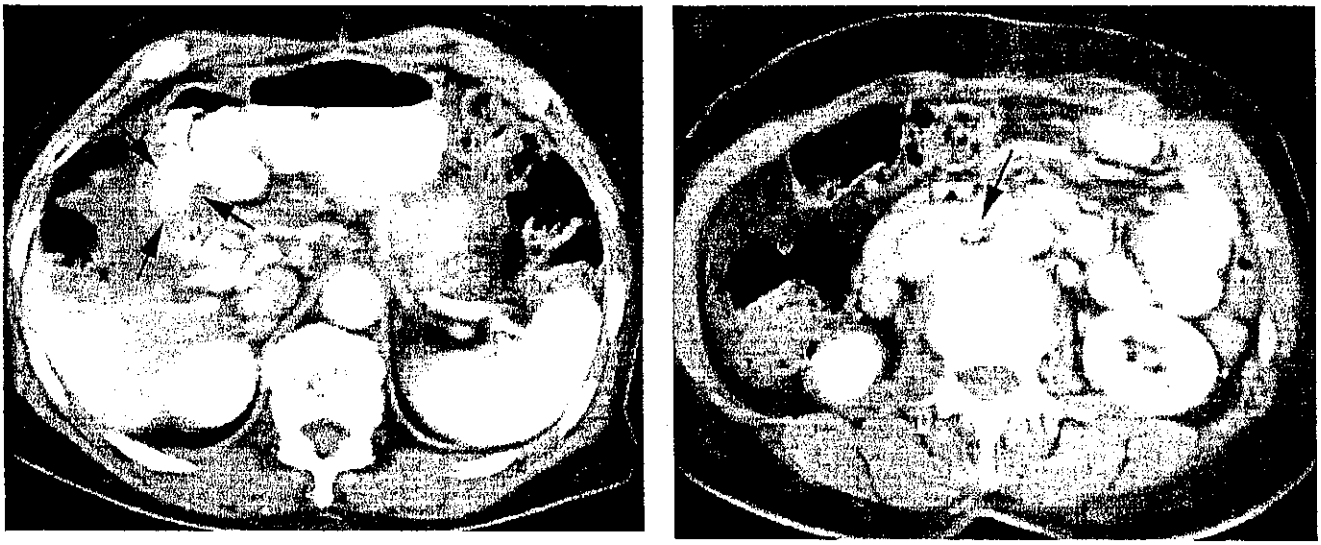

A B

Figure 6. An abdominal computed tomography (CT) scan after 6 courses of irinotecan (CPT-11) and mitomycin C (MMC) revealed the disappearance of the tumor in the antrum (black arrows) (A) and lymph node swelling at #16 (black arrow) (B).

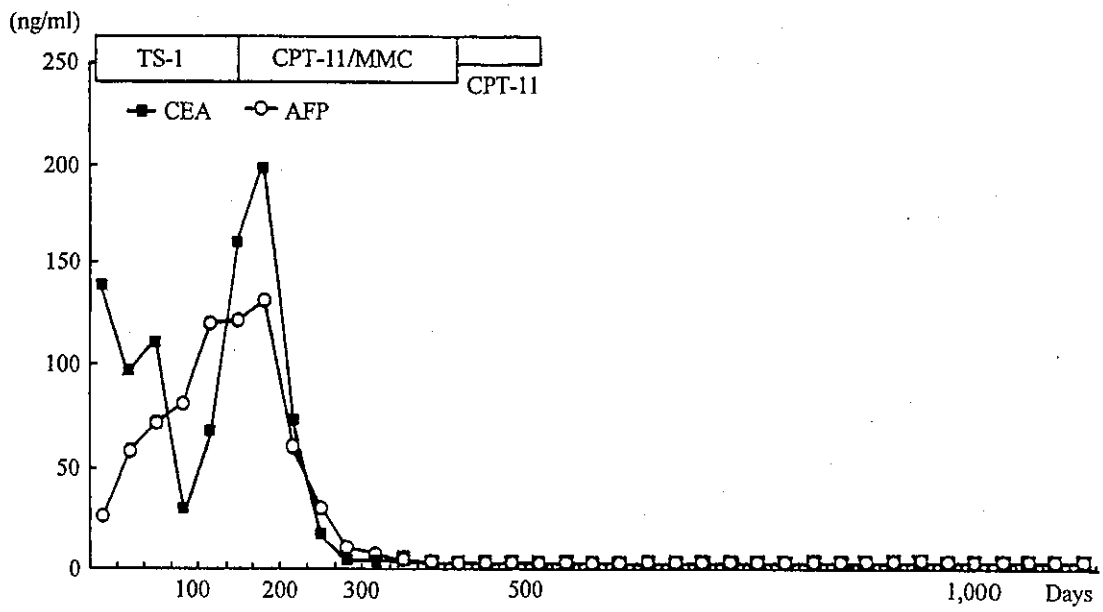

Figure 7. Patient's clinical course after admission to our department. CEA: carcinoembryonic antigen, AFP: alpha fetoprotein, TS-1: S-1 (tegafur · gimeracil · oteracil potassium), CPT-11: irinotecan, MMC: mitomycin C.

was chosen in the present case because Yamao et al reported that this therapy was effective against advanced gastric cancer, and there was little cross-resistance with 5-FU based regimens (13). The present patient achieved CR after 6 courses of CPT-11/MMC chemotherapy. Second line therapy with CPT-11/ MMC was very effective, and well tolerated in the present case. To our knowledge, there have been no reports describing the effectiveness of CPT-11/MMC chemotherapy for AFPGC. We speculate that one of the reasons why the present case achieved CR was that liver metastasis was not seen at the diagnosis although widespread lymph node metastases were seen. The other reason might be that we tried to treat this patient with MMC. Takahashi showed that a combination of MMC and AFP antibody has an appreciable inhibitory effect on AFPGC tumor growth (14). Moreover, as to CPT-11, Shimada et al recently reported two

cases with AFPGC with liver metastases successfully treated with CPT-11 (100 mg/body) plus low-dose cisplatin (10 mg/body) and described that this combination might be worth trying as 1st line chemotherapy for this disease (15). Thus, both CPT-11 and MMC may have promising antitumor activity against AFPGC. Further studies on the effectiveness of CPT-11/MMC chemotherapy for AFPGC are certainly necessary.

There have been several case reports of advanced gastric cancer in which CR was achieved after first or 2nd line chemotherapy with different regimens; CDDP/5-FU, 5-FU/adriamycin/MMC, MMC/5-FU/OK-432 (16), and S-1/CDDP (17). Recently, Shimada et al (18) described that CPT-11/low-dose CDDP regimen was recommended as a promising 2nd line chemotherapy for patients with metastatic gastric cancer resistant to 5-FU and 2 of their 21 patients achieved CR. However, the number of patients who achieved CR after 2nd line chemoptherapy was too small to make predictive analysis on effectiveness of 2nd line chemotherapy for gastric cancer patients in terms of age, sex, clinical stage, and microscopic and pathological features of gastric cancer. A large-scale control study is necessary in order to address the type of 2nd line chemotherapy and clinical outcome in patients with advanced gastric cancer.

To date, 17 patients with unresectable gastric cancer have received CPT-11/MMC chemotherapy at our institution. A phase II study of CPT-11/MMC chemotherapy is being conducted as the second line chemotherapy by Japan Clinical Oncology Group from which data should be analyzed. As with the present patient, there are some patients with unresectable gastric cancers who live for a long time, although such cases are very rare. Yamao et al (13) noted that 1 of 30 their patients who received 2nd line chemotherapy of CPT-11/MMC achieved CR. Thus, further studies on the combinations, administration method, and efficacy of the chemotherapies with novel drugs for advanced gastric cancer are necessary.

In conclusion, we report a patient with unresectable AFPGC that survived for more than four years after chemotherapy. Even in the 2nd line chemotherapy, CPT-11/MMC may contribute to the improvement in the prognosis of patients with unresectable gastric cancer, and this combination therapy is promising.

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ORIGINAL REPORT

Comparison of the Efficacy, Toxicity, and Pharmacokinetics of a Uracil/Tegafur (UFT) Plus Oral Leucovorin (LV) Regimen Between Japanese and American Patients With Advanced Colorectal Cancer: Joint United States and Japan Study of UFT/LV

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A B S T R A C T

Purpose
To compare the efficacy, toxicities, and pharmacokinetics of an oral regimen consisting of uracil/tegafur (UFT) and leucovorin (LV) between Japanese patients and patients in the United States with previously untreated metastatic colorectal cancer.

Patients and Methods
Forty-four Japanese patients and 45 patients in the United States were enrolled in concurrent nonrandomized phase II trials. UFT 300 mg/m^2/d and leucovorin 75 mg/d were administered orally for 28 days followed by a 7-day rest period. The total daily dose of each drug was divided into three equal doses. Treatment was repeated every 5 weeks until disease progression. Blood samples for the pharmacokinetic study were obtained after the initial dose on day 1 of the first course.

Results
The response rate for the Japanese patients and the patients in the United States was 36.4% (95% CI, 22.4% to 52.2%) and 34.1% (95% CI, 20.5% to 49.9%), respectively. The only major toxicity was diarrhea, and other toxicities were mild in both populations. The incidence of grade 3 or higher diarrhea in the Japanese and Americans was 9% and 22%, respectively. Although the area under the curve and maximum concentration of fluorouracil were found to be slightly higher in the Japanese patients than the patients in the United States, and area under the curve–adjusted body surface area appeared to be comparable between the two groups.

