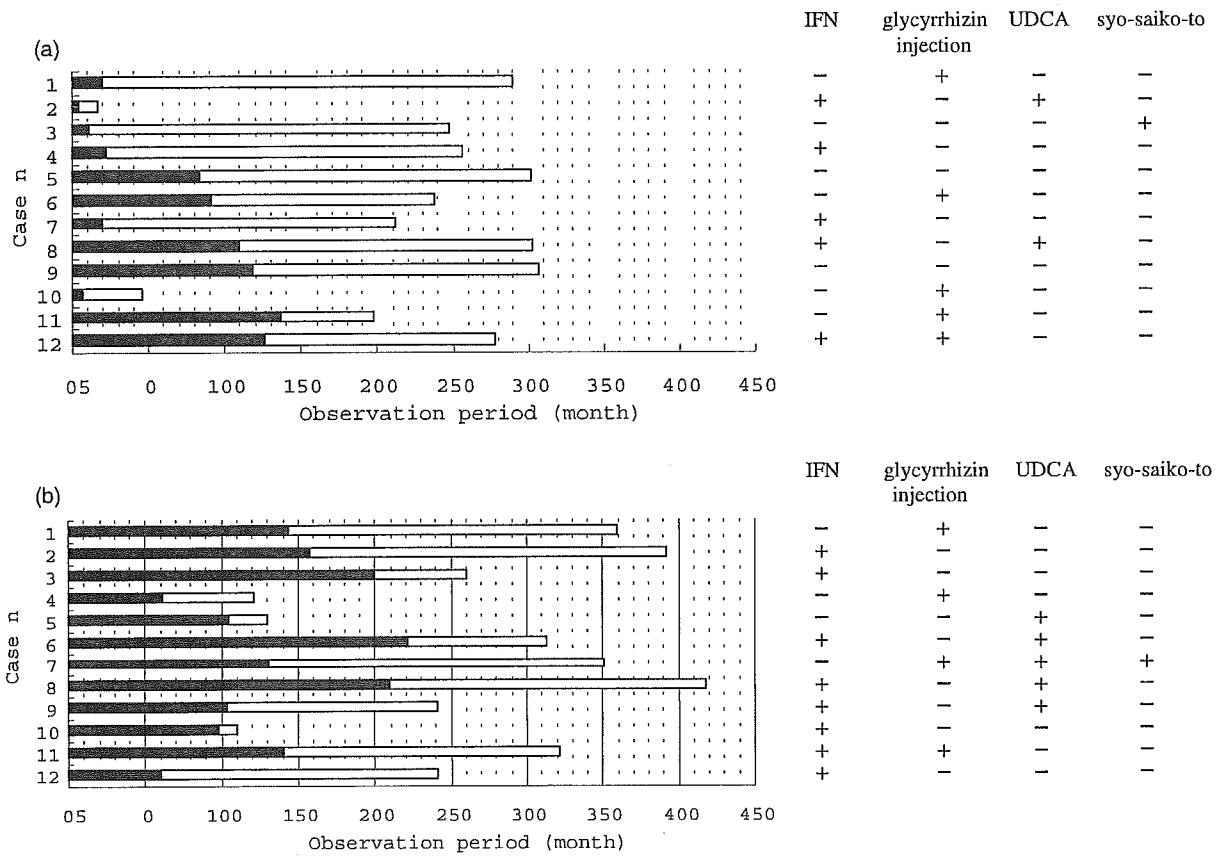

Figure 1 Seroconversion from (■) positive HBeAg to (□) positive anti-HBe in pair-matched groups by gender and age comprising (a) 12 genotype B and (b) 12 genotype C patients with chronic hepatitis B who were positive HBeAg at initial consultation. Given therapies for each patient are listed on the right. IFN, interferon; UDCA, ursodeoxycholic acid 300–600 mg/day; syo-saiko-to, Japanese herbal medicine containing glycyrrhizin.

Table 3 BCP and PC mutations in sera of 31 patients with chronic hepatitis B

	Genotype B ($n = 11$)	Genotype C ($n = 20$)	P
BCP (1762A/1764T) +/–	2/9	15/5	0.0068
1858C +/–	1/10	0/20	0.3548
PC (1896A) +/–	9/2	7/13	0.0233

BCP, basic core promoter; PC, precore.

Orito *et al.*[30] In our study, AST/ALT levels in genotype B patients were not statistically different to those in genotype C patients at initial consultation. This result is inconsistent with those obtained in other studies in Japan[32] and in Taiwan,[19] where genotype C patients had higher AST/ALT levels. This may be due to the relatively small number of examined cases in the present study.

There was no statistical difference in HBV viral load between genotype B and C patients. Orito *et al.* found a higher HBV viral load (5.87–5.02 LGE/mL) in 610 genotype C patients compared to 88 genotype B patients with type B chronic liver diseases.[30] Therefore many more cases with chronic hepatitis B seem to be needed to reach a final conclusion concerning this matter.

Histologically, genotype B patients had a slightly enhanced level of lobular necroinflammation than genotype C patients. In contrast, genotype C patients had more severe portal inflammation and fibrosis when compared to genotype B patients. These differences need to be more strictly reconfirmed by a larger number

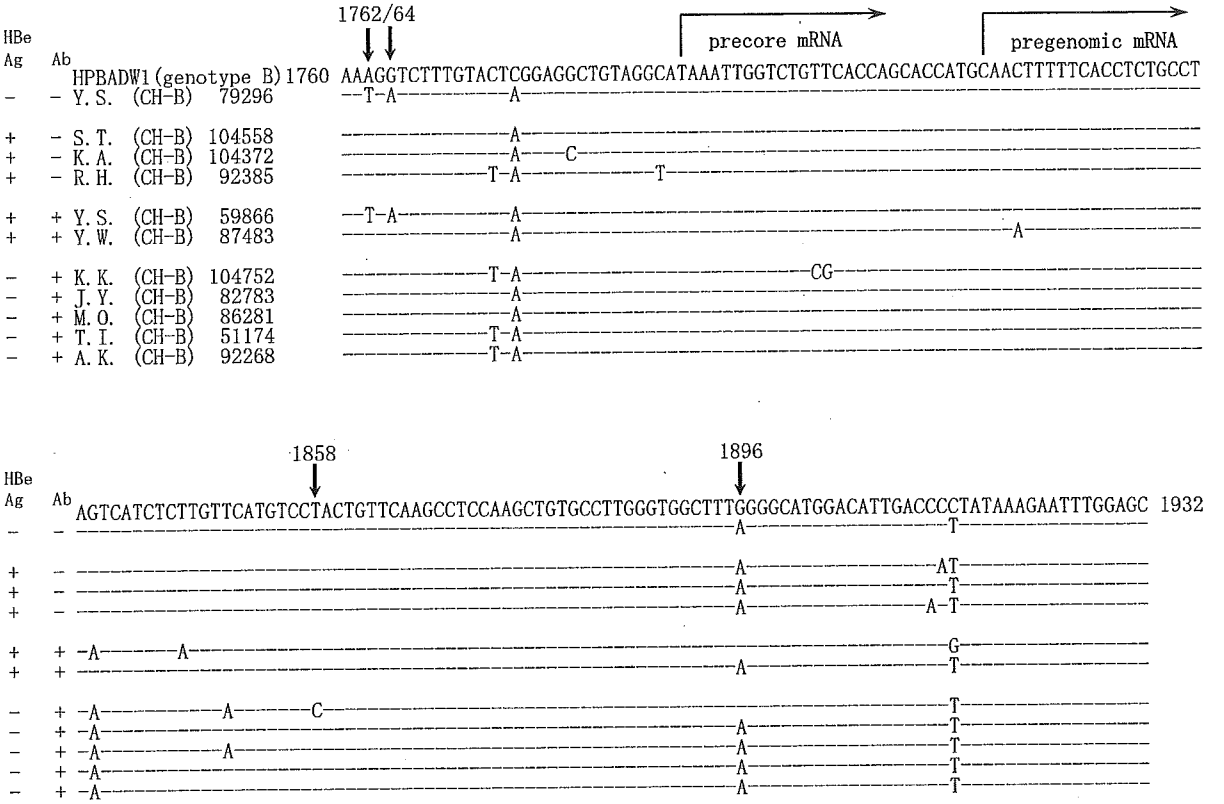

Figure 2 Nucleotide sequences of the basic core promoter and precore regions of genotype B hepatitis B virus (HBV) strains that were recovered from 11 patients with chronic hepatitis B. HPBADW1, reference sequence for genotype B HBV (uppermost line). These sequences are divided into four subgroups according to the HBeAg/anti-HBe status as shown in the left-hand columns. The nucleotide positions 1762, 1764, 1858 and 1896 are indicated by arrows. The precore and pregenomic mRNA are indicated by thin arrows.

of cases because the number of histologically examined genotype B patients was much fewer (10 cases) than that of genotype C patients (58 cases). There still remains a possibility that genotype B patients tended to have visited Niigata University Hospital due to the temporary flare-up of transaminases while genotype C patients did not have such a tendency.

Genotype B patients positive for HBeAg at initial consultation had significantly earlier seroconversion than their pair-matched genotype C patients (Fig. 1) despite the fact that they apparently had fewer therapeutic sessions. This difference may indicate that the outcome of chronic hepatitis B can be largely influenced by HBV genotype. It is an already established fact that HBeAg seroconversion usually precedes the emergence of PC mutation at nucleotide 1896.[31] More precisely, the emergence of PC mutation occurs independently but follows the HBeAg seroconversion according to the studies by Maruyama *et al.*[4,5] Moreover, the core promoter region of the HBV genome had also been proposed to be very strongly related to the disease severity and activity.[32] Thus, sequence analysis of these regions was thought to be necessary to further determine the genotypic difference in terms of HBeAg seroconversion.

