tetramer versus 2.1% by Nef138-2F-tetramer). The right chart depicts the result of double-tetramer-staining with Nef138-WT-tetramer and Nef138-2F-tetramer, showing Nef138-WT-CTL was stained by Nef138-WT-tetramer and was differentiated from Nef138-2F-CTL. (B) Serial changes in Nef138-10 sequence and Nef138-specific-CTLs of KI-161 infected by non-Nef138-2F strain. Top: the Nef138-10 sequence; bottom charts: results of double-staining assay with Nef138-WT-tetramer and Nef138-2F-tetramer. Numbers in each quadrant represent the frequency of tetramer-positive cells among total CD8+ cells. Right lower quadrant: frequency of Nef138-WT-tetramer-positive cells; left upper quadrant: frequency of Nef138-2F-tetramer-positive cells. Note the induction of Nef138-WT-CTL and reduction in their proportion after emergence of Nef138-2F mutation. Nef138-2F-CTLs were induced after emergence of Nef138-2F mutation but their proportion was relatively lower. (C) Serial changes in Nef138-10 sequence and Nef138-specific-CTLs of KI-144 infected by Nef138-2F variant. Note the induction of Nef138-2F-CTL. Nef138-WT-CTLs were never detected throughout the study.

Nef138-2F variant in HLA-A*2402 negative Japanese patients who were infected by sexual intercourse and reversion from Nef138-2F to wild type occurred very slowly over years. These might allow horizontal spread of Nef138-2F variant. Even if the transmission of this variant in Japanese patients is very frequent, our study included the five patients who did not have this variant initially and were considered as wild-type infection, and we provided longitudinal evidence of positive selection of Nef138-2F variant under the pressure of Nef138-WT-CTLs in those.

In conclusion, our study demonstrated that early antiretroviral treatment with five series of STI did not induce a sustained immune response. A high frequency of escape mutation in the immunodominant HLA-A*2402-restricted CTLs was found, which could be one of the causes of limited immune responses by STIs.

Acknowledgments

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Beneficial effects of living-donor liver transplantation on esophageal varices

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Background. Liver transplantation (LT) is known to improve bleeding esophageal varices (EVs) and portal hypertension. However, many issues related to EVs after LT remain unresolved, such as whether LT reduces blood supply to EVs, improves the diameter of unruptured EVs, or improves or worsens EVs. The aim of this retrospective study was to determine the effects of living-donor liver transplantation (LDLT) in patients with hepatic failure on EVs and inflow vessels to EVs and the factors associated with deterioration of EVs after LDLT. ***Methods.*** The study subjects were 35 patients with cirrhosis who underwent LDLT. Endoscopy and multidetector helical computed tomography (MDCT) were performed before and after LDLT. The diameter of the inflow vessel of EVs was measured by MDCT before and after LDLT, together with the LDLT-related reduction rate of the diameter of the gastric vein (RRGV). ***Results.*** Endoscopic examination showed improvement of EVs in 30 of 35 (86%) patients. RRGV improved in 17/35 (49%) patients, did not change in 13/35 (37%), and deteriorated in 5/35 (14%). The cause of RRGV deterioration seemed to be either the complication of portal vein or graft failure. In patients examined endoscopically at >1 year after LDLT, improvement of EVs was associated with significant changes in the rate of reduction of the major inflow vessel diameter and Child-Pugh score, compared with those who showed no improvement. ***Conclusions.*** LDLT results in improvement of EVs. EVs improved in 86% of the patients. Measurement of RRGV with MDCT is a good tool for prediction of EV improvement after LDLT.

Key words: living-donor liver transplantation, esophageal varices, inflow vessel of EVs, portal hypertension

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Introduction

The prognosis of patients with cirrhosis has improved markedly in recent years through advances in clinical management, such as long-term supplementation of branched-chain amino acids and a low protein and salt diet.[1,2] However, bleeding from esophageal varices (EVs) is still a major cause of death in patients with liver cirrhosis and portal hypertension, especially in those with end-stage hepatic failure.[3] Almost all patients who do not receive treatment after variceal bleeding develop recurrence of hemorrhage within 1 year after the initial bleeding episode.

