Randomized, Controlled Study on Adjuvant Immunochemotherapy with PSK® in Curatively Resected Colorectal Cancer

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A randomized, controlled trial of adjuvant immunochemotherapy with PSK® (Kureha Chemical Industry Co., Tokyo, Japan) in curatively resected colorectal cancer was studied in 35 institutions in the Kanagawa prefecture. From March 1985 to February 1987, 462 patients were registered. Four hundred forty-eight of those patients (97.0 percent) satisfied the eligibility criteria. The control group received mitomycin C intravenously on the day of and the day after surgery, followed by oral 5-fluorouracil (5-FU) administration for over six months. The PSK® group received PSK® orally for over three years, in addition to mitomycin C and 5-FU as in the control group. At the end of February 1990, the median follow-up time for this study was four years (range, three to five years). The disease-free survival curve and the survival curve of the PSK® group were better than those of the control group, and differences between the two groups were statistically significant (disease-free survival, $P = 0.013$; survival, $P = 0.013$). These results indicate that adjuvant immunochemotherapy with PSK® was beneficial for curatively resected colorectal cancer. [Key words: PSK®; Adjuvant immunochemotherapy; Colorectal cancer; Randomized, controlled study]

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In Japan, the incidence of colorectal cancer is only about a quarter of that seen in Western countries. However, it has tended to increase in recent years, and, if this tendency continues, the incidence of colorectal cancer in Japan will reach the same levels as in Western countries in the near future.

A first-choice treatment for colorectal cancer is surgery. However, curative results could only be obtained in under 60 percent of the cases by surgical intervention alone, and we are still not satisfied with the results. Various attempts have been made to improve the results of surgical treatment. One of these has been the concomitant use of anticancer drugs and immunomodulators with surgery, *i.e.*, adjuvant immunochemotherapy. However, even though tumor shrinkage effects have been observed with the use of immunochemotherapy in cases of colorectal cancer, there have been no sufficient studies to date in terms of the effect on prolonged survival times. Therefore, the benefit of the administration of PSK® (KRESTIN) in adjuvant immunochemotherapy of colorectal cancer was jointly studied in 35 institutions in the Kanagawa prefecture (Table 1).

PSK® is recognized as being an immunomodulator comprising a protein-bound polysaccharide extracted from mycelia of *Coriorus versicolor*. In

Table 1.
The Cooperative Study Group

Odawara City Hp.
Ashigarakami Prefectural Hp.
Kanagawa Cancer Center
Municipal Ida Hp. (City of Kawasaki)
Kanto Rosai Hp.
Kitasato Univ. School of Medicine
Keiyu General Hp.
Inadanoborito Hp.
Yokosuka Kyosai Hp.
Yokosuka Hokubu Kyosai Hp.
Sagamihara National Hp.
Yokohama National Hp.
Saiseikai Kanagawaken Hp.
Saiseikai Yokohamashi-Nambu Hp.
Yokohama Central Hp.
Showa Univ. School of Medicine, Fujigaoka Hp.
St. Marianna Univ. School of Medicine
St. Marianna Univ. School of Medicine, Toyoko Hp.
Teikyo Univ. Hp. at Mizonokuchi
Tokai Univ. School of Medicine
Tokai Univ., Oiso Hp.
Toshiba Rinkan Hp.
Second Hp. of Nippon Medical School
Hadano Red Cross Hp.
Hiratsuka Kyosai Hp.
Fujisawa City Hp.
Yamachika Hp.
Yamato City Hp.
Yokosuka City Municipal Hp.
Yokohama City Kowan Hp.
Yokohama City Municipal Hp.
Yokohama City Univ. School of Medicine (Surgery I)
Yokohama City Univ. School of Medicine (Surgery II)
Yokohama Red Cross Hp.
Yokohama Minami Kyosai Hp.

Hp. = Hospital; Univ. = University.