Recently, Sumi *et al.* found that genotype B patients were older than genotype C patients when they were restricted to those with advanced fibrosis (F3 and F4) and hepatocellular carcinoma.[33] They also noted that the cumulative HBeAg seroconversion rate in chronic liver diseases was significantly higher in genotype B patients than in genotype C patients. Our data concerning age and HBeAg seroconversion are consistent with their data, which were deduced from a far larger number of examined cases than ours. However, they noted that the lifelong risk of progression to advanced fibrosis and development to hepatocellular carcinoma may not differ among genotype B- and C-related chronic liver disease. Thus, further follow up of cases may be needed to verify the prognostic difference among genotype B- and C-related chronic liver diseases.

According to the obtained data concerning HBeAg seroconversion in the present study, genotype B HBV had earlier HBeAg seroconversion, higher incidence of PC mutation resulting in positive anti-HBe, whereas genotype C HBV had later HBeAg seroconversion, and higher incidence of BCP mutation resulting in positive HBeAg.

In European countries where the genotypes A and D HBV prevail, infection by genotype A HBV has been

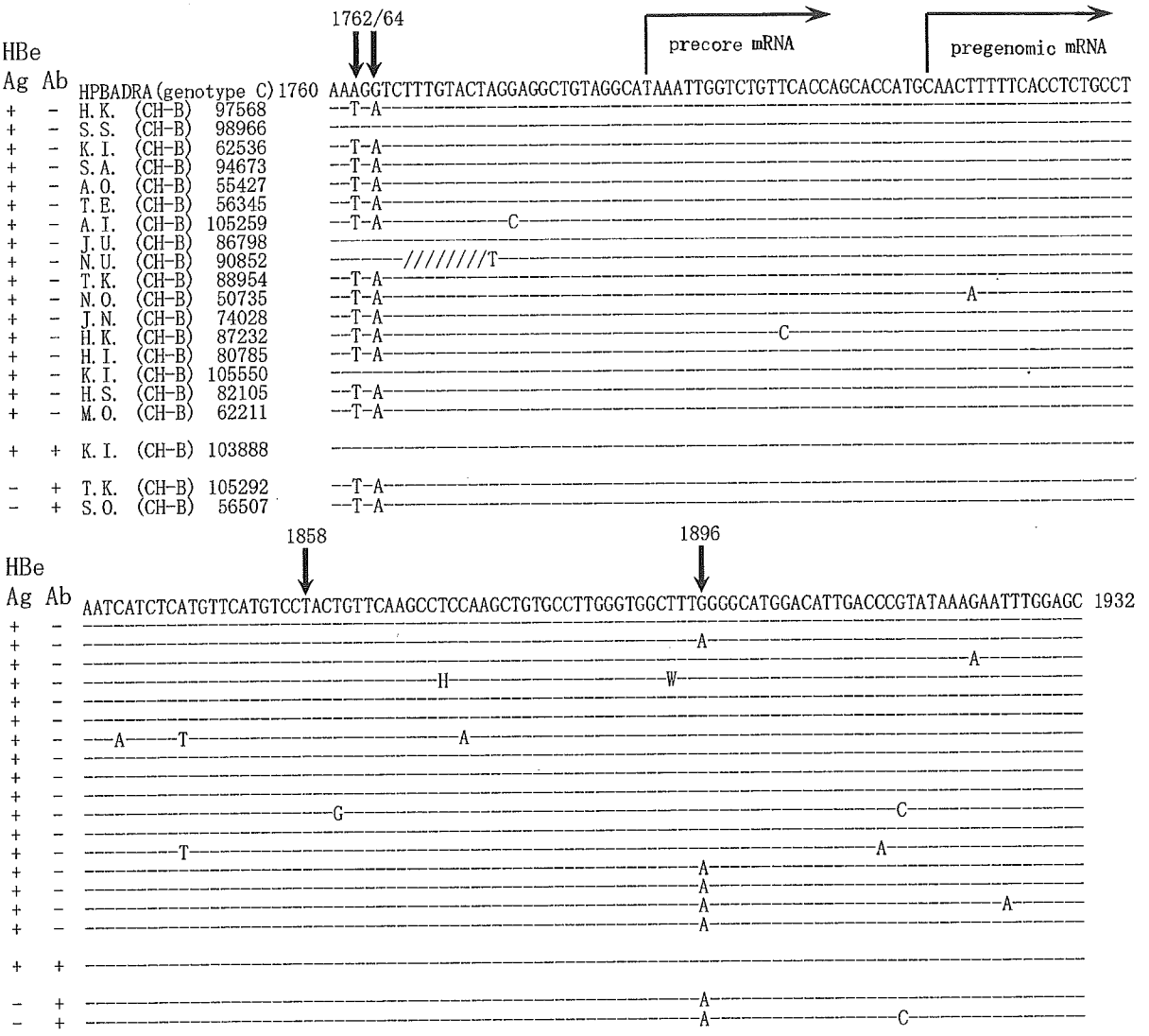

Figure 3 Nucleotide sequences of basic core promoter and precore regions of genotype C hepatitis B virus (HBV) strains that were recovered from 20 patients with chronic hepatitis B. HPBADRA, reference sequence for genotype C HBV (uppermost line). These sequences are divided into three subgroups according to the HBeAg/anti-HBe as shown in the left-hand columns. The nucleotide positions 1762, 1764, 1858 and 1896 are indicated by arrows. The precore and pregenomic mRNA are indicated by thin arrows. H, A/T/C; W, A/T.

closely related to the persistently positive HBeAg status.[18] Consequently, genotype A patients with chronic hepatitis B have often been thought to have more severe and prolonged inflammation in liver histology and a lower seroconversion rate when compared to genotype D patients. This can be explained by a single nucleotide replacement at position 1858 from T to C, which is complementary to the PC mutation site of 1896 in the stem-loop structure of the encapsidation signal of the HBV genome. In the present study, however, the majority of HBV strains had T instead of C at the nucleotide position 1858, irrespective of HBV genotypes (B or C). Thus, a difference we found in the incidence of PC mutation at 1896 between genotype B and C HBV cannot be explained by the 1858 nucleotide. Factors other than 1858C must be looked for to explain this difference in the incidence of PC mutation between genotype B and C HBV in Japan.

The significance of BCP and/or PC mutations in the HBeAg seroconversion, the persistence of HBV infection, or the replication of HBV has been studied, by many researchers, in relation to the disease activity.[34–42] The double mutation of BCP, 1762T/1764A may downregulate the PC messenger RNA transcription, resulting in a low HBeAg titer in blood.[39] Some investigators reported that this mutation might cause enhanced replication of the HBV particles by increasing the encapsidation efficiency[10] of the virus. Our results

highlighted a possible causative link between this peculiar mutation and the clinicopathological aspects of genotype C HBV infection that showed more severe portal inflammation and fibrosis, more delayed acquisition of anti-HBe, and more prolonged disease activities.[19,30,43] This possibility was further strengthened in the present study by the fact that portal hepatitis and fibrosis in genotype C patients were more severe than those in genotype B patients. Our study revealed exact differences in the incidence of both BCP and PC mutations between genotype B and C HBV, even though it had a cross-sectional nature. The genotype B HBV preferentially had PC mutation without BCP mutation, whereas most genotype C HBV strains in the present study possessed BCP mutation in the paucity of PC mutation.[43] Because PC mutation without BCP mutation follows the cease or markedly reduced production of HBeAg and core protein,[45] seroconversion from HBeAg to anti-HBe occurs with the stabilization of serum AST/ALT levels in most instances. In contrast, BCP mutation irrespective of PC mutation may be a key event to induce an enhanced, or at least unchanged rate of viral replication by more efficient encapsidation of the virus,[10] and is associated with the reduction of HBeAg production as previously described.[38] Consequently, this BCP mutation would give HBV a chance to persist longer and more easily in the host, therefore often inducing long-standing active inflammation and tissue damage, and eventually leading to liver cirrhosis and finally to hepatocellular carcinoma. The BCP mutation is recently reported to be an independent risk factor for development of HCC irrespective of HBV genotypes.[45] One genotype C HBV strain had a nucleotide deletion at nucleotide position 1767–1774 in the BCP, the same deletion has already been reported in Japanese patients with chronic hepatitis B.[46] These mutation and/or deletion events in the BCP region are a key phenomenon for viral persistence regardless of the PC mutation, which have been proven to associate more frequently with genotype C HBV infection in the present study.

The impact of this genotypic difference in chronic HBV infection must be emphasized because it is able to serve as one of the predicting factors for outcome and disease severity in chronic hepatitis B. It should also be extended to an attempt to analyze responses to known therapeutic modalities including steroids, interferon, or recently introduced lamivudine. The comprehensive understanding of the HBV genotype in future studies would help us in making more sophisticated therapeutic decisions and give us a perspective in the clinical course of chronic hepatitis B of any kind.

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Original Paper

Preoperative Human-Telomerase Reverse Transcriptase mRNA in Peripheral Blood and Tumor Recurrence in Living-Related Liver Transplantation for Hepatocellular Carcinoma

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ABSTRACT

Background/Aims: In this study we evaluated the potential role of preoperative h-TERT mRNA expression in peripheral blood as a tool for predicting prognosis and tumor recurrence after living-related liver donor transplantation (LRLDT).

Methodology: The study included patients with unresectable HCC who underwent LRLDT from July 1999 to May 2003.

Results: There was no significant difference between the survival curves of those patients who met the Milan criteria and those who did not. However, there was a statistically significant difference ($p=0.032$) between the survival curves of those patients with positive preoperative h-TERT mRNA expression, and those who either had an initially negative preoperative h-TERT mRNA or who converted from positive to negative after neoadjuvant immunochemotherapy.

Conclusions: In conclusion, the presence or absence of h-TERT mRNA in the peripheral blood may be a useful criterion in evaluating HCC patients for transplantation, as well as a valuable method of assessing anti-tumor therapy and tumor relapse.