Conclusion
The efficacy and pharmacokinetic parameters of UFT and LV are comparable in Japanese and American patients; however, a difference in toxicity profile, specifically diarrhea, was noted. This oral regimen of UFT and LV is considered to have similar activity against metastatic colorectal cancer and to have acceptable toxicity in patients in both countries.

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Colorectal cancer is the second most frequent cause of cancer deaths in the United States and most European countries, and its incidence has recently been increasing in Japan, where the number of deaths attributed to colorectal cancer now ranks third after lung cancer and gastric cancer. Colorectal cancer is therefore a major health problem worldwide, and the median survival time of patients with metastatic colorectal cancer treated with supportive care alone is approximately 4 to 6 months.[1] Systemic chemotherapy has recently been shown to prolong survival time, and median survival times now range from 17 to 21 months.[2,3]

Combination of irinotecan with fluorouracil (FU)/leucovorin (LV) as a first-line treatment for metastatic disease has

produced a survival benefit,[2-4] but recently there has been concern about the toxicity of the weekly bolus combination.[5] A randomized cooperative group study has yielded preliminary data that supports the continued role of intravenous(IV) FU and LV as the backbone of treatment for metastatic colorectal cancer.[6]

Uracil/tegafur (UFT) is a preparation composed of tegafur and uracil in a molar ratio of 1:4. Tegafur is a prodrug of FU and is mainly converted to FU in the liver.[7] In preclinical studies, the coadministration of uracil with tegafur enhanced the antitumor activity achieved with tegafur alone. Uracil strongly inhibits the degradation of FU to 2-fluoro-beta-alanine, thereby increasing the concentration of FU in plasma without increasing the toxicity resulting from 2-fluoro-beta-alanine.[8] LV is used to modulate FU biochemically, and has been widely adopted for the treatment of advanced colorectal cancer. Given the extensive use of LV with FU, the combination of UFT with oral LV was assessed for treatment of colorectal cancer, and administration schedules of UFT and oral LV were developed in phase I and II studies.[9-12] Those studies showed that the combination was very effective against metastatic colorectal cancer and had an acceptable safety profile. A randomized cross-over trial in advanced colorectal cancer showed that oral UFT/LV compared favorably with IV FU/LV in terms of toxicity and patient's preference, and that it prolonged FU exposure to a level comparable to the exposure achieved with continuous IV FU administration.[13]

The results of two large phase III studies on the UFT/LV regimen in Western countries were reported recently and demonstrated similar survival between IV FU/LV and UFT/LV in Western patients with metastatic colorectal cancer.[14,15] UFT was developed in Japan and is commonly used there, and the Japanese experience has demonstrated that UFT is well tolerated and displays evidence of antitumor activity in a variety of solid tumors.[16] Although several dosage regimens of UFT alone have been tried in colorectal cancer in Japan, there have been few studies on the combination of UFT plus LV in Japan.

We conducted the present study to determine whether the results of these phase III studies could be extrapolated to Japanese patients. If it showed equality of efficacy, safety, and pharmacokinetics in both Japanese and American patients, then the results of the Western phase III trials could be extrapolated to Japanese patients. This study was designed as an identical nonrandomized phase II analysis to evaluate the impact of ethnic factors on the efficacy and safety of a particular dosage and dose regimen in American and Japanese patients. The end point of the study was estimation of the efficacy, safety, and pharmacokinetic parameters of UFT/LV in American and Japanese patients with previously untreated metastatic colorectal cancer.

PATIENTS AND METHODS

Patient Selection

Patients had to be either Japanese or American nationals. The patients enrolled at the Japanese sites had to have been born in Japan and have lived more than 75% of their life in Japan, including the 2 years before enrollment. Patients enrolled at the US sites had to have been born in the United States and have lived more than 75% of their life there, including the 2 years before enrollment. Patients in both countries were entered into the study only if they fulfilled the following eligibility criteria: (1) histologic confirmation of colorectal carcinoma, (2) inoperable metastatic disease or recurrent metastatic disease after surgery, (3) measurable lesions, (4) age $\geq$ 20 years but $\leq$ 75 years, (5) performance status (PS) $\leq$ 2 on the Eastern Cooperative Oncology Group (ECOG) scale, (6) no prior chemotherapy for advanced disease (prior adjuvant chemotherapy for colorectal cancer must have been completed at least 6 months before enrollment), (7) adequate bone marrow function (absolute granulocyte count $\geq$ 1,500/μL and platelet count $\geq$ 100,000/μL), (8) adequate liver function (serum bilirubin level 1.5 mg/dL and serum transaminase levels $\leq$ 100 U/L), (9) adequate renal function (serum creatinine level $\leq$ 1.5 mg/dL), (10) no other severe medical conditions, (11) no other active malignancies, (12) no pregnant or breast-feeding women, and (13) provision of written informed consent.

Treatment Schedule

The dose of UFT was 300 mg/m^2/d for 28 days followed by a 7-day rest period. The total daily dose was divided into three doses administered orally every 8 hours (at approximately 7 AM, 3 PM, and 11 PM). UFT (Taiho Pharmaceutical Ltd, Tokyo, Japan) was supplied in the form of 100-mg capsules (100 mg tegafur). The daily dose of UFT was rounded up or down to the nearest 100 mg. If the capsule dose could not be divided equally, the highest dose was administered in the morning and the lower doses in the evening. Leucovorin (Lederle Laboratories, Wayne, NJ) was supplied as 25-mg tablets and administered orally at a dose of 75 mg/d. The total daily dose was divided equally into three doses administered concurrently with UFT. Patients consumed no food for an hour before and after taking the drugs. A course of therapy was defined as 28 consecutive days of treatment followed by a 7-day rest period, and courses were repeated every 5 weeks until disease progression or severe toxicity was observed. Patient compliance was verified by counting the remaining pills at the end of each course of treatment. Treatment was interrupted for grade 3 or higher granulocytopenia or thrombocytopenia, or grade 2 to 4 nonhematologic toxicity. If treatment was discontinued because of a grade 2 nonhematologic toxicity, UFT and LV were resumed at the same doses when the toxicity had completely resolved. Whenever grade 3 or 4 toxic effects occurred, the UFT dose was reduced by 50 mg/m^2/d in subsequent courses, but the dose of LV remained at 75 mg/d.

Evaluation

Patients were evaluated by appropriate investigations, including physical examination, chest x-ray, and computed tomographic scans of the abdomen and chest, before entry into the study to determine the extent of disease. A complete blood cell count, liver function tests, renal function tests, and urinalysis were performed at least once every 2 weeks during treatment. Appropriate investigations were repeated as necessary to evaluate the sites of marker lesions before every course or every other course

The tumor response of the lesions was evaluated according to WHO criteria.[17] National Cancer Institute common toxicity criteria were applied to evaluate the toxicity of this therapy.[18] The eligibility and suitability of the subjects for assessment and their response to treatment were reviewed extramurally.

Pharmacokinetics

On day 1, blood specimens were obtained immediately before drug administration and at 0.25, 0.5, 1, 1.5, 2, 3, 5, and 8 hours after drug administration. They were collected in heparinized tubes and centrifuged in a refrigerated centrifuge, and the plasma obtained was frozen at −20°C until analysis.

Concentrations of tegafur (FT) were determined by a validated high-performance liquid chromotography (HPLC) assay with ultraviolet detection based on the method reported by Muranaka et al.[19] A validated gas chromatographic-mass spectrometric assay method was used to quantitate FU and uracil in the plasma samples. The assay method used was based on a method published previously for FU[20] and was modified to include simultaneous quantitation of uracil as reported by Muranuka et al.[19] LV and 5-methyl tetrahydrofolate (5-MTHF) were determined by validated HPLC methods that were modifications of methods reported previously.[21,22] LV and 5-MTHF were extracted from plasma as described by Etienne et al[21]; however, LV was resolved from endogenous interference by gradient HPLC (mobile phase A, 40% acetonitrile-50% methanol in 25 mmol/L KH_2PO_4 [pH 2.3]; mobile phase B, 25 mmol/L KH_2PO_4 [pH 2.3]), and 5-MTHF was resolved from endogenous interference by isocratic HPLC with a mobile phase consisting of 5% acetonitrile-50% methanol in 25 mmol/L KH_2PO_4 (pH 2.3). Both LV and 5-MTHF were detected at 310 nm.[22]

For FT, FU, and uracil, the standard curves were linear ($R^2 > 0.991$) over the concentration range of 50 to 20,000, 1 to 5, and 20 to 5,000 ng/mL, respectively. Based on the analysis of quality control samples, the accuracy was −0.9% to 3.4% and the inter- and intrarun precision was 6.0% to 7.9% of the assays for FT; 5.7% to 11.0% and 2.0% to 8.6% for FU; and 2.8% to 8.0% and 1.2% to 8.7% for uracil. For both LV and 5-MTHF, the standard curves were linear ($R^2 > 0.992$) over the concentration range of 50 to 2,000 ng/mL; the accuracy was 1.5% to 3.0%, and the inter- and intrarun precision was 5.9% to 8.7% of the assay for LV, and 1.8% to 3.3% and 4.5% to 7.8% for 5-MTHF, respectively.