Among the various therapeutic modalities available today, pharmacological therapy has an established role in the prevention and management of acute variceal hemorrhage, based on its safety, availability, easy administration, and low cost.[4] Furthermore, endoscopic variceal ligation appears to be as effective as sclerotherapy in the control of acute variceal hemorrhage, and it has the advantage of avoiding some of the complications of sclerotherapy.[5] Sclerotherapy is widely used as the first-line treatment for acute bleeding because it provides control of hemorrhage in more than 90% of cases, with a rate of rebleeding of 15.5%–34.4%.[6] Shunt surgery, including a transjugular intrahepatic portosystemic shunt, is effective in reducing the risk of rebleeding, but carries the risk of precipitating or exacerbating encephalopathy.[7]

Liver transplantation (LT) should be considered for all patients with end-stage hepatic failure, as it not only deals with acute variceal hemorrhage but also restores normal portal circulation. Although variceal hemorrhage alone is not an indication for LT, the latter must be considered in those patients with poor liver function who present with acute variceal bleeding.[8] The best survival rates are reported in patients with EVs who receive LT (79% at 1 year and 71% at 5 years), with the greatest survival advantage in patients with Child-Pugh class C.[9]

It is important to ensure the improvement of EVs following the successful performance of LT, since variceal bleeding is a significant mortality risk factor.

It has already reported that bleeding EVs and portal hypertension improve following LT.[10–13] However, many issues related to EV after LT remain unresolved. For example, there is little or information on whether LT results in (1) reduction of blood supply to EVs, (2) improvement in the diameter of unruptured EVs, or (3) improvement or worsening of EVs.

The aim of the present retrospective study was to assess the benefits of living-donor liver transplantation (LDLT) on EVs by both endoscopy and multidetector helical computed tomography (MDCT). Endoscopic examination of EVs showed improvement of EVs in most patients after LDLT. MDCT showed that the main inflow vessels to EVs were the left gastric vein (LGV), posterior gastric vein (PGV), and short gastric vein (SGV). Measurement of the reduction rate of the diameter of gastric veins (RRGV) after LDLT varied among patients, ranging from 0% to >20%.

Methods

Patients

Ninety-five consecutive patients underwent adult-to-adult LDLT from January 1991 to April 2007 at Hiroshima University Hospital. Thirty-five of the patients were enrolled in this study, based on the fulfillment of the following criteria: (1) they underwent adult-to-adult LDLT because of end-stage hepatic failure, (2) they underwent assessment of EVs by endoscopy before and after LDLT, and (3) they underwent MDCT before and after LDLT and the diameters of the LGV and PGV were measured. Among the remaining 60 patients, 20 did not have end-stage hepatic failure, 17 did not have EVs before LDLT, and 23 patients did not undergo endoscopic examination after LDLT because of refusal or death. None of the enrolled patients was on any medications, such as β-blockers, and all abstained from alcohol drinking 6 months before LDLT.

Table 1 lists the characteristics of enrolled patients, including age, sex, background liver disease, portal vein (PV) pressure measured during surgery, tissue graft weight, graft volume, model for end-stage liver disease (MELD) score, Child-Pugh score, timing of endoscopy, and timing of MDCT examination. With regard to the graft used for LDLT, the right lobe was used for 32 recipients and the left lobe for three. In three patients, splenectomy was also performed at the time of LDLT. Before LDLT, written informed consent was obtained from each patient.