KEY WORDS: Liver transplantation; Hepatocellular carcinoma; Circulating cancer cell; h-TERT mRNA; Immunosuppression

ABBREVIATIONS: Human-Telomerase Reverse Transcriptase (h-TERT); Living-Related Liver Donor Transplantation (LRLDT); Hepatocellular Carcinoma (HCC); α-Fetoprotein (AFP); Donor-Specific Transfusion (DST)

INTRODUCTION

Tumor recurrence remains the major problem in liver transplantation performed for hepatocellular carcinoma (HCC) (1,2). One reason for tumor relapse is the dissemination of cancer cells from the native diseased liver into the patient's systemic circulation. Sato *et al.* (3) reported that the detection of α-fetoprotein (AFP) mRNA in peripheral blood using nested RT-PCR could be a useful prognostic predictor of HCC recurrence in this patient population. However, the specificity of this test was limited since AFP mRNA was detectable in the mononuclear cell fraction of dendritic cells and the cell source of this AFP mRNA was unclear.

Telomerase is a reverse transcriptase that has been implicated in the *de novo* synthesis of GGTTAG telomeric DNA on to chromosomal ends. This synthesis stabilizes genomic integrity, thus aiding in the immortalization of cancer cells. Investigators have shown that telomerase is reactivated in approximately 85% of malignant tumors of various types, but it remains inactive in most normal non-neoplastic somatic cells (4,5).

Human-telomerase reverse transcriptase (h-TERT) is a catalytic component of reverse transcriptase, and h-TERT expression is the rate-limiting step for telomerase activity. In a previous study, we reported that detection of h-TERT mRNA after immunomagnetic separation is a specific and sensitive tool to detect HCC cells. We postulated that our findings might warrant further investigation of h-TERT mRNA's use in detecting metastatic disease (6). In this study we evaluate the potential role of preoperative h-TERT mRNA expression in peripheral blood as a tool for predicting prognosis and tumor recurrence after living-related liver donor transplantation (LRLDT) in HCC patients.

METHODOLOGY

Quantitative h-TERET assay: Two mL of peripheral blood were drained and anticoagulated with EDTA before various treatments. The samples were immediately diluted with the same volume of modified PBS (PBS containing 0.5mM EDTA and 1% BSA) and separated into two aliquots (1 and 3mL) for negative and positive selection of epithelial cells,

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respectively. For the negative and positive selection, $8x10^7$ immunomagnetic beads covalently coated with anti-CD45 monoclonal antibodies (Dynabeads M450 CD45; Dynal A.S., Oslo, Norway), which react to panleukocytes, were added and gently rotated at 4°C for 30 min. The cells that bound to the beads were harvested using a magnetic field as an L-fraction. The cells in the supernatant were also collected by centrifugation at 3000 rpm for 5 min as an NL-fraction. For the positive selection, $2.4x10^7$ immunomagnetic beads covalently coated with Ber-EP4 monoclonal antibodies (CELLection Epithelial Enrich; Dynal A.S.) were mixed with 1mL of 3mL of the diluted blood. Ber-EP4 antibody recognizes various types of normal and malignant epithelial cells including approximately 67% of HCC. Incubation and collection were performed in the same manner as described in the negative selection. The cells in the supernatant were collected as an NE-fraction. The harvested cells in a magnetic field were resuspended with the 1-mL residual of the diluted sample, and the cells were harvested again. The same procedure was performed once more, and final harvested cells were collected as an E-fraction. Finally, the cells of the E- and L-fractions were washed twice with 1mL of modified PBS to clear up RBCs and resuspended in 300μL of modified PBS.

RT-PCR: Total RNA of each fraction was isolated using IsoGen LS (Wako, Osaka, Japan) according to the manufacturer's instructions and resuspended in 20μL of water treated with diethyl pyrocarbonate. After denaturation, cDNA synthesis was performed by incubating 200pmol of random hexaoligonucleotides and 7 units of RAV-2 reverse transcriptase (Takara, Otsu, Japan). Three μL of synthesized cDNA were subjected to PCR reaction after diluting the products to 60μL with diethyl pyrocarbonate-treated water. For amplification of h-TERT cDNA, nested PCR was executed. In the first PCR, 369-bp fragments from a 1435-1803 nucleotide position (GenBank accession number AF018167), which is not involved in reported alternatively spliced regions, were amplified in 10μL of a reaction mixture containing 5pmol of each primer (5'-AGGTGTACGGCTTCGTGCG-3' and 5'-TGCTCCAGACACTCTTCCGGTAG-3'), 0.2mM deoxynucleoside triphosphates, and 0.5 units of Taq DNA polymerase (Takara). Amplification was performed using a Thermal cycler MP (Takara) with 35 cycles as follows: denaturation at 98°C for 15s; annealing at 65°C for 30s; and extension at 72°C for 30s with a final extension at 72°C for 5 min. Three μL of 100-fold diluted first PCR products were subjected to a second PCR using primers 5'CAGGCACAACGAACGCCG-3' and 5'-CCTGAGCAGCTCGACGACGTAC-3', which amplify 235-bp fragments at a 1496-1730 nucleotide position. The second PCR program was the same as that of the first PCR, except for the annealing temperature at 67°C.

A 491-bp fragment of α chain of IL-2r cDNA at 422-912 nucleotide position was amplified in a 10-μL reaction mixture using primers 5'-AATGCACAAGCTCTGCCACTC-3' and 5'-GGCCACTGCTACCTGGTACTC-3'. The amplification cycles consisted of 35 cycles, denaturation at 94°C for 30s, annealing at 65°C for 30s, and extension at 72°C for 30s. As for h-TERT positive fractions, 3μL of 100-fold diluted first PCR products were subjected to a second PCR performed in the same manner as the first PCR. A 626-bp fragment of β-actin cDNA specific for active β-actin gene was amplified in the same manner as the previous report. Nested PCR for amplification of AFP messages was performed in the same way as the previous report using the cDNA prepared for h-TERT evaluation. After separation through 6% polyacrylamide gels, all PCR products were visualized by ethidium bromide staining.

Patients: The pre- and postoperative peripheral blood samples of 11 patients with unresectable HCC who underwent LRLDT in our institution from July 1999 to May 2003 were examined for the presence of h-TERT mRNA. Five patients were female; six patients were male. Ages ranged from 39 to 68 years with a median age of 55±8.3 years. Six patients had stage A HCC and deviated from the criteria set forth by Mazzaferro *et al.* (7), while the rest of the patients fell within the limitations of the Milan criteria. Median follow-up was 28 months with a rage of 15 to 60 months.

In addition to LRLDT, four patients were also offered three phases of medical therapy. Neoadjuvant immunochemotherapy consisted of 5-FU, Adriamycin, and Interferon β, as previously described (9). As adjuvant chemotherapy, patients were given 10mg of Adriamycin once a week. "Sandwich chemotherapy", also described previously (8), consisted of intraportal delivery of 250mg of 5-FU for three consecutive days, starting on Day 7 to 14 after LRLDT.

In total, three patients received postoperative sandwich chemotherapy. One patient did not receive neoadjuvant immunochemotherapy because of his poor preoperative condition. One patient did not receive sandwich chemotherapy because of a severe postoperative infection.

FK506 and prednisolone were used for immunosuppression following LRLDT. Additionally, donor blood was transfused via the recipient portal vein immediately after reconstruction of the hepatic artery. Thereafter, for eight weeks on a weekly basis, a donor-specific transfusion (DST) of 50-100mL was infused via the portal vein. Ten patients underwent repeated DST via the portal vein in an effort to reduce the level of required immunosuppressants (8,10-12).

Histologic analyses of the resected specimens were performed. The effects of neoadjuvant immunochemotherapy and adjuvant chemotherapy on the outcomes of patients who underwent LRLDT for HCC were examined. h-TERT mRNA expression in the peripheral blood was analyzed.

RESULTS

Histologic Analysis

All six resected tumors from patients who did not fit the Milan criteria had vascular invasion of vp 1-3,

and vv1 upon histologic analysis. Of those five patients who fell within the Milan criteria, the resected specimen from the h-TERT mRNA positive patient had vascular invasion, while the other four patients including Milan criteria had no vascular invasion. Tumor size ranged from 22mm to 280mm. Numbers of tumor nodules in the resected specimens ranged from two to 35.

Expression of h-TERT mRNA and Survival

The outcomes of each of the eleven patients are shown in **Figure 1**. h-TERT mRNA was detected preoperatively in five patients. Preoperative h-TERT mRNA disappeared in two of the four patients who received neoadjuvant immunochemotherapy. One of these patients still survives without recurrence for 60 months The other, patient died after seven months due to sepsis but was tumor-free and without evidence of Hepatitis B and C recurrence at the time of death. The other two patients who still had detectable levels of h-TERT mRNA after neoadjuvant immunochemotherapy died seven and eight months, respectively, after their LRLDT secondary to disseminated disease (pulmonary metastases). The sixth patient who fell within the Milan criteria did not receive neoadjuvant immunochemotherapy because of his poor preoperative status. Two months after LRLDT, he developed lung and graft metastases and ultimately died ten months after transplant.

These results were evaluated, isolating the effects of the Milan criteria and of h-TERT mRNA expression on survival **(Figures 2 and 3)**. There was no significant difference between the survival curves of those patients who met the Milan criteria and those who did not. However, there was a statistically significant difference (p=0.032) between the survival curves of those patients with positive preoperative h-TERT mRNA expression, and those who either had an initially negative preoperative h-TERT mRNA or who converted from positive to negative after neoadjuvant immunochemotherapy.