The plasma concentration-time data following administration for five analyses were analyzed by a noncompartmental method using the computer program WINNonlin (version 3.1; Pharsight Co, Apex, North Carolina). The peak plasma concentration (C_{max}) and the time to reach the peak concentration (T_{max}) were recorded directly from the experimental observations. The area under the plasma concentration-time curve (AUC) from time 0 to T, AUC_{0-T}, where T is the time of the last measurable concentration, was calculated by the trapezoidal method. No weighting factor was used, and the slope of the terminal phase of the plasma profile, K, was determined by log-linear regression of at least three data points, which yielded a minimum mean square error. The absolute value of K was used to estimate the terminal half-life ($t_{1/2}$) according to the formula $t_{1/2} = \ln 2/K$. AUC from time 0 to infinity, AUC_{0-inf}, was determined by summing the areas from time 0 to the time of the last measured concentration, calculated using conventional trapezoidal and log-trapezoidal methods, and the extrapolated area. The extrapolated area was determined by dividing the final concentration by the slope of the terminal log-linear phase. Since a terminal log-linear phase was not identified in a minority of the patients, the $t_{1/2}$ and AUC_{0-inf} values of these patients could not be calculated.

Statistical Methods

Response rates of metastatic colorectal cancer between 16.6% to 21.5% for UFT alone and between 18% and 43% for UFT plus LV have been reported in some phase II studies. The sample size in this study was calculated based on a target activity level of 31.5% and minimum activity level of 20%, to ensure the lower limit of the 90% CI, and thus the number of patients required in each country was 44. Time to progression was measured from the start of treatment to the date of progression. Progression time was censored at the close out date if progressive disease was not observed.

The equivalence of AUC and C_{max} in analyses was concluded if the 90% CI with a power of 80% for difference of logarithmic means between Japanese and American patients was entirely within the range of −0.36 to 0.36.

This trial was approved by the institutional review board of the clinical oncology program at all hospitals participating in this study.

Results

Eighty-nine patients with advanced metastatic colorectal carcinoma were entered onto the trial between November 1998 and September 2000. Forty-four patients were entered in Japan, and all were assessable for toxicity and response. Forty-five patients were entered in the United States, and 44 were assessable for response. One patient in the United States was judged ineligible because no target lesions were available. The 44 patients in the United States consisted of 30 white patients, four Hispanic patients, and 10 black patients.

The characteristics of the eligible patients are listed by country in Table 1. Almost all patients in both groups had good performance status. Almost all patients in both groups had undergone surgery, and five (11%) Japanese patients and 10 (23%) patients in the United States (hereafter, "American patients") had received FU-based adjuvant chemotherapy. The characteristics of the 88 eligible Japanese and American patients were matched for age, sex, performance status, and prior therapy.

The actual daily doses of UFT according to body-surface area (BSA) are shown in Table 2. Because the mean BSA of the American patients was larger (mean: American, 1.92 m^2; Japanese, 1.57 m^2), the median actual daily dose of UFT received by the American patients was 600 mg, as opposed to 500 mg by the Japanese patients. The median number of courses of treatment was four in both countries.

Response

All 44 Japanese patients had measurable lesions. Fourteen patients had a partial response, and two patients had a complete response (response rate, 36.4%; 95% CI, 22.4% to 52.2%; Table 3). Two patients were not assessable because of early withdrawal due to toxicity. The response rate by metastatic site was 35% (six of 17 patients) in the lung, 30% (eight of 27 patients) in the liver, and 18% (two of 11

Table 1. Characteristics of Eligible Patients

	Japan (n = 44)		United States (n = 44)*	
	No. of Patients	%	No. of Patients	%
Age, years				
Median	59		60	
Range	26-73		44-88	
Sex				
Male	26	59	29	66
Female	18	41	15	34
PS (ECOG)				
0-1	42	95	44	100
2	2	5	0	0
Primary lesions				
Colon	24	55	34	77
Rectal	20	45	10	23
Prior therapy				
Surgery	43	98	44	100
Adjuvant chemotherapy	5	11	10	23
Radiotherapy	0	0	4	9
Others	1	2	2	5
Body surface area, m^2				
Mean	1.57		1.92	
Range	1.19-1.92		1.53-2.63	

Abbreviations: PS, performance status; ECOG, Eastern Cooperative Oncology Group
*Race/ethnicity: white patients, 30 (68%); Hispanic patients, four (9%); black patients, 10 (23%)

Table 3. Objective Response

	Japan (n = 44)	United States (n = 44)
Complete response	2	0
Partial response	14	15
Stable disease	11	18
Progressive disease	15	8
Not assessable	2	3
Overall response rate	16/44	15/44
%	36.4	34.1
95% CI	22.4 to 52.2	20.5 to 49.9

patients) in the lymph nodes. Of the 44 American patients with measurable metastatic lesions, 15 patients had partial responses, and there were no complete responses (response rate, 34.1%; 95% CI, 20.5% to 49.9%; Table 3). Three patients were not evaluated because of early withdrawal due to toxicity, early death as a result of disease progression, and a target lesion that was too small. The response rate by metastatic site was 71% (five of seven patients) in the lung, 24% (eight of 34 patients) in the liver, and 25% (two of eight patients) in the lymph nodes.

The median time to progression at the close of this trial (August 2001) was 127 days (range, 21 to 703+ days) among the Japanese patients and 142 days (range, 19 to 512 days) among the American patients.

Toxicity

Forty-four Japanese patients and 45 American patients were assessable for toxicity. Table 4 shows the highest grade of toxicities during all treatment courses according to patient. Although the incidence of grade 3 and 4 hematologic toxicities in the American patients was almost the same as in the Japanese patients, the incidence of all grades of thrombocytopenia was higher in the American patients than in the Japanese patients. Diarrhea occurred in 38.6% of the Japanese and 68.9% of the American patients ($P = .006$). The incidence of grade 3 and 4 diarrhea was also higher in the American patients (22.2%) than in the Japanese patients (9.1%). Five American patients exhibited grade 3 or 4 dehydration due to diarrhea. All-grade and grade 3 and 4 nausea and vomiting were higher in the American patients than in the Japanese patients. However, the incidences of the grade 3 or 4 toxicities were not high, even among the American patients. All-grade and grade 3 or 4 stomatitis/mucositis occurred more often in the Japanese patients (34.1% and 4.5%, respectively) than in the American patients (17.8% and 0%, respectively). Hand-foot syndrome

Table 2. Body Surface Area and UFT Dose

			Japan (n = 44)		United States (n = 44)			
BSA (m^2)	Daily Dose of UFT (mg)	Divided Doses (mg)	No. of Patients	%	No. of Patients	%	No. of Patients for Pharmacokinetic Study (n = 43)	%
BSA ≤ 1.49	400	200-100-100	15	34	0	0	0	0
1.5 ≤ BSA ≤ 1.83	500	200 200 100	26	59	17	39	18*	42
1.84 ≤ BSA ≤ 2.16	600	200 200-200	3	7	20	45	19	44
2.17 ≤ BSA ≤ 2.5	700	300 200 200	0	0	5	11	4	9
2.51 ≤ BSA	800	300 300 200	0	0	2	5	2	5

Abbreviations: UFT, uracil/tegafur; BSA, body surface area
*One patient, who was judged ineligible because no target lesions were available, was added for pharmacokinetic analysis.

Table 4. Toxicity

	Japan (n = 44)		United States (n = 45)	
	All Grades (%)	Grade 3/4 (%)	All Grades (%)	Grade 3/4 (%)
Anemia	47.7	4.5	31.1	2.2
Neutropenia	34.1	0	22.2	0
Thrombocytopenia	6.8	2.3	31.1	0
Diarrhea	38.6	9.1	68.9	22.2
Nausea	29.5	0	64.4	4.4
Vomiting	18.2	0	31.1	4.4
Stomatitis/mucositis	34.1	4.5	17.8	0
Hand foot syndrome	0	0	2.2	0
Bilirubin	59.1	4.5	46.7	4.4
AST	38.6	2.3	30.6	5.6
ALT	38.6	4.5	33.3	0

was rarely observed in either group. The incidence of grade 3 and 4 abnormal bilirubin and AST and ALT values was almost the same in both groups (2.3% to 5.6%).

Almost all grade 3 or 4 toxicity occurred before the end of the second course of treatment. All 14 patients (Japan, three patients; United States, 11 patients) in both countries who exhibited any grade 3 and 4 toxicity were hospitalized for treatment. Two Japanese patients discontinued the UFT/LV regimen because of grade 3 hyperbilirubinemia, grade 4 elevation of AST/ALT, and grade 2 transient cerebral ischemia that was judged to have no relation to the drug. One American patient discontinued treatment because of grade 3 diarrhea with dehydration. However, it was not deemed necessary to discontinue treatment in any of the other patients who experienced grade 3 or 4 toxicities. These patients were able to continue treatment after a temporary interruption or decrease in the dose of the drug. Of the 193 courses administered, 183 (95%) were given at 75% or more of the protocol-defined dose of UFT among the Japanese patients. Of the 221 courses administered, 210 (95%) were given at 75% or more of the protocol-defined dose of UFT among the American patients. There were no treatment-related deaths. This regimen was well tolerated and could be repeated as planned in both countries.

Pharmacokinetics

Blood specimens for pharmacokinetic analysis were available from 44 patients in Japan and 43 patients in the United States, and a summary of the results along with the numbers of patients available for each parameter is provided in Table 5. A patient was judged nonassessable for pharmacokinetic analysis if three or more blood specimens were missing.