Endoscopic examination for assessment of EVs

Endoscopic examination was performed by four experienced operators before and after LDLT using a model GIF XQ240 endoscope (Olympus Optical, Tokyo, Japan). The varices-related endoscopic findings were evaluated according to the general rules proposed by the Japanese Research Society for Portal Hypertension.[14] In brief, the form of the varices (F factor) was classified as small straight (F1), enlarged tortuous (F2), or large coil-shaped (F3). The red color (RC) sign of the mucosal area covering the varices (red-colored blood visualized underneath a very thin vascular wall)

Table 1. Characteristics of patients before LDLT

Age (years)[a]	51 (27–69)
Sex (male/female)	25/10
Original diagnosis (HCV/HBV/Alcoholic/AIH/PBC)	17/10/4/3/1
With hepatocellular carcinoma	17
Portal vein pressure (mmHg)[a]	24 (20–30)
MELD score[a]	13 (10–35)
Child-Pugh score[a]	9 (5–14)
Graft (right/left)	32/3
Graft weight (g)[a]	568 (350–1142)
Graft volume (ml)[a]	727 (381–1221)
Timing of endoscopic examination (post-LDLT, month)[a]	10 (1–56)
Timing of MDCT examination (post-LDLT, month)[a]	5 (1–28)
Form of esophageal varices (F1/F2/F3)	15/17/3
Red color sign of esophageal varices (RC0/RC1/RC2/RC3)	24/8/2/1
Inflow vessels (LGV/PGV)	27/8
Diameter of inflow vessels (LGV/PGV/SGV)[a] (mm)	5.87/4.71/3.20

LDLT, living donor liver transplantation; HCV, hepatitis C virus; HBV, hepatitis B virus; AIH, autoimmune hepatitis; PBC, primary biliary cirrhosis; MELD score, model for end-stage liver disease score; MDCT, multidetector helical computed tomography; LGV, left gastric vein; PGV, posterior gastric vein; SGV, short gastric vein; RC, red color sign

[a] Values are median (range)

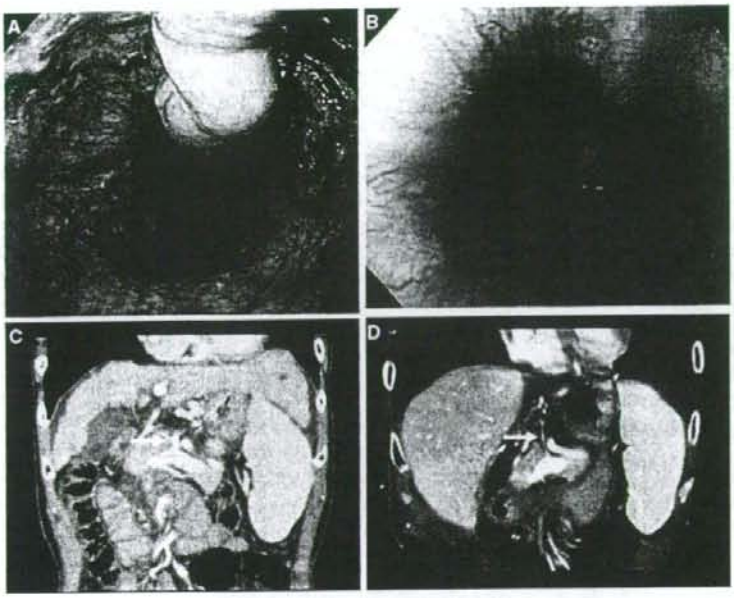

Fig. 1A–D. Endoscopic view of esophageal varices (EVs) demonstrating improvement after living-donor liver transplantation (LDLT) (case 2). The form of EVs before LDLT was F3 (**A**) and improved to F1 at 12 month after LDLT (**B**). Narrowing of the left gastric vein was seen on multiplanar reconstruction images of multidetector computed tomography (MDCT) after LDLT. The diameter of the left gastric vein (LGV; *arrow*) in this patient was 15.4 mm before LDLT (**C**), and decreased to 5.9 mm at 7 months after LDLT (*arrow*) (**D**), with a rate of reduction of the LGV of 61.6%

was classified according to the criteria of the Japanese Research Society for Portal Hypertension[14] as negative (RC0), localized (RC1), between localized and entire circumference (RC2), and entire circumference (RC3). Endoscopy-based improvement in EVs represented improvement in the F or RC factor as determined 10 (range, 1–56) months after LDLT.