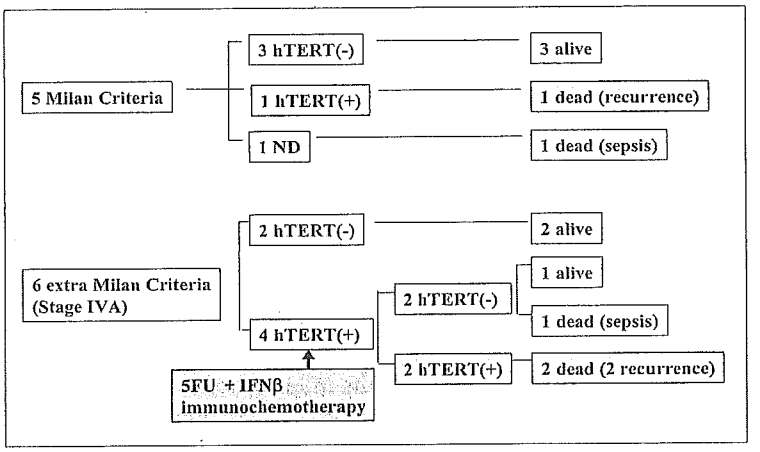

FIGURE 1 Outcomes of the survival of in eleven HCC patients who underwent LRLDT concerning Milan criteria and the expression of h-TERT mRNA.

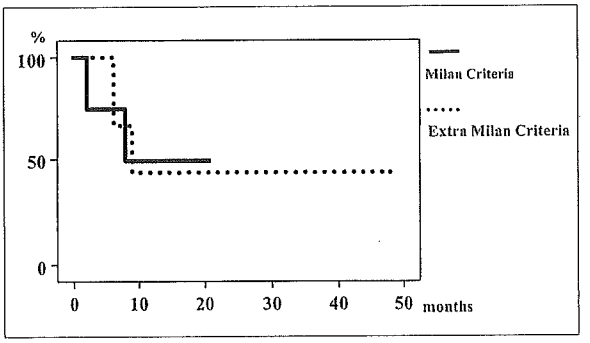

FIGURE 2 Comparison of survival curve between Milan criteria met group and extra Milan criteria group.

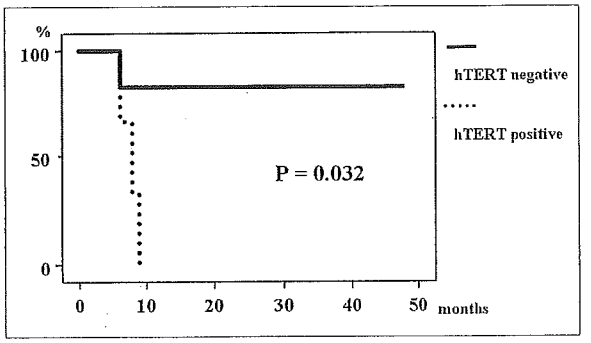

FIGURE 3 Comparison of survival curve between h-TERT mRNA positive and negative patients.

DISCUSSION

Because of the high rates of tumor recurrence, initial experiences with liver transplantation for HCC were disappointing. In an effort to codify selection criteria for these patients, investigators looked at the effect of different pathologic features, such as tumor size, number of nodules, intrahepatic vascular involvement and regional lymph node involvement, on patient survival. These factors were found retrospectively to have bearing on patient outcomes. Mazzaferro *et al.* applied these findings in a prospective trial and elucidated a set of specific tumor features favorable to survival that are now widely accepted as the Milan criteria (7). Since Mazzaferro's report, however, researchers have continued to search not only for ways to further refine these criteria but also for new factors that would improve patient selection. This has been particularly true in Japan where almost all liver transplantation is performed using living donors.

Two important reasons for tumor recurrence are the presence of clinically undetectable circulating cancer cells and the need for immune suppression after transplantation. Circulating cancer cells are believed to escape from the native, diseased liver and are often largely ignored because of a lack of detection techniques. With more precise preoperative detection of these errant cells, clinicians would presumably have a more accurate method of predicting prognosis after LRLDT for HCC. We chose to study h-TERT mRNA, believing it to be more specific than AFP mRNA as a marker for the presence of circulating cancer cells. In this study, all patients with h-TERT mRNA present in

their peripheral blood preoperatively had tumor relapse soon after LRLDT. Those two patients whose h-TERT mRNA expression disappeared after neoadjuvant immunochemotherapy did not develop tumor recurrence, much like the group that had negative h-TERT mRNA expression from the outset. Given these finding, h-TERT mRNA might prove valuable not only in the study of preoperative treatment modalities and in postoperative patient surveillance, but also in the evaluation of prospective LRLDT patients with HCC.

It has also been reported that neoadjuvant chemotherapy or adjuvant chemotherapy can prolong survival after liver transplantation for even those HCC patients outside of the Milan criteria. For this reason, in this study we used neoadjuvant immunochemotherapy and postoperative "sandwich chemotherapy" with our patients. It was our belief that this regimen could potentially reduce or eliminate the preoperative circulating cancer cells, thus preventing the development of postoperative metastatic disease.

Besides the presence of circulating microscopic disease, the need for immune suppressing drugs after transplantation can contribute to higher rates of tumor recurrence in HCC patients. Tumors are found to grow at an accelerated rate since immunosuppressive drugs can suppress the host immunity usually important in controlling the growth of micrometastases (1). In an effort to decrease the amount of immunosuppressant required, we performed intra- and postoperative transfusions of DST via the portal vein (12). In a previous study, we found that with DST, steroids could be withdrawn within one month of LRLDT. DST created a macrochimerism of donor CD56+NKT cells within the graft of HCC patients. We postulated that these cells might, in effect, act against host tumor cells as a kind of micro-transplant (8,10,11). If so, use of such techniques could potentially expand the indications for transplantation to those patients with more advanced HCC.

In conclusion, the presence or absence of h-TERT mRNA in the peripheral blood may be a useful criterion in evaluating HCC patients for transplantation, as well as a valuable method of assessing anti-tumor therapy and tumor relapse. Additionally, in liver transplant candidates who deviate from the Milan criteria, neoadjuvant immunochemotherapy and adjuvant chemotherapy may improve what might otherwise be poor prognoses by eliminating circulating microscopic disease.

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Leukocytapheresis Is Effective in Inducing But Not in Maintaining Remission in Ulcerative Colitis

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Goals and Background: Ulcerative colitis (UC) is a chronic inflammatory bowel disease characterized by dense infiltration of lymphocytes, plasma cells, neutrophils, and monocyte-macrophages into the colonic mucosa. Leukocytapheresis is a procedure for selectively removing white blood cells from withdrawn blood. It is used for the treatment of several autoimmune diseases. This study was performed to evaluate the effectiveness of leukocytapheresis for inducing and maintaining remission in corticosteroid-resistant UC, as compared with corticosteroid-responsive UC.

Study: Forty-five patients with active UC who were treated with a dose of 1 mg/kg per day or more of prednisolone given systemically for at least 2 weeks were evaluated. Twenty patients (6 males, 14 females) in whom improvement was induced only by high doses of prednisolone were allocated as the corticosteroid-responsive group. The other 25 patients (11 males, 14 females) who did not respond to the above-mentioned dose of prednisolone therapy were allocated as the corticosteroid-resistant group and received leukocytapheresis therapy once a week for 5 weeks. Of patients who had a remission, the corticosteroid-responsive group continued to have the conventional therapy and the corticosteroid-resistant group were given leukocytapheresis once every 4 weeks for at least 2 years as maintenance therapy.

Results: Remission was induced by 5 weeks of leukocytapheresis in 23 of the 25 (92%) patients with corticosteroid-resistant active UC. The number of days required to achieve remission of UC was fewer in patients who received leukocytapheresis than in those who did not. Follow-up study of the patients who had remission showed similar relapse rates at 2 years in the patients who received leukocytapheresis and those given high doses of prednisolone alone.

Conclusions: Leukocytapheresis is an effective treatment of acute corticosteroid-resistant UC but does not prevent the recurrence of UC.

Key Words: leukocytapheresis, maintenance, therapy, ulcerative colitis

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Ulcerative colitis (UC) is a nonspecific inflammatory disease that diffusely affects the mucosa of the colon and rectum. Microscopically, active UC is characterized by marked infiltration of the colorectal mucosa by leukocytes, including lymphocytes, plasma cells, monocytes/macrophages, and neutrophils. Inflammatory substances produced by these leukocytes, such as proinflammatory cytokines, free radicals, leukotrienes, and platelet activating factor, are thought to damage the colorectal mucosa in UC.[1–4] Many kinds of drugs, including 5-aminosalicylic acid (5-ASA), sulfasalazine, corticosteroid hormones, and immunosuppressive agents, are used to treat UC, and most are effective for inducing remission and preventing relapse.[5] However, lack of an adequate response to steroid treatment remains an important problem.[6] Recently, three techniques for leukocytapheresis, leukocyte apheresis with a leukocyte removal filter (Cellsorba EX, Asahi Medical Co, Ltd, Tokyo, Japan), centrifugal leukocyte apheresis (Multi Component System, Haemonetics Co., Braintree, MA), and granulocyte and monocyte absorption apheresis (Adacolumn, Japan Immunoresearch Laboratories, Takasaki, Japan), are used in Japan to treat moderate to severe and corticosteroid-resistant UC. Several studies have evaluated the efficacy of leukocytapheresis for the treatment of active UC.[7–10] To our knowledge, however, no study has reported whether leukocytapheresis is effective for the maintenance therapy for UC. This case-control study was designed to assess the effectiveness of leukocytapheresis in inducing and in maintaining remission in steroid-resistant UC compared with steroid-responsive UC in which remission was induced only by high doses of prednisolone.