For each parameter, the 90% CIs for difference of logarithmic means between Japanese patients and American patients are shown Table 6. The $AUC_{0\text{-}8\ hours}$ and C_{max} for each compound, except for LV, were not equivalent with a power of 80% between the countries. The mean $AUC_{0\text{-}8\ hours}$ and C_{max} for FT and uracil in the Japanese patients tended to be higher than in the American patients. The same tendency was also observed with regard to FU, which is an active form of UFT (Table 5). The mean $AUC_{0\text{-}8\ hours}$ and C_{max} of FU in the Japanese patients were 1.36 and 1.61 times as large as in the American patients. The plasma FU over time curves of both groups are shown in Figure 1. The mean $AUC_{0\text{-}8\ hours}$ and C_{max} for LV were similar between the countries. The plasma LV over time curves of the Japanese and American patients are shown in Figure 2. Furthermore, the mean $AUC_{0\text{-}8\ hours}$ and C_{max} for 5-MTHF, which is a metabolic product of LV, also tended to be higher in the Japanese patients than the American patients (Table 5).

Data was obtained from blood specimens collected during the 8 hours after a single dose of UFT/LV in the morning on day 1. As shown in Table 2, the total daily dose of UFT was decided according to the patient's BSA, but the morning dose of UFT was not (the morning dose of UFT was the same in all patients receiving 400 to 600 mg as the total daily dose). We therefore examined the AUC of FU according to BSA (Fig 3). The subjects consisted of 81 patients (Japanese, 44 patients; American, 37 patients) who were administered 200 mg as the initial dose of UFT on day 1 of the first course. In the group with a BSA from 1.50 to 1.83 m^2, the AUCs of the 18 American patients were distributed from 31.0 ng · h/mL to 494.1 ng · h/mL, and the AUCs of the 26 Japanese patients (52.2 to 418.2 ng · h/mL) were distributed within the American patients' range. In addition, in the group with a BSA from 1.84 to 2.16 m^2, a similar tendency was observed. The distribution of the AUCs for FU were similar among patients from both countries within the same BSA range. The AUCs of uracil- and LV-adjusted BSA also showed the same findings between the countries. Therefore, the pharmacokinetic parameters of UFT/LV appear to be comparable between Japanese and American patients.

DISCUSSION

Two large phase III studies were recently performed to compare an oral regimen of UFT and LV with conventional intravenous FU/LV therapy in patients with previously untreated metastatic colorectal carcinoma.[14,15] In both trials, the oral UFT/LV provided a safer, more convenient alternative to the standard bolus intravenous FU/LV regimen for metastatic colorectal cancer and resulted in similar survival. These large trials were performed in Western countries only, and thus we conducted the present study to determine whether the results of those phase III studies could be extrapolated to Japanese patients. The significance of this study lies in its comparison between patients living in

Table 5. Pharmacokinetic Parameters After Administration of UFT and LV

	$AUC_{0\text{-}8h}$ (ng·h/mL)	C_{max} (ng/mL)	T_{max} (h)	$t_{1/2}$ (h)
FT				
Japan				
Mean	41,063.2	9,158.7	0.8	6.5
SD	10,376.9	1,910.1	0.4	1.8
No. of available patients	44	44	44	44
United States				
Mean	23,857.8	5,470.7	1.3	5.4
SD	7,469.2	2,013.7	1.0	1.3
No. of available patients	43	43	43	40
Uracil				
Japan				
Mean	5,989.5	6,867.2	0.8	0.2
SD	3,255.1	3,772.3	0.4	0.1
No. of available patients	44	44	44	39
United States				
Mean	3,610.6	3,409.2	1.2	0.2
SD	3,218.7	3,305.3	0.8	0.1
No. of available patients	39	43	43	24
FU				
Japan				
Mean	223.1	245.0	0.7	0.3
SD	154.8	192.6	0.4	0.1
No. of available patients	44	44	44	44
United States				
Mean	164.0	152.2	1.1	0.6
SD	118.4	154.2	0.9	0.8
No. of available patients	43	43	43	41
LV				
Japan				
Mean	2,659.8	473.6	2.2	7.0
SD	1,156.5	214.0	0.6	1.9
No. of available patients	44	44	44	44
United States				
Mean	2,241.2	436.3	2.3	7.7
SD	942.2	293.1	1.1	2.6
No. of available patients	42	43	43	38
5-MTHF				
Japan				
Mean	2,046.7	468.0	2.3	3.1
SD	889.7	193.0	0.9	1.4
No. of available patients	43	44	44	36
United States				
Mean	1,498.5	337.8	2.8	3.9
SD	544.9	116.4	1.7	1.3
No. of available patients	37	43	43	26

Abbreviations: UFT, uracil/tegafur; LV, leucovorin; AUC, area under the curve; C_{max}, maximum concentration; T_{max}, time to maximum concentration; $t_{1/2}$, half-life; FT, tegafur; SD, standard deviation; FU, fluorouracil; 5-MTHF, 5-methyl tetrahydrofolate

two different countries, not in comparisons between patients of different races. This is the only trial in the literature to compare the efficacy, toxicity, and pharmacokinetics of UFT/LV between patients in two different countries.

The response rate in this trial was 36.4% in the Japanese patients and 34.1% in the American patients. The patient characteristics in both groups were almost identical, and the response rates were very similar, suggesting no difference between Japanese and American patients in regard to the efficacy of a combination of UFT and LV. These response rates are also compatible with the results (response rate, 18% to 43%) of other phase II studies of UFT/LV for colorectal cancer in Western countries.[11,12,23-27] The response rates in this trial and other phase II trials of UFT/LV were generally higher than with UFT alone in metastatic colorectal cancer in Japan (21.5%; 14 of 65 patients)[16] and the United Kingdom (16.6%; six of 36 patients).[28] However, the doses, schedules, eligibility criteria, and response criteria in the

Table 6. 90% CI for Difference in Logarithmic Means of Each Pharmacokinetic Parameter Between Japanese and Patients in the United States

Compound	PK Parameter (ng·h/mL)	Difference From Logarithmic Mean	90% CI
FT	$AUC_{0\text{-}8}$	0.5678	0.4601 to 0.6756
	C_{max}	0.5677	0.4529 to 0.6825
FU	$AUC_{0\text{-}8}$	0.3906	0.1293 to 0.6519
	C_{max}	0.7193	0.3722 to 1.0664
Uracil	$AUC_{0\text{-}8}$	0.7598	0.4515 to 1.0680
	C_{max}	1.0602	0.7145 to 1.4060
LV	$AUC_{0\text{-}8}$	0.1795	0.0265 to 0.3324
	C_{max}	0.1665	−0.0143 to 0.3472
5-MTHF	$AUC_{0\text{-}8}$	0.2762	0.1250 to 0.4275
	C_{max}	0.2899	0.1528 to 0.4270

Abbreviations: PK, pharmacokinetic; FT, tegafur; AUC, area under the curve; C_{max}, maximum concentration; FU, fluorouracil; LV, leucovorin, 5-MTHF, 5-methyl tetrahydrofolate

trials differed, rendering a comparison of efficacy difficult. Nevertheless, the data support addition of the effect of LV to UFT, the same as with FU and LV, and this additional effect appears to be the same in different countries.

The incidence and degree of toxicity did not differ much between the countries, except for gastrointestinal toxicities and thrombocytopenia. The incidences of all grades of diarrhea, nausea and vomiting, and grade 3/4 diarrhea were higher in the American patients than in the Japanese patients. McCollum et al[29] have reported that treatment-related toxicity differs between the black and white patients receiving FU-based treatment, with white patients experiencing statistically significantly higher rates of diarrhea, nausea and vomiting, and stomatitis. The same tendency, except for stomatitis, was also observed among American patients in the present study. In particular, for diarrhea, the incidence of grade 3/4 diarrhea was 29% (nine of 31 patients) in the white patients, 0% (zero of 10 patients) in the black patients, and 25%(one of four patients) in the Hispanic patients. This difference in the incidence and degree of diarrhea between the Japanese and American patients in our study may be a reflection of racial/ethnic differences, although this has not been verified. However,

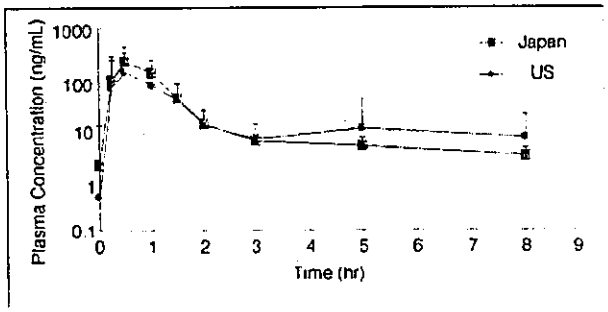

Fig 1. Plasma fluorouracil concentration versus time curve

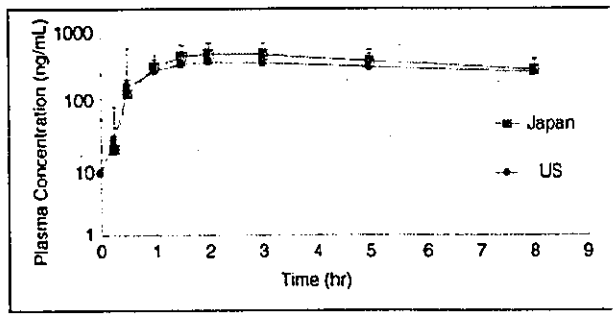

Fig 2. Plasma leucovorin concentration versus time curve

diarrhea was manageable with antidiarrheal drugs or by temporary interruption of UFT/LV, and few patients in either group had to cease treatment because of diarrhea. Furthermore, a difference in the incidence of thrombocytopenia between the two countries was observed in the present study, although McCollum et al[29] reported no difference in thrombocytopenia according to race. However, in our trial, the grade was 1 for all American patients in which the incidence of thrombocytopenia was higher. Moreover, the mean nadir of thrombocytes between the countries did not differ (Japanese patients, 189,000/μL; American patients, 198,000/μL). Therefore, no significant difference in thrombocytopenia was apparent between the countries. UFT administration is only rarely complicated by hand-foot syndrome,[13-15] and similarly was rare in both groups in this trial. Although some differences in toxicities in both countries were observed in this trial, the incidence and degree of toxicities in both countries were mostly consistent with the results of the other phase II trials of UFT/LV.[11,12,23-27]