MDCT examination for assessment of inflow vessels

An MDCT examination was performed before and after LDLT by two experienced radiologists. At each examination, the trunk diameters of LGV, PGV, and SGV, the main suppliers of portal blood to EVs, were measured (Fig. 1). The gastric vein with the largest diameter was considered the inflow vessel. We used the rate of reduction of the diameter of the gastric vein (RRGV) to assess the effect of LDLT on inflow vessels. RRGV (%) was calculated using the formula: [(diameter of GV before LDLT – diameter of GV after LDLT) / diameter of GV before LDLT] × 100. MDCT-based improvement after LDLT was considered when RRGV measured at 5 (range, 1–28) months after LDLT was ≥20%. On the other hand, values less than 20% were considered to represent no change in the diameter of the inflow vessels, while RRGV values less than 0% reflected deterioration of the inflow vessels.

Databases of all images obtained in this study (online atlases) were used as reference libraries for our intelligent workstation.

Data analysis

Differences between patients with improvement or no change in the F score, RC sign, and RRGV were examined for statistical significance by the Mann-Whitney *U* test and χ-squared test where appropriate. A *P* value less than 0.05 was considered statistically significant. Univariate analysis was used to determine those factors associated with failure of improvement of EVs after LDLT. All *P* values less than 0.05 by two-tailed tests were considered significant. The tested factors included age, sex, background disease, PV pressure, MELD score, Child-Pugh score, right or left of graft, graft weight, graft volume, time of endoscopic examination after LDLT, alanine aminotransferase after LDLT, PV thrombosis, alcohol intake after LDLT, and the rate of reduction of major inflow vessel diameters. All statistical analyses were performed using the SPSS program (version 7.5, SPSS, Chicago, IL, USA).

Results

Improvement of EVs after LDLT

Changes in EVs were assessed by endoscopy. Among the four major factors that describe the status of EVs [i.e., location (L), form (F), and red color (RC) sign], we focused on F and RC since these two factors correlate with the status of EVs (ruptured or intact EVs).

Table 2. Esophageal varices grade (examined by endoscopy) and inflow vessel diameter (determined by CT examination) in 35 patients before and after LDLT