MATERIALS AND METHODS

All patients in this study were admitted to Niigata University Hospital between 1995 and 2000 and gave informed consent for the study before therapy. Forty-five patients with active UC who were treated with a dose of 1 mg/kg or more per day of prednisolone given systemically for at least 2 weeks were evaluated. Twenty patients (6 males, 14 females) showing a clinical improvement in response to high doses of prednisolone continued to be given prednisolone systemically and were allocated as the corticosteroid-responsive group. The other 25 patients (11 males, 14 females) who did not respond to the above-mentioned dose of prednisolone therapy were allocated as the corticosteroid-resistant group and received leukocytapheresis therapy besides prednisolone. Most of the patients in both groups concurrently received 5-ASA

or sulfasalazine. Any other drugs, such as immunosuppressants or antimetabolites, were contraindicated by the protocol. UC was diagnosed on the basis of clinical, endoscopic, and histopathologic findings. All patients had active UC (24 total colitis and 21 left-sided colitis). Clinical disease activity was moderate to severe according to Truelove and Witts' disease severity index.[11] The average dose of prednisolone in the month before study entry ranged from 404 to 1468 mg. The clinical features of the subjects, mean daily dose of prednisolone at baseline, and total cumulative dose of prednisolone given during the month before the study are shown in Table 1. T1

Patients with corticosteroid-resistant UC received one of two kinds of leukocytapheresis: the first 11 patients received leukocytapheresis with a leukocyte removal filter (LCAP), starting in 1995, and the next 14 patients received centrifugal leukocytapheresis, starting in 1997. LCAP was performed with a Plasauto apheresis unit equipped with a Cellsorba leukocyte removal filter (Cellsorba EX, Asahi Medical Co, Ltd). Extracorporeal removal of peripheral leukocytes by LCAP is effected by adherence of leukocytes to the polyester non-woven fabric in the filter. Blood (3000 mL) was processed at a blood flow rate of 50 mL/min. Centrifugal leukocytapheresis was performed using a centrifugal separation apparatus (Multi Component System, Haemonetics Co). At each session, leukocyte-rich fractions of the buffy coat layers were extracorporeally removed from 2000 to 2400 mL of patients' peripheral blood, obtained via a cubital vein. Each patient received one session of apheresis per week for 5 consecutive weeks for the treatment of active UC.

The subjects of this study were broadly divided into 1) patients who received induction therapy and 2) patients who received maintenance therapy. Induction therapy with prednisolone therapy alone was given to 20 steroid-responsive patients (conventional therapy group), and 12 of 20 who had remission received the conventional maintenance therapy. Induction therapy with LCAP leukocytapheresis or with centrifugal leukocytapheresis was done in 11 patients and 14 patients in corticosteroid-resistant group, respectively. Twenty-three patients who had remission in response to either method of leukocytapheresis subsequently received leukocytapheresis once every 4 weeks as maintenance therapy. The patients were followed up for at least 2 years. Relapse rates were calculated and compared with those in the 12 patients in whom remission was induced only by treatment with high doses of prednisolone. The dosage of 5-ASA or sulfasalazine was not modified during the study in patients without side effects. To minimize the risk of steroid-related side effects, the dose of prednisolone during remission after leukocytapheresis or treatment with prednisolone alone could be gradually reduced in accordance with the status of UC during maintenance therapy.

The endpoint of this study was defined as the time of relapse of UC. Remission and clinical improvement were assessed on the basis of the following symptoms, evaluated according to Lichtiger's score: diarrhea, nocturnal diarrhea, visible blood in stools, fecal incontinence, abdominal pain, general well-being, abdominal tenderness, and need for antidiarrheal drugs.[12] Remission was defined as less than 4 bowel movements daily, no nocturnal diarrhea, no visible blood in stools for 3 days or longer, no fecal incontinence, no abdominal pain, excellent or very good general well-being, and no abdominal tenderness. Improvement was defined as clinical improvement in many clinical symptoms within 5 days after intensive treatment with prednisolone.[10] Relapse was defined as the presence of visible blood in stools for 3 or more consecutive days or the need for additional treatment to control symptoms. No patient was lost to follow-up.

The clinical features of the subjects are shown in Table 2. T2 The dose of prednisolone at relapse and the average cumulative dose of prednisolone given per month from remission until study endpoint were assessed for each patient in each group.

Statistical analysis was done with the use of log-rank tests and the Kaplan-Meier method. The results for prednisolone are presented as mean ± SD. Comparisons were made using unpaired Student t tests. P values of less than 0.05 were considered to indicate statistical significance.

TABLE 1. Characteristics of the Patients With Refractory Ulcerative Colitis

	Conventional	LCAP	Centrifugal
No.	20	11	14
Male:female	6:14	5:6	6:8
Mean age (yr)	33.0 ± 16.6	37.9 ± 15.2	29.5 ± 9.6
Disease duration (mo)	50.2 ± 60.3	68.4 ± 55.7	36.9 ± 50.1
Disease extent (left-sided:entire)	9:11	5:6	7:7
Inflammatory type (nonpseudopolyposis: pseudopolyposis)	8:12	4:7	7:7
Severity (severe:moderate)	7:13	3:8	5:11
Preceding administration of PSL (mg/mo)	404 ± 538	1325 ± 625	1468 ± 1426
PSL doses at base line (mg/kg)	1.2 ± 0.6	1.2 ± 0.6	1.3 ± 0.5
Remission rate (after 5 wk)	4/20 (20%)	10/11 (90.9%)	13/14 (92.8%)

RESULTS

Leukocytapheresis procedures were well tolerated by all patients. During active-stage therapy, clinical remission was achieved within 5 weeks in 10 of 11 patients by LCAP and in 13 of 14 patients by the centrifugal leukocytapheresis. Total remission rates after 5 sessions of leukocytapheresis were

TABLE 2. Characteristics of Patients With Refractory Ulcerative Colitis Who Received Maintenance Therapy

	Conventional	LCAP	Centrifugal
No.	12	10	13
Age (yr)	26.0 ± 12.0	33.9 ± 14.6	28.5 ± 9.09
Severity (severe:moderate)	4:8	3:7	4:9
Disease extent (left-sided:entire)	4:8	4:6	7:6
Inflammatory type (nonpseudopolyposis: pseudopolyposis)	5:7	4:6	7:6

92%. The time to remission after initial treatment was shorter in the leukocytapheresis group than in the prednisolone alone therapy group, in which 12 of the 20 patients had remission; 8 of the 12 patients had remission within 80 days and the other 4 after 80 days (Fig. 1). The period from the first procedure to the absence of blood in stools negatively correlated with the dose of prednisolone received at baseline (Fig. 2). However, follow-up study showed that patients in remission gradually had relapse during maintenance therapy in the leukocytapheresis group as well as in the prednisolone alone group (Fig. 3). Relapse rates in the groups became similar at 2 years. The dose of prednisolone received at the time of relapse was higher in the leukocytapheresis group than in the prednisolone alone group (Fig. 4). However, the average cumulative dose of prednisolone per patient for each month from remission until study endpoint was lower in the steroid-refractory patients who received leukocytapheresis for maintenance therapy than in the steroid-responsive patients (Fig. 5). Most patients with early relapse had diffuse and deep ulcers (pseudopolyposis). There were no serious side effects in this trial. Headache occurred during leukocytapheresis in 2 patients, and a fever of 38°C persisted for 1 day in 1 patient.

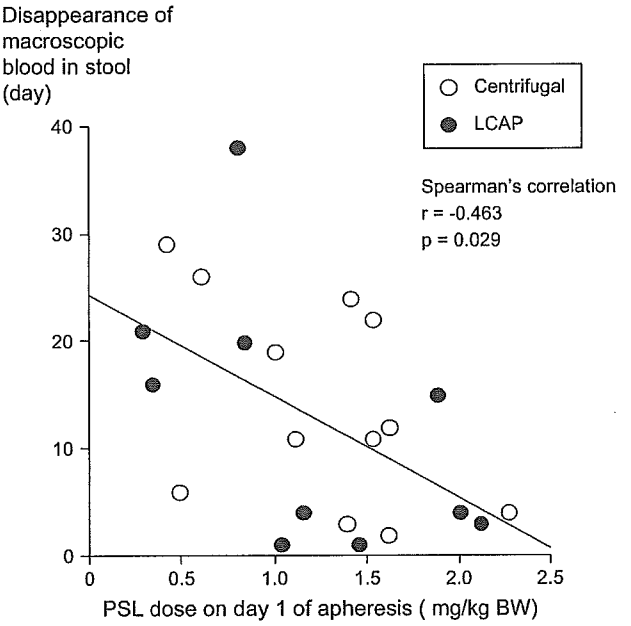

FIGURE 2. Correlation between the period taken until remission and doses of prednisolone taken at the baseline.

DISCUSSION

Leukocytapheresis has been used to treat autoimmune diseases such as multiple sclerosis, dermatomyositis, rheumatoid arthritis, and Crohn's disease.[13–16] Most patients with active UC respond to conventional treatment with 5-ASA, sulfasalazine, and corticosteroids. Nonetheless, some patients with UC are resistant to corticosteroid treatment, whereas others are steroid dependent and have relapse when the dose is reduced.[17] Such patients usually require immunosuppressive drugs, such as azathioprine, cyclosporine, 6-mercaptopurine, or methotrexate.[6] However, immunosuppressive therapy can cause serious side effects, including agranulocytosis, alopecia, and pancreatitis. More effective treatments capable of curing corticosteroid-resistant UC without serious complications, thereby eliminating the need for surgery, are needed. Recently, improved techniques for leukocytapheresis, producing remission rates of 60% to 80% even in patients with corticosteroid-resistant active UC, have become available in Japan. Our

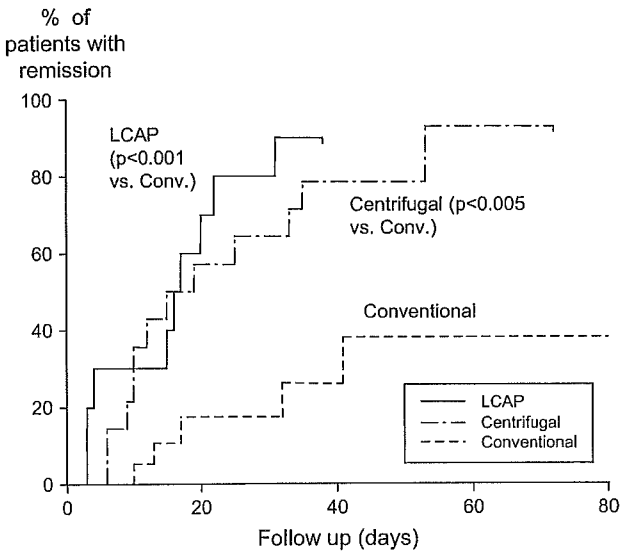

FIGURE 1. Induction rates of remission in active UC patients receiving prednisolone alone therapy, LCAP and centrifugal leukocyte apheresis.