The mean AUC and C_{max} of FT and uracil in the present study were slightly higher in the Japanese patients than the American patients, and the AUC and C_{max} of FU, which is an active form of UFT, were also slightly higher in the Japanese patients than the American patients. The mean

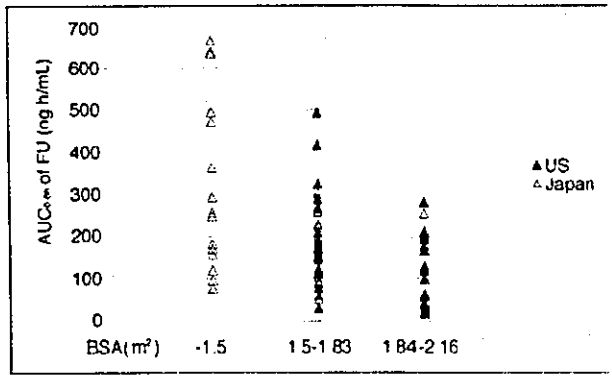

Fig 3. Area under the curve ($AUC_{0\text{-}8}$) of fluorouracil (FU) according to body surface area (BSA)

AUC values of FU in two other studies designed administering UFT 200 mg plus LV 30 mg in Western patients were 96.0[30] and 118 ng·h/mL,[31] respectively. Moreover, Borner et al[13] reported that the mean AUC value of FU on day 8 after administration of oral UFT 300 mg/m^2/d plus oral LV 90 mg/d to European patients was 113 μmol/L × min (244.8 ng·h/mL). The actual values for the mean AUC of FU obtained in those studies are similar to our data in American patients (164.0 ± 118.4 ng · h/mL) and similar to the results obtained in the Japanese patients (223.1 ± 154.8 ng·h/mL) in the present trial. Bolus intravenous FU has been reported to result in a significantly higher AUC and C_{max} of FU, indicating higher FU exposure than with oral UFT, and large phase III studies have confirmed higher toxicity and equal efficacy of intravenous FU in comparison to oral UFT.[14,15] These findings indicate that high FU exposure may not be necessary for tumor response, but may translate into toxicity.

We performed an analysis of covariance on the AUC of FU adjusted for certain characteristics (BSA, UFT dose, performance status, complications) and found that the most significant factor was BSA (P = .0029). Because the dose of UFT administered in the pharmacokinetic study was not decided according to BSA, we examined the AUC of FU according to BSA. The result was that the distributions of the AUCs of FU were similar among patients in both countries in the same BSA range. Therefore, the AUCs of FU are thought to be comparable between Japanese and American patients. However, the reasons for the difference in diarrhea between the patients in the two countries having almost the same pharmacokinetic profile could not be identified, except for the possibility of racial influences.

5-MTHF is a metabolic product of LV, and 5-MTHF and LV are known to enhance the antitumor activity of FU. Our results also showed a slightly higher AUC and C_{max} of LV and 5-MTHF in the Japanese patients than in American patients. Meropol et al[30] has reported that there is no interaction between UFT and LV and that the plasma concentration of FU is unaffected by LV. This finding may suggest that the plasma concentration of FU is regulated by uracil, not LV, through inhibition of dihydropyrimidine dehydrogenase.

The results of the present study indicate that UFT/LV is equally active in Japanese and American patients. However, a difference in toxicity profile, especially diarrhea, was noted. Although the AUC and C_{max} of FU were found to be slightly higher in the Japanese patients than in the American patients, AUC-adjusted BSA appeared to be comparable between the countries. This oral regimen of UFT and LV was considered to have similar activity against metastatic colorectal cancer and to have acceptable toxicity in patients in both countries.

Authors' Disclosures of Potential Conflicts of Interest

The authors indicated no potential conflicts of interest.

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GASTROENTEROLOGY

Complement regulatory proteins in normal human esophagus and esophageal squamous cell carcinoma

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Abstract

Background: Altered expression of three complement regulatory proteins, decay-accelerating factor (CD55), membrane cofactor protein (CD46) and homologous restriction factor 20 (CD59) has been identified in human gastrointestinal malignancies, but their expression in esophageal cancer has not been described. Therefore the purpose of the present paper was to study the distribution of these proteins in human normal and malignant esophageal mucosa.

Methods and results: In the normal esophageal mucosa, CD55 predominantly stained on the cell membrane of squamous epithelium in the superficial and prickle cell layers, whereas CD46 most intensely stained on the cell membrane in the basal and parabasal cell layers. In contrast to this reciprocal expression of CD55 and CD46, CD59 was broadly distributed on the cell membrane in all layers. In the esophageal squamous cell carcinoma, CD55 staining was intense in the stroma but was negligible in the cancer cells. In contrast, CD46 and CD59 stained almost uniformly on the tumor cell membrane. There was a significant difference in the intensity of the staining of CD55 and CD46 among cells in various layers of normal esophageal mucosa and esophageal carcinoma cells, but not in the staining of CD59. Similar expression patterns of the three complement regulatory proteins in carcinoma cells and in normal epithelium in the basal and parabasal cell layers were observed.

Conclusions: These observations on the expression of the three complement regulatory proteins would help understanding of the host immune responses involving the complement system against esophageal squamous cell carcinoma.

Key words: CD59/ homologous restriction factor 20, complement regulatory proteins, decay-accelerating factor, esophageal squamous cell carcinoma, membrane cofactor protein.

INTRODUCTION

The development of malignant tumors elicits cell-mediated and humoral factor-mediated immune responses against tumor cells as host defense mechanisms. Among the humoral factor-mediated responses, the complement system is a major effector pathway. The complement system has classically been implicated as a defense against infection by microorganisms and parasites, but it also participates in host immune responses to cancer.[1–5] Cancer cells as well as normal host cells express membrane-bound molecules that regulate complement activation to protect the cells from complement-mediated damage.[6–13] Among these molecules, decay-accelerating factor (CD55) inhibits the formation of C3/C5 convertases and promotes their catabolism;[14,15] membrane cofactor protein (CD46) is a cofactor for factor I-mediated cleavage of C3b and C4b;[16] and homologous restriction factor 20 (CD59) inhibits the formation of terminal complement complex by preventing the binding of C9 to C5b-8.[17,18] We and others have previously found altered expression of these com-

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plement regulatory proteins in gastrointestinal epithelial cells in various pathological conditions including malignant transformation.[9,10,13,19,20] However, their expression in normal esophagus and esophageal carcinoma has not been described except for that of CD55 in the normal esophagus.[6] In the present study, we immunohistochemically examined the distribution of CD55, CD46 and CD59 in normal human esophageal mucosa and esophageal squamous cell carcinoma.

METHODS

Tissues

Tissue specimens were obtained from 15 patients (two women and 13 men, mean age: 60 years) who had surgically resected esophageal squamous cell carcinoma. Of the 15 cancers, four were histologically well differentiated, 10 were moderately differentiated and one was poorly differentiated. The tumor, node, metastasis (TNM) stage of the esophageal cancers[21] was stage IIB, $n = 2$; stage III, $n = 8$; stage IVA, $n = 3$; and stage IVB, $n = 2$. Endoscopic biopsy specimens of normal-appearing esophageal mucosa were obtained from eight patients who underwent diagnostic endoscopy for gastrointestinal symptoms (two women and six men, mean age: 56 years). Histologically normal mucosa was obtained also from the uninvolved portions of surgically resected esophagus from 17 patients who underwent esophageal resection for esophageal squamous cell carcinoma (three women and 14 men, mean age: 60 years). The study was conducted according to the guidelines of the Declaration of Helsinki and with the approval of the local ethical committee. Informed consent was obtained from each patient.

Immunohistochemistry

The tissue specimens were fixed in a periodate–lysine–paraformaldehyde fixative[22] and cryostat sections 6 μm thick were stained using an indirect peroxidase-labeled antibody method. The following monoclonal antibodies diluted with phosphate buffered saline (PBS) containing 1% bovine serum albumin (Sigma Chemical, St Louis, MO, USA) were used as primary antibodies: 1C6 antibody to CD55 (IgG1 isotype);[15] J4-48 antibody to CD46 (IgG1 isotype, Immunotech, Marseille, France);[23] and 1F5 antibody to CD59 (IgG1 isotype, a gift from Prof. Hidechika Okada, Nagoya City University, School of Medicine, Nagoya, Japan).[18] As negative controls, normal mouse IgG1 (Dako, Glostrup, Denmark) and PBS were used instead of the primary antibodies. The sections were incubated with each of the primary antibodies for 2 h at 20°C.