	Endoscopy				MDCT									
		EVs		Time from		Major inflow		LGV		PGV		SGV		Time from
No	Judge	Before	After	LT	Judge	vessel	RRGV	Before	After	Before	After	Before	After	LT
1	I	F2RC0	F0RC0	5	I	LGV	71.9	10.7	3.0	ND	ND	3.2	3.0	5
2	I	F3RC1	F1RC0	12	I	LGV	61.6	15.4	5.9	ND	ND	4.4	3.6	7
3	I	F2RC0	F1RC0	23	I	LGV	48.7	8.2	4.2	ND	ND	3.4	3.2	5
4	I	F2RC0	F0RC0	16	I	LGV	45.4	8.2	4.5	ND	ND	ND	ND	4
5	I	F2RC1	F1RC0	56	I	LGV	44.5	4.0	2.2	ND	ND	3.2	2.7	28
6	I	F1RC0	F0RC0	24	I	LGV	42.0	10.0	5.8	ND	ND	3.2	3.0	7
7	I	F2RC3	F1RC0	5	I	LGV	40.4	6.7	3.9	ND	ND	3.1	2.9	1
8	I	F1RC0	F0RC0	5	I	PGV	40.0	ND	ND	5.0	3.0	4.4	3.6	15
9	I	F2RC0	F0RC0	12	I	LGV	37.9	5.8	3.6	ND	ND	ND	ND	5
10	I	F2RC1	F1RC0	1	I	LGV	35.0	6.6	4.3	ND	ND	3.2	3.0	17
11	I	F3RC0	F1RC0	30	I	PGV	31.9	ND	ND	7.6	5.2	4.6	4.0	10
12	I	F1RC1	F0RC0	6	I	LGV	26.9	13.3	9.7	ND	ND	5.2	4.7	7
13	I	F2RC2	F0RC0	10	I	LGV	25.8	12.0	8.9	ND	ND	7.2	3.2	5
14	I	F3RC1	F1RC1	2	I	PGV	25.7	ND	ND	4.4	3.2	3.4	3.2	15
15	I	F1RC0	F0RC0	14	I	LGV	25.3	4.9	3.6	ND	ND	ND	ND	9
16	I	F2RC1	F1RC0	1	I	LGV	24.2	14.5	11.0	ND	ND	3.2	3.0	10
17	I	F1RC0	F0RC0	2	I	LGV	20.1	4.0	3.0	ND	ND	ND	ND	10
18	I	F1RC0	F0RC0	11	NC	PGV	19.3	ND	ND	3.1	2.5	2.6	2.3	6
19	I	F2RC2	F0RC0	15	NC	LGV	18.3	4.9	4.0	ND	ND	3.2	2.8	4
20	I	F1RC0	F0RC0	1	NC	LGV	15.5	5.8	4.9	ND	ND	5.2	4.7	5
21	I	F1RC0	F0RC0	11	NC	PGV	15.3	ND	ND	13.0	11.0	ND	ND	2
22	I	F2RC0	F0RC0	16	NC	LGV	13.1	5.9	5.1	ND	ND	3.1	2.9	2
23	I	F1RC0	F0RC0	8	NC	LGV	12.3	5.4	4.7	ND	ND	ND	ND	5
24	I	F1RC0	F0RC0	7	NC	LGV	8.1	5.3	4.8	ND	ND	ND	ND	2
25	I	F2RC0	F1RC0	43	NC	LGV	8.1	4.2	3.8	ND	ND	3.2	3.1	9
26	I	F2RC0	F0RC0	3	NC	PGV	8.0	ND	ND	9.4	8.6	5.3	4.0	2
27	I	F1RC0	F0RC0	11	NC	LGV	7.7	6.7	6.2	ND	ND	5.2	4.2	6
28	I	F2RC0	F0RC0	29	NC	LGV	7.2	9.7	9.0	ND	ND	ND	ND	4
29	I	F1RC0	F0RC0	21	NC	LGV	5.6	5.3	5.0	ND	ND	ND	ND	7
30	NI	F2RC1	F2RC0	27	NC	LGV	4.2	6.6	6.3	ND	ND	4.4	3.6	10
31	I	F1RC0	F0RC0	37	D	LGV	−2.3	3.8	3.9	ND	ND	ND	ND	2
32	NI	F2RC1	F2RC1	32	D	PGV	−7.5	ND	ND	3.2	3.4	ND	ND	8
33	NI	F1RC0	F1RC0	37	D	PGV	−14.3	ND	ND	3.2	3.7	2.9	3.0	15
34	NI	F2RC0	F2RC0	3	D	LGV	−14.4	5.8	6.6	ND	ND	3.1	3.1	12
35	NI	F1RC0	F1RC0	15	D	LGV	−20.5	3.4	4.1	ND	ND	ND	ND	5

CT, computed tomography; EVs, esophageal varices; I, improved; NI, not improved; NC, no change; D, deterioration; ND, not detected; LT, liver transplantation

Before LDLT, F3 varices were observed in three patients, F2 varices in 17, and F1 varices in 15 (Table 2). After LDLT, EVs improved from F3 to F1 in three patients, from F2 to F1 in six, from F2 to F0 in eight, and from F1 to F0 in 13. Thus, the F factor improved in 30 of 35 (86%) patients after LDLT. Figure 1 shows a representative case, illustrating improvement of EVs, F3 varices improved to F1 varices, without any other treatment apart from LDLT. On the other hand, the remaining five (14%) patients did not show any improvement in the F factor after LDLT (Fig. 2).