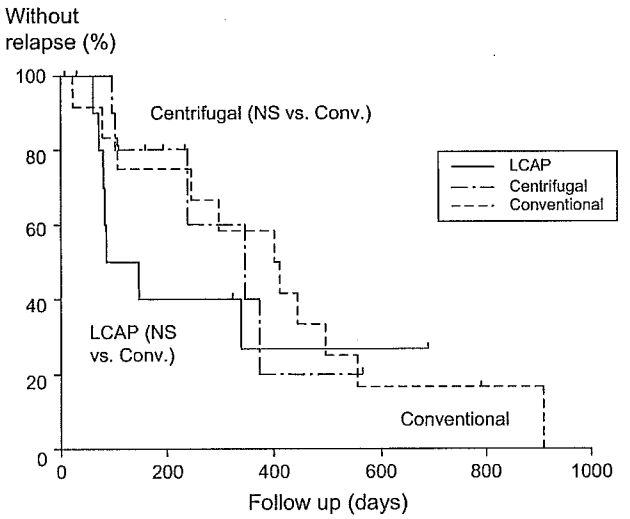

FIGURE 3. Kaplan-Meier of relapses during the follow-up period in patients induced into remission. Endpoint is defined as the time when patients had a relapse.

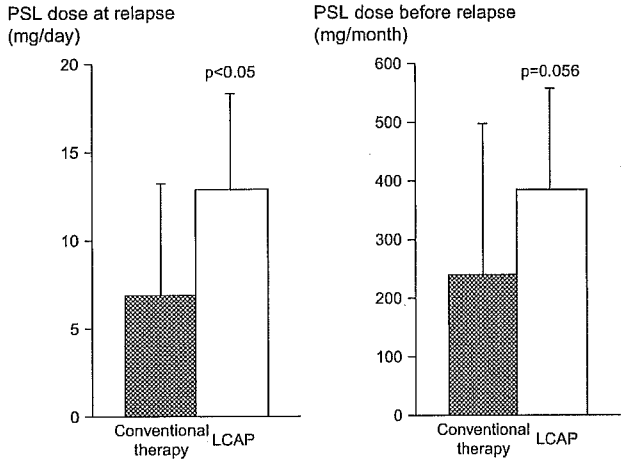

FIGURE 4. Doses of prednisolone taken at relapse in patients treated with leukocytapheresis and in those with prednisolone alone.

results of leukocytapheresis treatment of active UC are consistent with those of previous reports.[7–10] To date, serious complications have not been reported.

Many studies have suggested that UC may be caused by an imbalance between proinflammatory and immunosuppressive cytokines or between helper T lymphocytes and regulatory T lymphocytes (or both).[18–20] Medical treatments for inflammatory bowel diseases are thought to modulate such immunologic imbalances, eg, anti-tumor necrosis factor (TNF) monoclonal antibody against TNF-α, and interleukin (IL)-10 as an immunosuppressive cytokine.[21,22] Leukocytapheresis is considered an immunomodulatory treatment that deletes activated helper T lymphocytes responsible for the pathogenesis of UC or mobilizes new lymphocytes from bone marrow. Lymphocytes in peripheral blood markedly decrease on the day after leukocytapheresis and return to the baseline level in several days (personal communication).

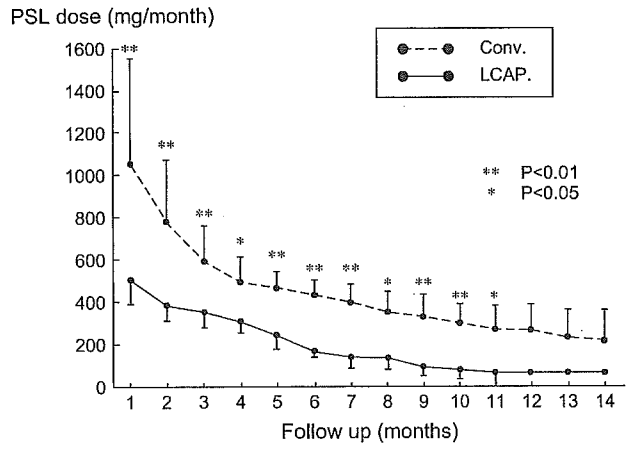

FIGURE 5. Average doses of accumulated prednisolone given for each month from the remission until the endpoint in the leukocytapheresis group and in the prednisolone alone group.

The effectiveness of lymphocytapheresis for Crohn's disease remains controversial. Several uncontrolled trials reported that lymphocytapheresis improves the clinical course of patients with Crohn's disease unresponsive to conventional treatment. However, Lerebours et al reported that lymphocytapheresis slightly decreases the incidence of corticosteroid dependence but does not prevent early relapse.[16] Our study also showed that maintenance therapy with leukocytapheresis once every 4 weeks did not prevent relapse of UC and did not alter the short-term natural history of UC. One study has reported that granulocyte and monocyte absorptive apheresis is useful for maintenance therapy in patients with UC.[23] However, most patients in that study were receiving 6-mercaptopurine for maintenance therapy and were corticosteroid-naive patients. As the patients in our study were not received immunosuppressants or antimetabolites as 6-mercaptopurine, this inconsistency might imply the usefulness of 6-mercaptopurine for the maintenance therapy for UC.

In UC, bacterial flora in the intestinal canal can activate helper T lymphocytes in the colonic mucosa and ileal pouch after proctocolectomy and ileoanal anastomosis, eventually leading to relapse and pouchitis.[24,25] Therefore, leukocytapheresis may be useful for inducing remission of active UC without serious complications, but not for long-term maintenance therapy. The reason why the period from the first procedure to remission negatively correlated with the daily dose of prednisolone at baseline is unknown. The combination of leukocytapheresis with prednisolone may easily induce apoptosis of white blood cells because Fas-ligand-positive CD4 T cells in the colonic mucosa are increased in UC.[26,27] Further study should be done to clarify the reasons why leukocytapheresis is effective for the treatment of active UC, but not as maintenance therapy (albeit this conclusion remains tentative and must be verified in future studies). We think that leukocytapheresis may be a way to avoid or to delay surgery in some of the patients with steroid-refractory UC.

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Research Article

Protection against malaria by anti-erythropoietin antibody due to suppression of erythropoiesis in the liver and at other sites

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Summary We have previously reported that erythropoiesis commences in the liver and spleen after malarial infection, and that newly generated erythrocytes in the liver are essential for infection of malarial parasites as well as continuation of infection. At this time, erythropoietin (EPO) is elevated in the serum. In the present study, we administered EPO or anti-EPO antibody into C57BL/6 (B6) mice to modulate the serum level of EPO. When mice were infected with a non-lethal strain (17NXL) of *Plasmodium yoelii* (blood-stage infection of 10^4 parasitized erythrocytes per mouse), parasitemia continued for 1 month, showing a peak at day 17. Daily injection of EPO (200 IU/day per mouse) from day five to day 14 prolonged parasitemia, whereas injection of anti-EPO antibody (1.5 mg/day per mouse) every second day from day five to day 28 decreased it. Erythropoiesis was confirmed in the liver, spleen and bone marrow by the appearance of nucleated erythrocytes (TER119+). When anti-EPO antibody was injected by the same protocol into mice infected with a lethal strain (17XL) of *P. yoelii*, all mice showed decreased parasitemia and recovered from the infection. These results suggest that the use of anti-EPO antibody after malarial infection may be of therapeutic value in severe cases of malaria.

Key words: antibody, anti-erythropoietin, erythropoietin, liver, malaria, parasitemia.

Introduction

In a series of recent studies,[1–3] we observed that erythropoiesis commences in the liver and spleen of mice infected with malaria and that newly generated erythrocytes in the liver become good targets for malarial parasite infection. Nucleated erythrocytes (reticulocytes), rather than mature denucleated erythrocytes (conventional erythrocytes) become the target of malarial parasites. Indeed, when mice were irradiated (6 Gy) to suppress erythropoiesis in the liver, they showed a low level of parasitemia.[3] In other words, the potential of erythropoiesis in the liver is a key factor for continuation of malarial infection. At this time, erythropoietin (EPO) increased in the sera of these mice.

In light of these findings, in the present study we modulated the serum level of EPO by the administration of EPO (upregulation) or anti-EPO antibody (downregulation). Because irradiation decreases the level of immunity, we administered anti-EPO antibody to suppress erythropoiesis. Depending on the magnitude of erythropoiesis in the liver and at other sites, the level of malarial infection increased or decreased. Taken together with our previous findings that erythropoiesis in the liver is essential for continuation of malarial infection,[2] the decreased erythropoiesis in the liver by the administration of anti-EPO antibody seems to have a potential use in the clinical therapy of malaria.

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Materials and methods

Mice and parasites

Eight-week-old C57BL/6 (B6) mice were used. The mice were maintained at the animal facility of Niigata University (Niigata, Japan) under specific pathogen-free conditions. *Plasmodium yoelii* 17NXL (non-lethal strain) and *P. yoelii* 17XL (lethal strain), generous gifts from Dr S. Waki (Gunma Prefectural College of Health Science, Maebashi, Japan), were used. Parasites were maintained by routine *in vivo* passages in bone marrow cells.[3] Mice were infected by an i.p. injection of 10^4 parasitized erythrocytes per mouse. Parasitemia in the blood was observed by Giemsa staining every 3 days, and mice were killed at the indicated days after infection. Lymphocytes were obtained from the liver, spleen and bone marrow. All experimental procedures were approved by the Committee on Animal Research of Niigata University.