After washing with PBS, the sections were reacted with horseradish peroxidase-labeled Fab' fragments of rabbit antimouse immunoglobulins, prepared as described,[24] for 1 h at 20°C. After washing with PBS, the sections were reacted with 50 mmol/L Tris-HCl buffer, pH 7.6, containing 0.02% 3,3'-diaminobenzidine and 0.005% hydrogen peroxide. The stained sections were counterstained with methyl green or Mayer's hematoxylin, dehydrated with butanol or ethanol and mounted in Canada balsam (Sigma Chemical).

Tissues of colon cancer and normal colonic mucosa were used as control tissues for the immunohistochemical staining; staining of colon cancer cells was evaluated for a positive control for CD55 and CD59 staining,[9] and staining of normal colonic epithelial cells was evaluated for CD46 staining.[9,10] The expression of the antigens in the normal and neoplastic epithelia was evaluated blindly according to an arbitrarily defined score: 2+, specific staining was comparable to the staining of the control colonic tissue; 1+, specific staining was detectable but in less than that of the positive control; and –, faint or no antigen was detectable.

Statistical analysis

Correlation between expression of the complement regulatory proteins and histology of esophageal tissues was analyzed by using χ^2 test for independence.

RESULTS

Normal human esophagus

In the normal esophageal mucosa, marked differences among the patterns of expression of CD55, CD46 and CD59 were observed. CD55 was intensely stained on the plasma membrane of cells of squamous epithelium in the superficial and prickle cell layers in all cases, whereas cells in the basal and parabasal cell layers had little or no staining (Fig. 1a). A reversed pattern of expression was observed for CD46, which was most intensely stained on the cell membrane in the basal and parabasal cell layers, and the staining progressively decreased toward the superficial cell layer (Fig. 1b). In contrast to the restricted expression of CD55 and CD46, CD59 was broadly distributed and intensely stained on the cell membrane in all layers (Fig. 1c). In the underlying stromal compartment, these complement regulatory proteins were stained throughout stromal cells and vessels (Fig. 1). Additionally, CD55 exhibited fibrillar staining in the stroma (Fig. 1a). No apparent difference of the expression of the three complement regulatory proteins was noted between normal-appearing esophageal mucosa obtained by endoscopic biopsy from patients without esophageal cancer, and histologically normal mucosa obtained from the uninvolved portions of surgically resected esophagus from esophageal cancer patients.

Esophageal squamous cell carcinoma

In the esophageal squamous cell carcinoma, differences in the staining patterns of the three complement regulatory proteins were apparent. CD55 staining was intense in the stroma but was negligible or only focally present in the cancer cells (Fig. 2a). In the areas of kera-

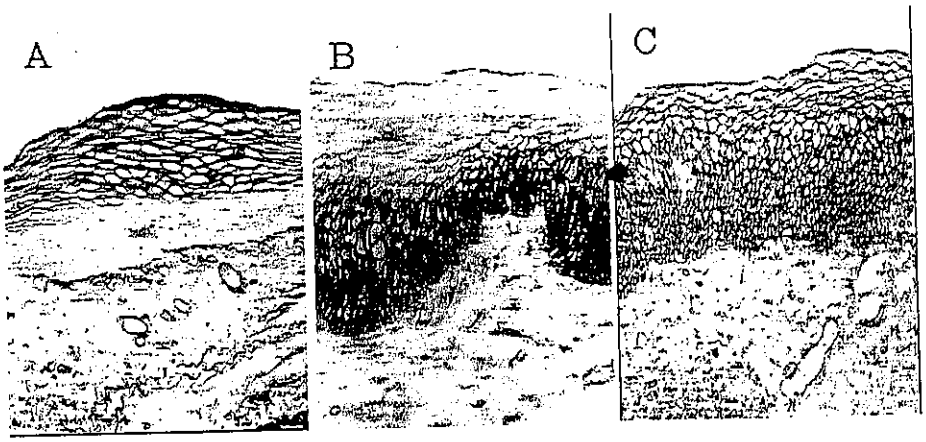

Figure 1 Immunohistochemical localization of complement regulatory proteins in normal esophageal epithelium. In the normal epithelium, (a) CD55 is stained predominantly on the superficial and prickle cell layers, whereas (b) CD46 is expressed mainly on the basal/parabasal cell layers and the staining progressively decreases toward the superficial cell layer. (c) CD59 was broadly distributed and intensely stained on the cell membrane in all layers.

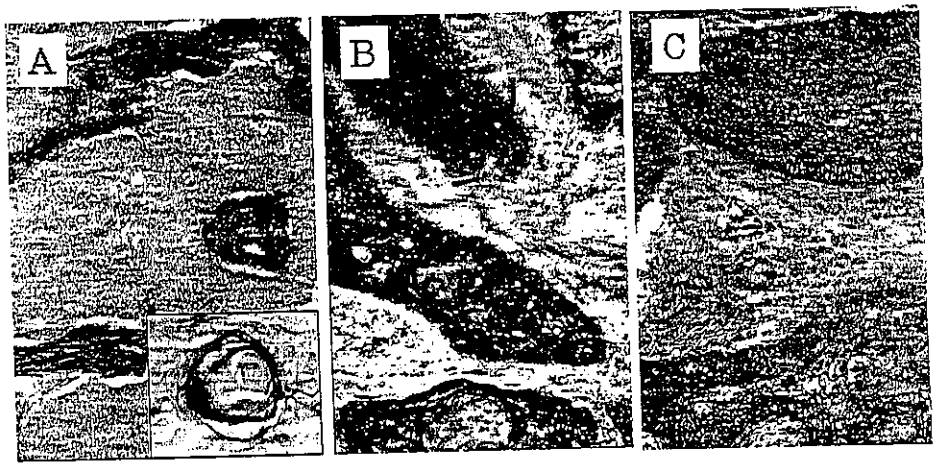

Figure 2 Immunohistochemical localization of complement regulatory proteins in esophageal squamous cell carcinoma. (a) CD55 staining is intense in the stroma but is negligible or only focally observed in the cancer cells. (a, insert) In areas of the keratinization, CD55 staining is observed along lamellar structures in the outside area of the keratin, which seem to be flattened keratinized cancer cells. (b) In contrast, CD46 is stained almost uniformly and intensely on the tumor cell membrane, whereas its staining in the stroma is weak. (c) CD59 staining is observed uniformly in the cancer cell membrane, and the stroma is also stained almost to the same degree as in cancer cells.

tinization, CD55 staining was observed along lamellar structures in the outside area of the keratin, which seemed to be flattened keratinized cancer cells (Fig. 2a, insert). In contrast to CD55 staining, CD46 staining was almost uniform and intense on the tumor cell membrane, whereas in the stroma it was weak (Fig. 2b). CD59 staining was present uniformly on the cancer cell membrane, and the stroma was also stained almost to the same degree as in cancer cells (Fig. 2c).

The intensity of the staining of the complement regulatory proteins in normal esophageal epithelium and esophageal carcinoma is summarized in Table 1. There was a significant difference in the intensity of the staining of CD55 and CD46 among cells in various layers of normal mucosa and carcinoma cells, but not in the staining of CD59. Similar expression patterns of the three complement regulatory proteins in carcinoma cells and in normal epithelium in the basal and parabasal cell layers were observed. There was no apparent correlation between the staining intensity of the three complement regulatory proteins and the histological type or the TNM stage of esophageal cancers (data not shown).

DISCUSSION

In the present study we defined the immunohistochemical distribution of the complement regulatory proteins, CD55, CD46 and CD59 in normal esophageal squamous epithelium and esophageal squamous cell carcinoma. Although the expression of CD55 in the normal esophageal mucosa has been reported,[6] the precise distribution of CD55 and the expression of CD46 and

Table 1 Expression of complement regulatory proteins in normal esophageal mucosa and esophageal squamous cell carcinoma

		–	1+	2+	Total	χ^2 test
CD55						
Normal	Superficial layer	0	7	18	25	
	Prickle layer	1	23	1	25	
	Basal, parabasal layer	25	0	0	25	
Carcinoma	Carcinoma cell	14	1	0	15	$P < 0.0001$
CD46						
Normal	Superficial layer	20	4	1	25	
	Prickle layer	17	5	3	25	
	Basal, parabasal layer	0	8	17	25	
Carcinoma	Carcinoma cell	0	2	13	15	$P < 0.0001$
CD59						
Normal	Superficial layer	0	3	22	25	
	Prickle layer	1	5	19	25	
	Basal, parabasal layer	0	6	19	25	
Carcinoma	Carcinoma cell	0	0	15	15	NS

2+, specific staining was comparable to the staining of the control colonic tissue; 1+, specific staining was detectable but less intense than that of the positive control; –, faint or no antigen was detectable; NS, not significant.

CD59 have not been described. We found that CD55 and CD46 had a restricted distribution, and CD55 was present in the superficial and prickle cell layers, whereas CD46 predominantly stained in the basal and parabasal cell layers. In contrast, CD59 was broadly distributed throughout the squamous epithelial layers. In the esophageal squamous cell carcinoma, carcinoma cells retained the expression of CD46 and CD59, but CD55 expression was greatly reduced.