With regard to the RC sign before LDLT, RC3 was observed in one patient, RC2 in two patients, RC1 in eight, and RC0 in 24 patients. After LDLT, the RC sign improved from RC3 to RC0 in one patient, from RC2 to RC0 in two, and from RC1 to RC0 in six. The RC factor was RC1 and RC0 before LDLT in two and 24 patients, respectively, and remained unchanged postoperatively. Thus, the RC factor improved or remained at RC0 in 33 of 35 (94%) patients and did not change in two of 35 (6%) patients (Fig. 3).

Gastric varices were identified in six of 35 patients. In all six patients, the F factor improved from F1 to F0, while the RC sign before LDLT was RC0 and did not change after LDLT (data not shown).

Reduced blood flow to EVs after LDLT based on MDCT assessment

For objective assessment of post-LDLT improvement of EVs, we investigated the effects of LDLT on blood flow to EVs by measuring the diameter of EV inflow vessels. The major inflow vessel of EVs was determined by MDCT imaging. The major inflow vessel was LGV in 27 patients, PGV in eight patients, and SGV in no patients. Before LDLT, the median diameter of LGV was 5.87 mm, that of PGV was 4.71 mm, and that of SGV was 3.2 mm. After LDLT, the median diameter of LGV was 4.69 mm, that of PGV was 3.54 mm, and that of SGV was 2.70 mm. In order to analyze the change of the inflow vessel after LDLT, we calculated the rate of reduction of the inflow vessel diameter of the gastric vein (i.e., RRGV) by measuring the diameters of major inflow vessels before and after LDLT. As shown in Table 2, RRGV was <0% in five patients, 0%–10% in seven, 10%–20% in six, 20%–30% in six, 30%–40% in three, 40%–50% in six, and >50% in two patients. These results indicate that LDLT resulted in attenuation of blood flow to EVs in 17 of 35 (49%) patients, no change in 13 of 35 (37%) patients, and worsening in 5 of 35 (14%) patients.

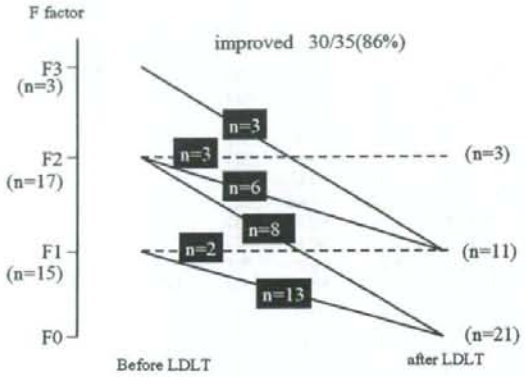

Fig. 2. Improvement of F factor of EVs. The EVs were examined endoscopically before and after LDLT. The form of EV was categorized into F0, F1, F2, or F3. *Solid line*, improved patients; *broken line*, no change in patients

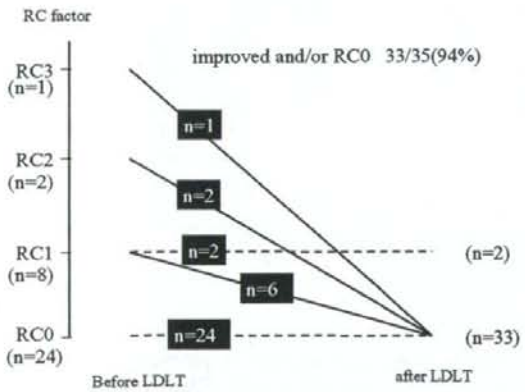

Fig. 3. Improvement of red color (*RC*) sign of EVs. EVs were examined endoscopically before and after LDLT. The RC sign was categorized into RC0, RC1, RC2, or RC3

Relationship between endoscopic EVs and MDCT findings

Since the amount of blood inflow to EVs affects the shape and properties of EVs, we evaluated the relationship between improvement of endoscopic findings and the narrowing of the inflow vessel by MDCT. Seventeen patients showed improvement in both EVs and inflow vessel diameter. On the other hand, 12 patients showed improvement of EVs on endoscopic variables (F and RC) but no improvement in inflow vessel diameter. One patient showed endoscopic improvement of EVs and deterioration of the inflow vessel. Another patient showed no changes in either EVs or the inflow vessel diameter. Finally, four of 35 (11.4%) patients showed no change of EVs and deterioration of the inflow vessel (Table 3).