Cell preparation

Hepatic mononuclear cells were isolated by a previously described method.[4] Briefly, the liver was removed, pressed through a 200-gauge stainless steel mesh, and suspended in Eagle's MEM (Nissui Pharmaceutical, Tokyo, Japan) supplemented with 5 mmol/L HEPES and 2% heat-inactivated newborn calf serum. After being washed once with medium, cells were fractionated by centrifugation in 15 mL of 35% Percoll solution (Amersham Pharmacia Biotech, Piscataway, NJ,

USA) for 15 min at 440 *g*. The pellet was resuspended in erythrocyte lysing solution (155 mmol/L NH_4Cl, 10 mmol/L $KHCO_3$, 1 mmol/L EDTA-Na and 170 mmol/L Tris, pH 7.3). Splenocytes and bone marrow were obtained by forcing the spleen and bone marrow through stainless steel mesh. Splenocytes and bone marrow cells were used after mature erythrocyte lysing.

Immunofluorescence tests

Surface phenotypes were identified by immunofluorescence tests.[2] FITC-, phycoerythrin (PE)- or biotin-conjugated reagents of mAbs were used and biotin-conjugated reagents were developed with tricolor-conjugated streptavidin (Caltag Laboratory, San Francisco, CA, USA). The mAbs were anti-CD3 (145–2C11) and anti-erythrocyte (TER119) (BD PharMingen, San Diego, CA, USA). Cells were analysed by FACScan (BD Biosciences, Mountain View, CA, USA). To prevent non-specific binding of mAbs, CD16/32 (2.4G2; BD PharMingen) was added before staining with labelled mAb.

Erythropoietin

Recombinant EPO and anti-EPO antibody were kindly provided by Chugai Pharmaceutical (Tokyo, Japan).[2] Anti-EPO antibody was polyclonal rabbit antibody against human EPO; 1 mg anti-EPO antibody can neutralize approximately 10^4 IU EPO in both humans and rodents. EPO (200 IU/day per mouse) was injected i.p. from day five to day 14 after malarial infection, whereas anti-EPO antibody (1.5 mg/day per mouse) was injected i.p. every second day from day five to day 28. Serum titres of EPO (mU/mL) were measured by radio-immunoassay (SRL, Tokyo, Japan).

Results

Ten thousand parasitized erythrocytes (non-lethal strain 17NXL of *P. yoelii*) were injected i.p. into C57BL/6 (B6) mice and parasitemia was enumerated in the peripheral blood (Fig. 1a). Parasitemia appeared at day five to day seven and continued up to day 27, showing a peak at day 17. The serum level of EPO increased gradually and reached a plateau at day seven (Fig. 1b). Subsequently, the haematocrit (%) gradually decreased from 50 to less than 30%. Nucleated cells were then isolated from the liver, spleen and bone marrow, and surface markers were analysed by immunofluorescence tests (Fig. 1c). Non-nucleated erythrocytes (conventional red blood cells) were ruptured by a lysing buffer. Two-colour staining for TER119 (erythrocyte marker) and CD3 (T-cell marker) was conducted. Nucleated erythrocytes were identified in the liver and spleen beginning at day 14 after infection (indicated by arrows). In the case of the bone marrow, nucleated erythrocytes were detected before malarial infection, but this proportion increased after infection (from day seven).

To modulate the serum level of EPO, either EPO or anti-EPO antibody was injected into malaria-infected mice (a non-lethal strain) (Fig. 2a,b). EPO (200 IU/day per mouse) was injected i.p. daily from day five to day 14 after malarial infection, whereas anti-EPO antibody (1.5 mg/day per mouse) was injected i.p. every second day from day five to day 28. The administration of EPO was found to prolong parasitemia, whereas that of anti-EPO antibody decreased parasitemia. The serum titre of EPO and haematocrit (%) were then measured (Fig. 2b,c). The titre of EPO increased with the administration of EPO (beyond 1000 mU/mL), but that of EPO decreased with the injection of anti-EPO antibody (undetectable or less than 200 mU/mL). The decrease in haematocrit was found to be suppressed by anti-EPO antibody. The appearance of nucleated erythrocytes was augmented by EPO but suppressed (indicated by arrows) by anti-EPO antibody (Fig. 2e). An immunofluorescence test was conducted at day 14. In Figure 2e, the control data were derived from mice without malarial infection.

In a final part of our experiments, we used mice infected with a lethal strain (10^4 parasitized erythrocytes per mouse) of *P. yoelii* (17XL) (Fig. 3). All mice infected with the lethal strain died by day 10. However, when mice were injected every second day with anti-EPO antibody from day five to day 28, parasitemia decreased and the mice recovered from the infection.

Discussion

In this study, we demonstrated that the serum levels of EPO determine the severity of malarial infection in mice. To modulate the level of EPO in sera, we used EPO and anti-EPO antibody. In mice injected with EPO, parasitemia increased, whereas in those injected with anti-EPO antibody, parasitemia decreased. The most striking evidence was that mice administered with anti-EPO antibody survived infection with even the lethal strains, showing only a low level of parasitemia during the infection. Malaria-induced anaemia augments erythropoiesis and generated, nucleated erythrocytes become good targets for malaria parasites. In contrast, the suppression of erythropoiesis by anti-EPO antibody has a potential to stop this blood-stage infection. We emphasize that anti-EPO antibody was effective on not only the non-lethal strain but also the lethal strain of the parasite.

There have been several reports in which immature erythrocytes were found to be better targets for malarial parasites than mature erythrocytes.[5–9] Our previous[1–3] and present data support these findings. In the case of human malaria, CD36 antigens (sequestrin) on erythrocytes are known to mediate adherence and subsequent infection of *Plasmodium falciparum*.[10,11] These CD36 antigens are expressed on immature erythrocytes. Therefore, newly generated erythrocytes may be important in human malarial infection as well as in rodent malaria. Appling these findings, we were able to achieve protection against malaria by treatment with the anti-EPO antibody. Although the antibody suppressed erythropoiesis in the liver and at other sites, the level of mature erythrocytes was partially influenced by this treatment. During a 2 week treatment with the anti-EPO antibody, the haematocrit fell to as low as 30% (from 45%). The anaemia induced by the antibody was not serious in mice; in contrast, malaria-induced anaemia was serious because both immature and mature erythrocytes were affected.[12]

The present results remind us of previous data indicating that a major factor involved in malarial protection might be innate immunity mediated by extrathymic T cells in the liver (TCRint cells).[1–3,13–15] Nucleated erythrocytes infected with malarial parasites still express MHC antigens on their cell surface.[2] Certain autoantigens or malarial antigens inserted into MHC might be recognized by TCRint on extrathymic