Several studies have described the expression of these complement regulatory proteins in the skin[6,25–30] and uterine cervix,[31,32] which, like the esophagus, are covered with stratified squamous epithelium. In the normal skin epidermis, a somewhat different pattern of expression from that in the esophagus has been noted; CD59 was mainly present in the basal cell layer, and conflicting results have been reported on the distribution of CD55.[6,25,29,30] In contrast, the reported patterns of expression of the three complement regulatory proteins in the normal ectocervix[31,32] are almost the same as those we have observed in the esophageal mucosa. In the normal ectocervical squamous epithelium, CD55 has been found in superficial and intermediate squamous epithelial cells, whereas CD46 was present predominantly in immature cervical epithelium (i.e. basal and parabasal cells). The reciprocal patterns of expression of CD55 and CD46 in the squamous epithelium of the ectocervix are believed to be related to cellular maturity,[31,32] and this may be the case also in the squamous epithelium of the esophagus. CD59 staining was broadly distributed throughout the squamous epithelium in the ectocervix,[31,32] as in the esophagus, and a developmental gradient of expression was not observed.

In the esophageal squamous cell carcinoma, we found that cancer cells were almost devoid of CD55 staining, whereas CD46 and CD59 uniformly stained on the plasma membrane of cancer cells. These findings might indicate that esophageal carcinoma cells are resistant to complement-mediated immune attack by regulating complement activation at the level of C3 convertases and preventing the formation of the cytolytic membrane attack complex (i.e. C5b-9), but further studies such as staining for complement deposition are needed to clarify this issue.

Reduction of CD55 expression in esophageal squamous cell carcinoma is in accordance with findings in squamous cell carcinoma of other tissues, except for squamous cell carcinoma of human skin.[28] Cutaneous squamous cell carcinoma has been reported to express CD55, whereas squamous carcinoma cells of the cervix[31,32] and lung[3] reportedly have the same expression pattern as in the esophagus (i.e. CD55 was negative but CD59 and CD46 were positive). The epidermis of skin is slightly different from other organs covered with squamous epithelium because of the presence of a horny cell layer with keratinizaion; this anatomical difference might be one of the factors that account for the difference of expression pattern of the complement regulatory proteins between skin and other tissues in both normal and malignant conditions.

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Letters to the editor

Pneumocystis carinii pneumonia in patients with ulcerative colitis

Pneumocystis carinii pneumonia (PCP) causes pneumonia in immunocompromised patients suffering from diseases such as leukemia, malignant lymphoma, and other cancers, and those treated with corticosteroids and/or immunosuppressive agents. However, it has rarely been reported in patients with ulcerative colitis (UC). We report three patients with UC who developed PCP during corticosteroid and/or azathioprine (AZA) administration.

Case 1 was a 26-year-old woman with an 8-month history of ulcerative colitis. She had hematochezia, and colonoscopy showed severe pancolitis. She had a blood transfusion and was treated with prednisolone. at a dose of 60 mg/day. Her disease was unresponsive to therapy with prednisolone. AZA at 100 mg/day was added. Her symptoms improved gradually, but AZA was stopped on the day 34 after the beginning of administration because of leukocytopenia. The following day she had a high-grade fever. Laboratory data were as follows: white blood cell count (WBC), 2500/mm^3; lymphocyte count (Ly), 650/mm^3; hemoglobin (Hb), 10.3 g/dl; and platelet count (Plt), 24.1 × 10^4/mm^3. Arterial blood gases in room air were: pH 7.44, PCO_2, 36.0 mmHg; and PO_2, 56.0 mmHg. The CD4/CD8 ratio in peripheral blood was decreased, at 0.5. X-ray and computed tomography (CT) of the chest revealed bilateral "ground-glass" shadows (Fig. 1).

Case 2 was a 68-year-old woman with an 11-year history of left-sided ulcerative colitis. She presented with acute colitis. We started pulse steroid therapy. The response was excellent and she entered the remission stage on clinical tests. She developed a high fever 2 days after the start of maintenance therapy with prednisolone at a dose of 7.5 mg/day. Laboratory data were as follows: WBC, 6200/mm^3; Ly, 990/mm^3; Hb, 10.5 g/dl; and Plt, 35.9 × 10^4/mm^3. Arterial blood gases in room air were: pH 7.47, PCO_2, 32.7 mmHg; and PO_2, 70.0 mmHg. The CD4/CD8 ratio in peripheral blood was normal (1.1). Chest X-ray and CT showed bilateral "ground-glass" shadows.

Case 3 was a 29-year-old man with a 4-month history of ulcerative colitis. He presented with acute colitis exacerbation. Colonoscopy showed total colitis. We changed the corticosteroid from prednisolone, 40 mg/day, to betamethasone, 4 mg/day. AZA, 100 mg/day, was added and the response was satisfactory. Then we began to reduce betamethasone gradually, to 1.5 mg/day. His clinical course was excellent and he was discharged. Four months later, he was readmitted with a high fever. Laboratory data were as follows: WBC, 3400/mm^3; Ly, 880/mm^3; Hb, 11.0 g/dl; and Plt, 15.6 × 10^4/mm^3. Arterial blood gases in room air were: pH, 7.39; PCO_2, 41.0 mmHg; and PO_2, 91.0 mmHg. Chest X-ray was normal, but chest CT revealed faint "ground-glass" shadow in the right lung (Fig. 2).

In all three patients, polymerase chain reaction (PCR) for detection of *P. carinii* in sputa was positive. and we diagnosed PCP. Cytomegalovirus antigen in peripheral blood was negative in all the patients. After the diagnosis of PCP, they were treated

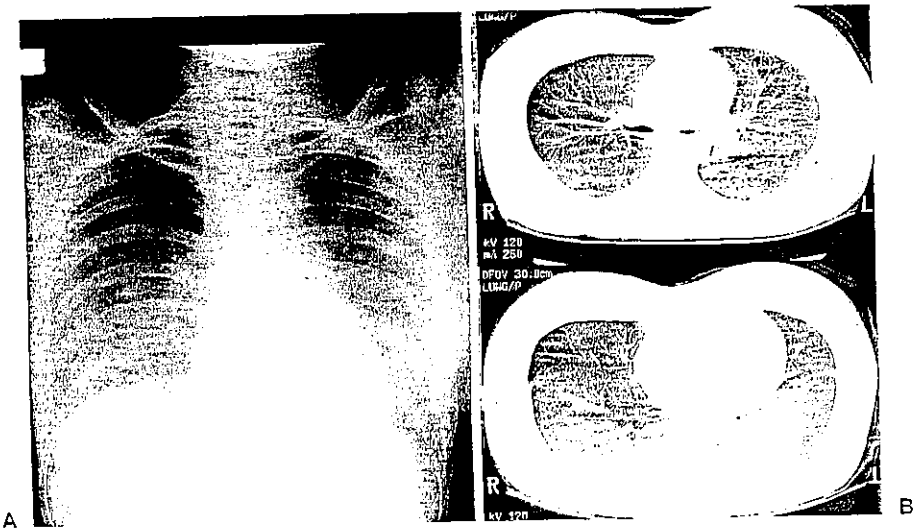
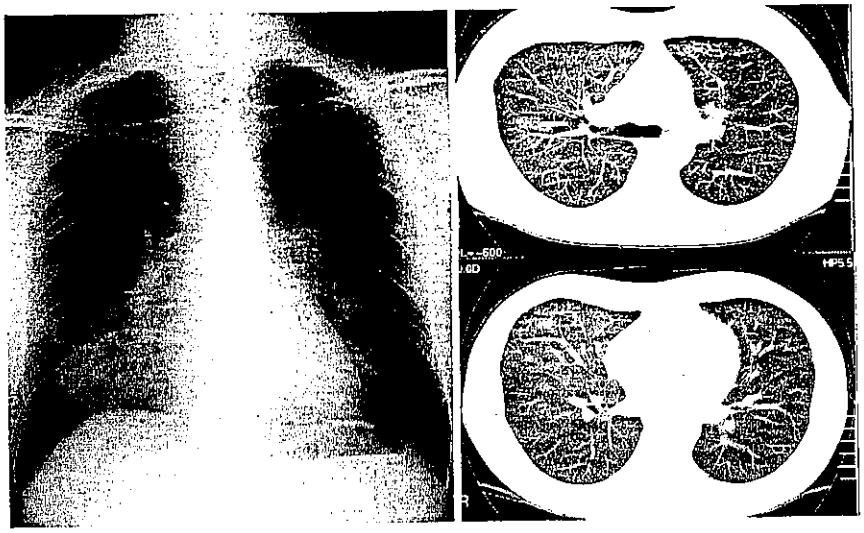


Fig. 1. A Chest X-ray shows interstitial shadows in bilateral middle and lower lung fields. B Chest computed tomography (CT) reveals diffuse "ground-glass" opacity in bilateral lung fields. with a small amount of pleural effusion. Both images are from case 1. taken on the same day



A B

Fig. 2. A Chest X-ray shows almost normal findings. B Chest CT shows faint "ground-glass" opacity in the right lung. Both images are from case 3, taken on the same day

with intravenous trimethoprim-sulfamethoxazole (TMP-SMX) and improved quickly.