Clinical features of patients without improvement in EVs after LDLT

We attempted to identify the mechanisms of portal hypertension in the five patients who showed no improvement in EVs after LDLT. The first patient (case 33) showed recurrence of HCV infection. Two months after LDLT, this patient was treated with peginterferon α-2b plus ribavirin for 9 months. However, the treatment was discontinued due to anemia and general

Table 3. Relationship between improvement of esophageal varices by endoscopy and improvement of inflow vessel diameter by MDCT

Improvement of both EVs and inflow vessel diameter	49% (17/35)
Improvement of EVs but no change in inflow vessel diameter	34% (12/35)
Improvement of EVs and deterioration of inflow vessel diameter	3% (1/35)
No changes in EVs and inflow vessels	3% (1/35)
No change in EVs and deterioration of inflow vessel diameter	11% (4/35)

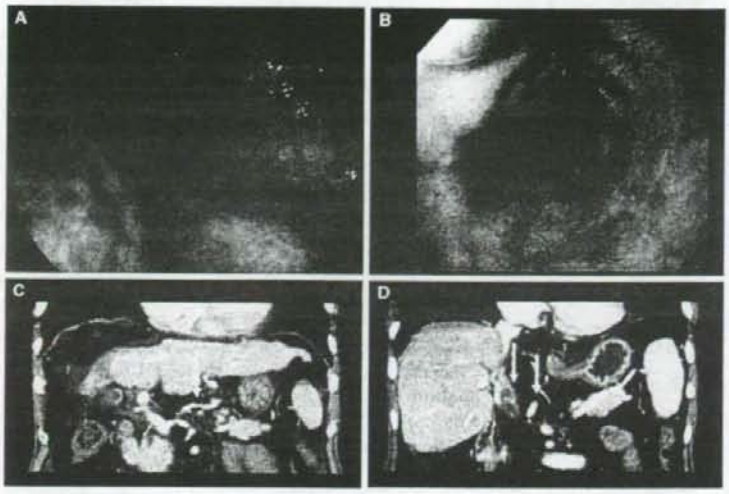

Fig. 4A–D. Endoscopic view of the EVs demonstrating no change after LDLT (case 30). The form of EVs before LDLT was F2 (**A**), and remained as F2 at 27 months after LDLT (**B**). Narrowing of the LGV on MDCT was not seen after LDLT. The diameter of the LGV in this patient was 6.6 mm before LDLT (**C**; *arrow*, LGV), and decreased to 6.3 mm at 10 months after LDLT (**D**), with a rate of reduction of the LGV of 4.2% (*left arrow*, portal vein thrombosis; *right arrow*, LGV)

fatigue. After discontinuation of interferon treatment, the hepatitis worsened, and eventually the patient acquired liver cirrhosis. The second patient (case 35) suffered from autoimmune hepatitis and developed stricture of the portal vein 1 year after LDLT. The third patient (case 30) had alcoholic liver cirrhosis and developed PV thrombosis 6 months after LDLT. The PV thrombosis did not improve despite anticoagulant therapy (Fig. 4). The fourth (case 32) had hepatocellular carcinoma (HCC) with HCV. He had recurrence of HCC in the transplanted liver 1 year after LDLT. HCC occupied a large area of the liver, which caused hepatic failure. The fifth patient (case 34) had alcoholic liver cirrhosis, and she continued to drink alcohol after LDLT. In these cases, portal hypertension recurred and EVs did not improve after LDLT.