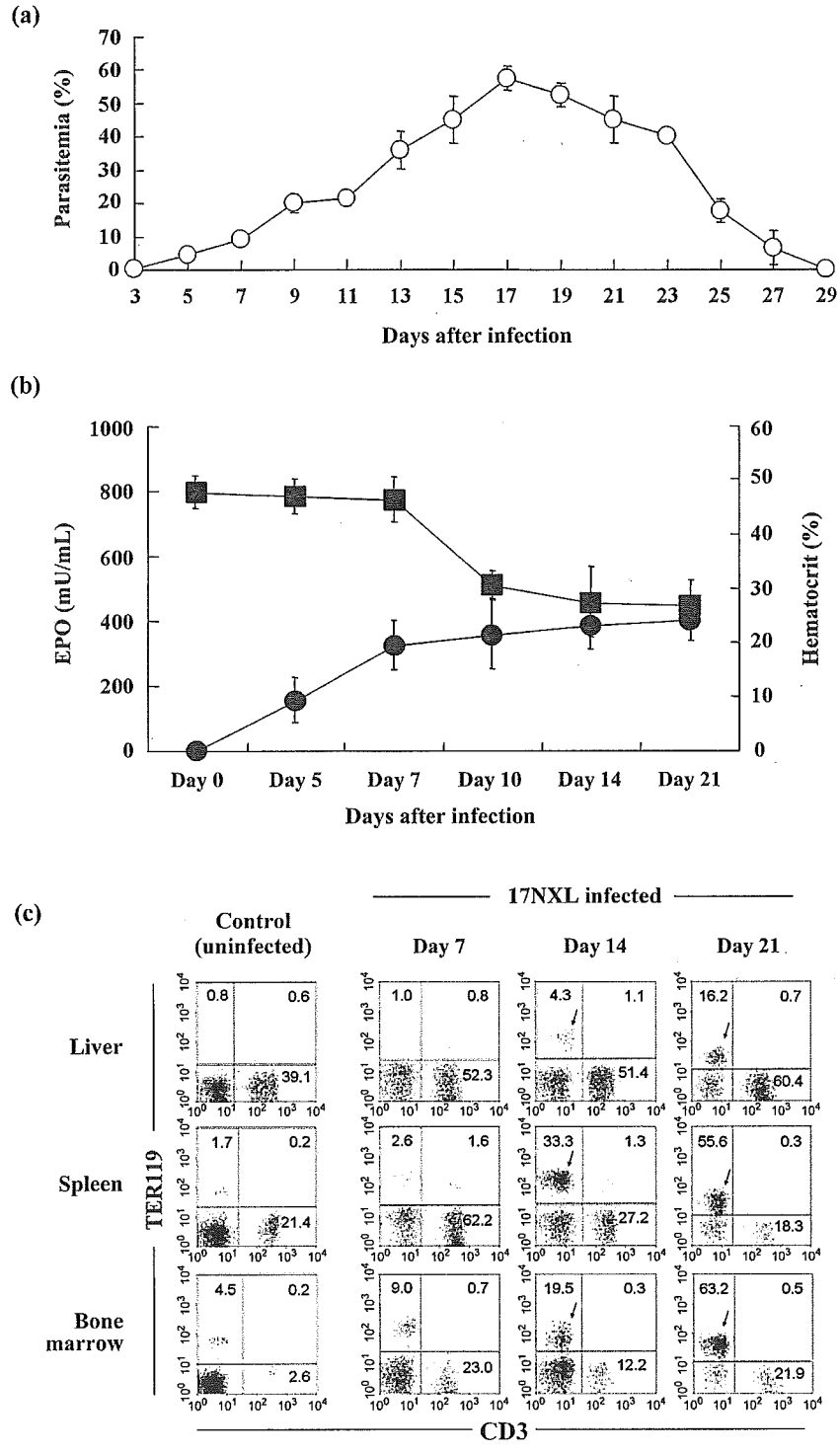

Figure 1 Time-kinetics of parameters after the injection of the non-lethal strain (17NXL) of *Plasmodium yoelii*. (a) Parasitemia (%); (b) serum level of erythropoietin (EPO; ●) and haematocrit (■); and (c) induction of erythropoiesis in the liver, spleen and bone marrow of mice. Mice were infected with 10^4 parasitized erythrocytes and various parameters were enumerated. The mean and one SD were produced from four separate experiments in (a) and (b). In (c), representative results of four experiments are shown. Numbers in this figure indicate the percentages of fluorescence-positive cells in corresponding areas. Erythropoiesis is indicated by arrows.

T cells, and such interaction would induce innate immunity. In any case, decreased production of nucleated erythrocytes in the liver and at other sites influences subsequent malarial infection. The use of anti-EPO antibody after malarial infection may be of therapeutic value in severe cases of malaria.

Acknowledgements

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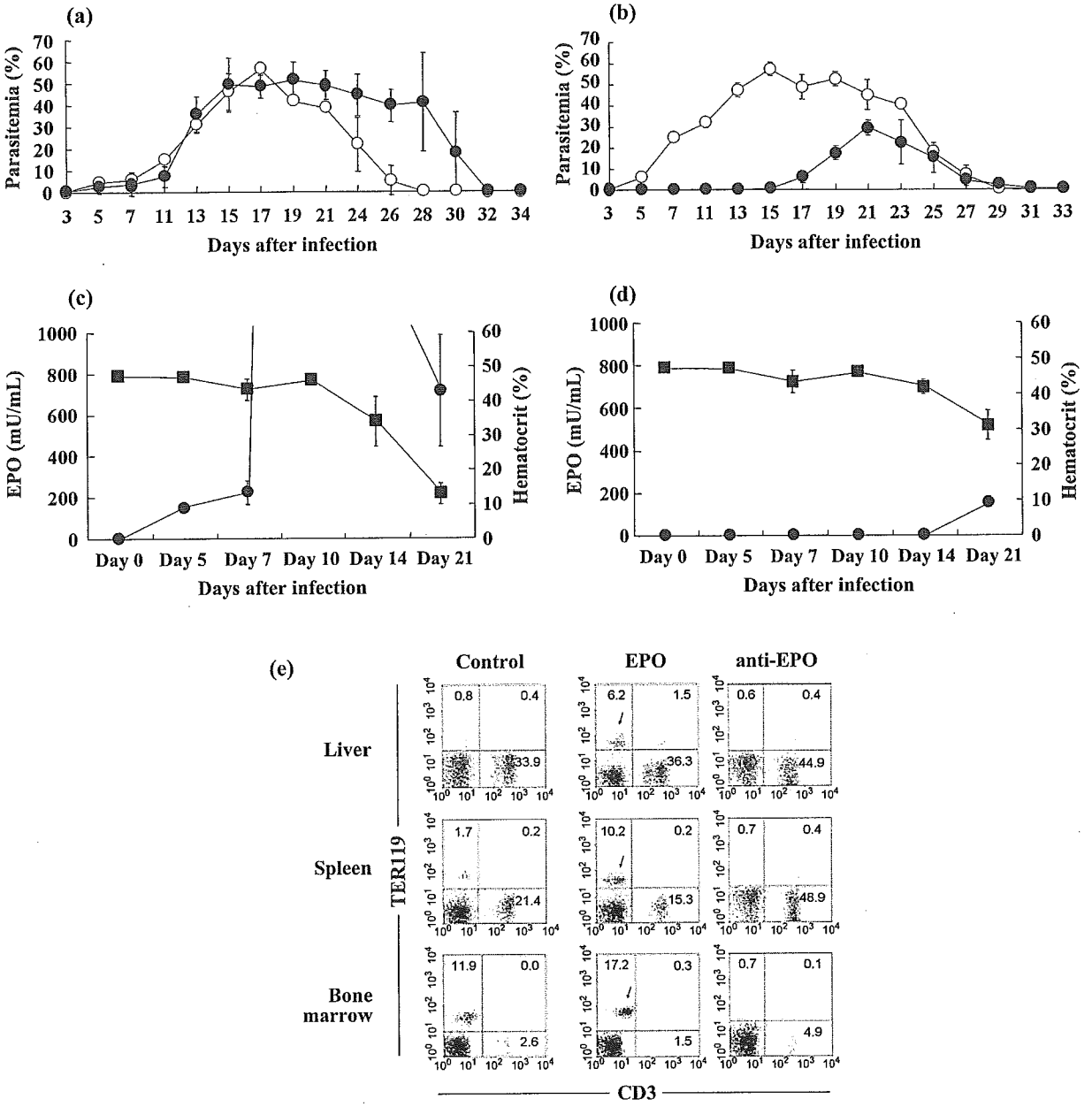

Figure 2 Modulation of malarial infection by the administration of erythropoietin (EPO) or anti-EPO antibody. (a,b) Parasitemia; (c,d) serum level of EPO (●) and haematocrit (■); and (e) erythropoiesis. Mice were infected with a non-lethal strain of *Plasmodium yoelii*. These mice were injected with either EPO or anti-EPO antibody. The mean and one SD were produced from four separate experiments in (a–d). In (e), representative results of four experiments are shown. Numbers in this figure indicate the percentages of fluorescence-positive cells in corresponding areas. Erythropoiesis is indicated by arrows. (a) ○, 17NXL only; ●, 17NXL + EPO. (b) ○, 17NXL only; ●, 17NXL + anti-EPO.

Note added in proof

After completion of this paper, we encountered the article by Chang *et al.* (2004). The conclusion of that paper is quite similar to the conclusion of our paper; namely, that anti-EPO antibody reduced parasitemia in mice infected with malaria, raising the possibility of its clinical therapeutic value for malaria patients. However, in some respects, our experimental results extended those of Chang *et al.*'s paper. For example, we also used a lethal strain of mouse malaria in B6 mice, and we revealed that treatment with anti-EPO antibody modulated the erythropoiesis in the liver where malarial parasites invaded into newly generated erythrocytes.

Chang KH, Tam M, Stevenson MM. Modulation of the course and outcome of blood-stage malaria by erythropoietin-induced reticulocytosis. *J. Infect. Dis.* 2004; **189**: 735–43.

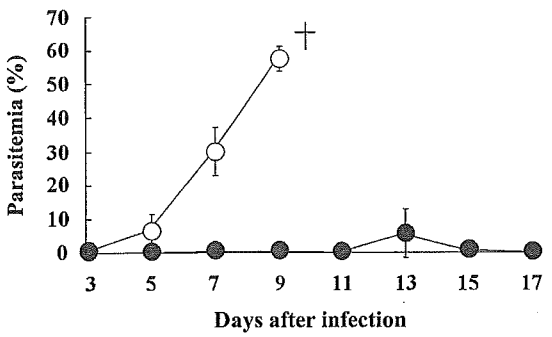

Figure 3 Protection against a lethal strain of *Plasmodium yoelii*. Mice were infected with the lethal strain (17XL) of *P. yoelii*. These mice were also administered every second day with anti-EPO antibody (1.5 mg/day per mouse) from day five to day 28. The mean and one SD were produced from four separate experiments. ○, 17XL only; ●, 17XL + anti-EPO antibody. Death is indicated by the † symbol.

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肝細胞癌の腫瘍マーカー “アルファフェトプロテイン(AFP)” その量から質への評価の変遷

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はじめに

アルファフェトプロテイン(α-fetoprotein：AFP)は肝細胞癌(HCC)の血清学的診断法として広く用いられているが，本来胎児血中における主要な生理的蛋白である。すなわち，胎生13週においてその濃度は300 mg/dLにおよび，同時期での血清アルブミンの濃度(約500 mg/dL)に匹敵する量が産生されている[1]。しかしながら，この蛋白の存在意義については，免疫抑制能や担体蛋白としての意義などいくつかの説が提唱されてはいるが，依然明らかにはされていない[2)-21)]。

この蛋白がHCCにおいて再生産される事実は，1963年旧ソ連のAbelevによりラットの移植腹水HCC系において見出され，これらの報告を契機に，HCCの血清免疫学的診断法として広く普及するに至った[22)23)]。また，この事実が発見された当時は，その測定法(マイクロオクタロニー法などの免疫沈降法)の感度(10,000 ng/mL程度)が低かったため，きわめてHCCに特異的であると考えられていた[24)-26)]。しかし，ラジオイムノアッセイなどの測定法の高感度化にともない，劇症肝炎のみならず肝硬変(LC)などの慢性肝疾患においても上昇を認める事実が次第に明らかになり，その特異性の低下が指摘されるに至った[27)-29)]。

本稿では，HCC診断におけるAFPの診断的意義を，その濃度ならびに質的変化である糖鎖変異(実際的にはL3分画によるフコシル化AFP分画の面)より検討し，その意義を述べる。また，最後に，AFPの多分岐型を含めた糖鎖変異と疾患特異性についても概説する。