The symptoms of PCP worsen without appropriate therapy. As chest X-rays are often normal, as shown in our case 3, or show a faint ground-glass shadow, chest CT is very useful for detecting abnormalities at the early stage of PCP.[1] This modality usually reveals bilateral diffuse ground-glass opacity. Diagnostic identification of *P. carinii* is based on the microscopic detection of the organism in pulmonary materials. However, it is difficult to do such an invasive examination as bronchoscopy when the patient's condition is worsening. Noninvasive diagnosis of *P. carinii* infection using PCR of patient's sputa is of great benefit.

For the prevension of PCP in patients with UC who are receiving immunosuppressive therapy, prophylactic treatment with TMP-SMX should be considered.[2–4] Administration of prednisolone, at more than 16 mg/day over a 2-months period,[5] or at 20 mg/day over a 4-weeks period,[6] is suggested as the indication for preventive therapy. In the present case report, we have described three patients with UC who developed PCP during immunosuppressive therapy; one patient received pulsed steroid therapy, and two received AZA together with corticosteroids. Thus, we believe that prophylaxis has to be carried out in UC patients receiving immunosuppressive therapy with high doses of corticosteroid, as well as in those receiving combination therapy with corticosteroids and immunosuppressants such as AZA, considering the benefit of such prophylaxis and the high mortality of PCP.

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A pregnant patient with fulminant hepatic failure was found to carry a novel missense mutation in the argininosuccinate synthetase gene

Classical citrullinemia (CTLN1; OMIM no. 215700) caused by mutations of the argininosuccinate synthetase (*ASS*; EC6.3.4.5) gene on chromosome 9q34 differs from adult-onset type II citrullinemia (CTLN2; OMIM no. 603471), which is caused by mutations of citrin encoded by *SLC25A13* on chromosome 7q21.3. Plasma citrulline/arginine levels enable the distinction of CTLN1 (2500 ± 1040/58 ± 31 nmol/ml) from CTLN2 with liver-specific *ASS* deficiency (521 ± 290/232 ± 167 nmol/ml). To date, 50 *ASS* mutations have been identified in CTLN1 patients, and differences among races have been noted.[1] Most CTLN1 patients are neonatal- or infantile-onset, but some adult-

Increased serum concentrations and surface expression on peripheral white blood cells of decay-accelerating factor (CD55) in patients with active ulcerative colitis

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Inflammatory stimuli induce expression and release of decay-accelerating factor (DAF), a complement-regulatory protein present on peripheral-blood cells. Therefore, in ulcerative colitis (UC), an inflammatory colonic disease in which activated leukocytes are involved, DAF may be released from leukocytes into the circulation. In this study we compared serum DAF concentrations and surface DAF expression on peripheral-blood cells in patients with UC with disease activity. Peripheral-blood samples were obtained from 60 patients with UC (30 with active and 30 with inactive disease) and 19 healthy volunteers. Serum DAF concentrations were determined by means of immunoassay, and surface DAF expression on blood cells was examined with the use of flow cytometry. Serum DAF concentrations in patients with active disease (mean 48.6 ng/mL) were significantly higher than those in patients whose disease was in remission (33.3 ng/mL; $P = .0003$) and those in healthy controls (32.3 ng/mL; $P = .0007$). Surface DAF expression on neutrophils, CD14+ monocytes, and subsets of lymphocytes in patients with active UC was significantly increased compared with that in patients with UC in remission and in healthy controls. The increased serum DAF concentrations and surface DAF expression on leukocyte fractions in patients with active disease fell to significantly lower levels when the disease had gone into remission after medical therapy. Serum DAF concentrations are increased in UC patients in relation to disease activity. The likely source of increased DAF concentrations is peripheral-blood leukocytes that have been activated as part of the UC disease process. (J Lab Clin Med 2004;143:152-8)

Abbreviations: DAF = decay-accelerating factor; FITC = fluorescein isothiocyanate; HRP = horseradish peroxidase; IL = interleukin; SCR = short consensus repeat; TNF = tumor necrosis factor; UC = ulcerative colitis; WBC = white blood cell

Altered regulation of cell- and humoral factor–mediated immune responses against intestinal constituents may play a role in the development of UC.[1] The complement system is a major effector pathway in humoral factor–mediated immunity, and we have previously shown the activation and degradation of complement in mucosal lesions of active UC.[2] Autologous complement activation is regulated by complement-regulatory proteins to protect host cells. One of these proteins, DAF (CD55), is a glycosylphosphatidyl inositol–anchored protein that inhibits

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Table I. Characteristics of UC patients and controls

Characteristics	UC			Healthy controls (*n* = 19)
	Total (*n* = 60)	Active (*n* = 30)	Remission (*n* = 30)	
Age (yr)*	39.0 ± 2.0 (14-74)	38.2 ± 2.5 (14-62)	39.8 ± 3.1 (14-74)	35.8 ± 1.8 (27-59)
Sex (M/F)	32/28	14/16	18/12	11/8
Age at diagnosis (yr)*	31.8 ± 1.8 (9-71)	31.3 ± 2.2 (13-59)	32.2 ± 3.0 (9-71)	
Duration of disease (yr)*	7.2 ± 0.9 (0-30)	6.7 ± 1.3 (0-30)	7.6 ± 1.3 (0-26)	
Location of disease				
Proctitis	8 (13%)	3 (10%)	5 (17%)	
Left-sided colitis†	21 (35%)	13 (43%)	8 (27%)	
Pancolitis	31 (52%)	14 (47%)	17 (56%)	

*Data expressed as mean ± SEM (range).
†Inflammation up to the splenic flexure.

the formation and promotes the catabolism of C3 and C5 convertases.[3] We have found that DAF expression is markedly increased in the inflamed mucosa of UC patients[4] and that DAF is released into the stools of patients with active UC.[5] In the regulation of DAF expression, we have shown that inflammatory cytokines IL-4 and IL-1β markedly enhance the expression and release of DAF from cultured HT29 intestinal epithelial cells.[6] These inflammatory cytokines also activate polymorphonuclear leukocytes (neutrophils), and activated neutrophils express increased amounts of DAF molecules on their surfaces.[7] In addition, both resting and stimulated neutrophils have released DAF into culture supernatants.[8] Therefore DAF may be released from activated leukocyte into the circulation in patients with active UC. In this study, we examined serum DAF concentrations and cell-surface expression of DAF on peripheral-blood cells in patients with UC in relation to the severity of disease activity.

METHODS

Patients and study design. Peripheral-blood samples were obtained from 60 patients with UC (28 women, 32 men; mean age 39 years, age range 14-74 years) and 19 healthy volunteers (8 women, 11 men; mean age 36 years, range 27-59 years). The diagnosis of UC was based on history, clinical symptoms, and endoscopic and histologic findings. Thirty-one patients had pancolitis, 21 had left-sided colitis, and eight had proctitis. Disease activity was graded on the basis of clinical features and laboratory data in accordance with the criteria of Truelove and Witts[9,10] by 2 gastroenterologists (MM and HO) who had no knowledge of the DAF levels in serum and on the surfaces of peripheral-blood cells of the patients under evaluation. Remission was defined as a mild degree or better of clinical activity, without hematochezia; or better, WBC counts were not used in the evaluation. Thirty patients had active disease (19 moderate, 11 severe), and 30 had colitis in remission when blood samples were obtained. The patients' characteristics are presented in Table I. Serum samples were kept frozen at −80°C until use. We also obtained heparinized blood samples from 14 patients with active UC (11 moderate, 3 severe), 13 patients with inactive UC, and 14 healthy volunteers for the analysis of surface expression of DAF on blood cells by means of flow cytometry. We obtained blood samples from 7 patients with active UC when their disease went into remission after medical treatment. Two of the 7 were women and 5 were men (mean age 31 years, range 14–44 years); 6 had pancolitis and 1 had left-sided colitis; the colitis was severe in 2 and moderate in 5.

The study was conducted in accordance with the guidelines of the Declaration of Helsinki. Our local ethics committee approved the study protocol. The objective of the study was explained to each patient before the study, and written informed consent was obtained from each patient.

Determination of serum DAF protein. Serum samples were diluted with phosphate-buffered saline solution containing 1% bovine serum albumin (Sigma-Aldrich, St Louis, Mo), 0.05% Tween 20, and 1 mmol/L phenylmethylsulfonylfluoride with increased NaCl concentration (0.4 mol/L) to reduce nonspecific reactions as described.[11] Details of the immunoassay for the measurement of DAF have been described.[5,11-14] In brief, human DAF was purified from pooled human erythrocyte stroma, and mouse monoclonal antibodies to DAF were prepared.[15] Two of the mouse monoclonal antibodies (IgG_1), clones 1C6 and 4F11, were used. The 1C6 antibody is directed to the active site on the DAF molecule (ie, SCR 3), and the 4F11 antibody recognizes SCR4.[16] The 1C6 monoclonal antibody was labeled with HRP as described.[17] The wells of microtiter plates were coated with 4F11 monoclonal anti-DAF, and serially diluted serum samples were added to the wells. After washing, HRP-labeled 1C6 anti-DAF was added. After further washing, bound 1C6 antibody was detected with 2,2'-azino-di-3-ethylbenzo-thiazoline-6-sulfonic acid as substrate. Optical densities at 415 nm were measured with an automated ELISA plate reader. A calibration curve was obtained from several dilutions of known quantities of purified DAF, and the concentrations of serum DAF were calculated. Samples were analyzed in duplicate.