Underlying factors for failure of improvement in EVs after LDLT

In the next step, we used univariate analysis to identify those factors associated with failure of improvement in EVs after LDLT. Since this study was conducted retrospectively, it was difficult to make the interval of endoscopic examination equal among the cases. Therefore, we classified the cases into two groups according to the interval (≤1 year and >1 year) and evaluated the relationship between LDLT and EV recurrence. Univariate analysis identified 14 parameters associated with failure of improvement in EVs after LDLT.

In the ≤1 year group, age, sex, background disease, PV pressure, MELD score, Child-Pugh score, right or left graft, graft weight, graft volume, time of endoscopic examination after LDLT, alanine aminotransferase after LDLT, PV thrombosis, alcohol intake after LDLT, and the rate of reduction of major inflow vessel diameter were not significantly different between patients with and without improvement of EVs after LDLT (Table 4). On the other hand, in the >1 year group, there were significant differences in the rate of reduction of major inflow vessel diameter and Child-Pugh score between patients with and without improvement of EVs after LDLT (Table 4).

Discussion

Liver transplantation is a definitive and final treatment for patients with end-stage hepatic failure. Thanks to great progress in operative techniques and the development of new immunosuppressants and antiviral agents, the prognosis of patients with end-stage hepatic failure after liver transplantation has improved, with an estimated survival rate of 65%–90% at 3 years and 65%–70% at 5 years in liver transplant recipients.[15–17] However, patients with decompensated cirrhosis often develop various complications, such as ascites, hepatic encephalopathy, and esophageal varices.

The present study showed that LDLT per se improved EVs, as demonstrated endoscopically by improvement in the F factor in 30 of 35 (86%) patients and the RC factor in 33 of 35 (94%) patients (Figs. 2 and 3). The beneficial effects of LDLT on EVs were also demonstrated by improvement in the diameters of inflow vessels of EVs and the rate of reduction of the vein diameter. Matsutani et al.[18] reported that hepatofugal

Table 4. Characteristics of patients who showed improvement and no change in endoscopic esophageal varices after LDLT according to the interval to endoscopic examination

	Time of endoscopic examination (≤1 year) ($n = 19$)			Time of endoscopic examination (>1 year) ($n = 16$)		
	Improvement after LDLT ($n = 18$)	No change after LDLT ($n = 1$)	P value	Improvement after LDLT ($n = 12$)	No change after LDLT ($n = 4$)	P value
Age (years)[a]	52 (27–69)	46	NS	51 (38–63)	54 (32–66)	NS
Sex (male/female)	(13/5)	(0/1)	NS	(10/2)	(2/2)	NS
Original diagnosis (HCV/HBV/AIH/PBC/alcoholic)	(10/4/1/1/2)	(0/0/0/0/1)	NS	(5/6/1/0/0)	(2/0/1/0/1)	NS
With hepatocellular carcinoma	7	0	NS	9	0	NS
Portal vein pressure (mmHg)[a]	25 (20–40)	22	NS	36 (22–42)	38 (37–39)	NS
MELD score[a]	16.0 (10.1–35.7)	15.9	NS	18.3 (11.9–44.7)	17.5 (11.9–30.9)	NS
Child-Pugh score[a]	9 (5–14)	9	NS	10 (7–12)	12 (10–14)	0.049
Graft (right/left)	(17/1)	(1/0)	NS	(10/2)	(4/0)	NS
Graft weight (g)[a]	618 (350–1022)	762	NS	614 (398–1142)	612 (566–632)	NS
Graft volume (ml)[a]	718 (467–1104)	677	NS	766 (381–1221)	632 (620–770)	NS
Alanine aminotransferase (IU/l)[a] after LDLT	49 (19–519)	29	NS	46 (11–164)	(26–686)	NS
Portal vein thrombosis	0	0	NS	1	1	0.05
Alcohol intake after LDLT	0	1	NS	0	0	NS
RRGV[a]	24.3 (7.7–72.0)	–14.4	NS	42 (–2.4 to 48.8)	–10.2 (–20.6 to 4.3)	0.005

RRGV, reduction rate of gastric vein; NS, not significant
[a]Values are median (range)