1965, Kureha Chemical Industry's attention was attracted to the case of a gastric cancer patient who recovered so far as to be able to do daily work after being administered a hot water extract of so-called "Saru-no-koshikake," which is a kind of Basidiomycetes. Since then, experimental and clinical studies on the antitumor effects of Basidiomycetes have been carried out, and this Basidiomycetes extract was first commercially available on the market as KRESTIN® in 1977. The average molecular weight of PSK® is about 100 kilodaltons, as measured by ultracentrifuge analysis. The constituent major monosaccharide is glucose, with smaller amounts of other saccharides (Table 2), and structural analysis showed that the main glycoside portion is β-1,4-glucan. In addition, PSK® has branches at positions 3 and 6 at a rate of one branch per several residual groups of 1→4 bonds (Fig. 1). Liquid chromatographic analysis of PSK® after hydrochloric acid hydrolysis reveals that the protein portion of PSK® consists predominantly of acidic amino acids such as aspartic acid and glutamic acid, and also neutral amino acids such as valine and leucine, with basic amino acids including small amounts of lysine and arginine (Table 3).

METHODS

Patient Selection

Eligibility criteria were as follows: 1) primary carcinoma of the colon and rectum; 2) TNM classification: any TN1, 2, 3M0 or T4N0M0; 3) macroscopically curative resection; 4) under 75 years of age; and 5) informed consent obtained from patient or patient's family before registration in the study. (In Japan, there are many patients who are not informed about their carcinomas. In the cases of those patients, informed consents were occasionally obtained from their families in this study.)

Table 2.
Monosaccharide Composition in Sugar Portion of PSK®

Monosaccharide	Composition (%)
Glucose	74.6
Galactose	2.7
Mannose	15.5
Xylose	4.8
Fucose	2.4

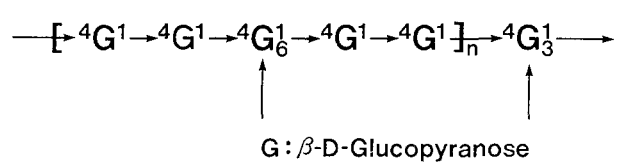

Figure 1. Estimated formula of saccharide portion of PSK®.

Table 3.
Amino Acid Composition in the Protein Portion of PSK®

Amino Acid	Composition (%)	Amino Acid	Composition (%)
Aspartic acid	13.2	Methionine	1.9
Threonine	4.5	Isoleucine	5.9
Serine	4.7	Leucine	13.4
Glutamic acid	14.4	Tyrosine	2.9
Proline	+	Phenylalanine	6.7
Glycine	7.8	Tryptophan	+
Alanine	9.2	Lysine	2.8
Cystine	+	Histidine	2.3
Valine	9.6	Arginine	0.7

+ = trace.

Patients were excluded if they had 1) received cancer therapy before resection, 2) duplicated or multiple carcinoma, 3) had severe complications, or 4) shown the following abnormal laboratory findings before resection: WBC < 4,000/mm^3, PLT < 100,000/mm^3, protein < 6.0 g/dl, albumin < 3.0 g/dl, A/G < 1, SGOT·SGPT < 100 U, urine protein: (+), and creatinine > 1.5 mg/dl.

Patient Registration and Randomization Procedures

A total of 462 patients with colorectal cancer were registered during the two-year period from March 1, 1985 to February 28, 1987. Of these, 448 patients (97.0 percent) satisfied the eligibility criteria (colon, 249 cases; rectum, 199 cases) (Fig. 2). Patients were registered using a centralized registration system by telephone.

The attending physician informed the center office (Tokai University) of the results of the operation immediately after each operation. Then each of the patients was assigned one of two different regimens by the permuted blocks that were stratified according to the location of the carcinoma (colon *vs.* rectum) and the institution.

Treatments

The control group received mitomycin C (6.0 mg/m^2) intravenously on the day of and the day after surgery, followed by oral 5-FU (200 mg per day) administration for more than six months.

The PSK® group received PSK® (3 g per day) orally for over three years, in addition to mitomycin C and 5-FU as in the control group (Fig. 3).

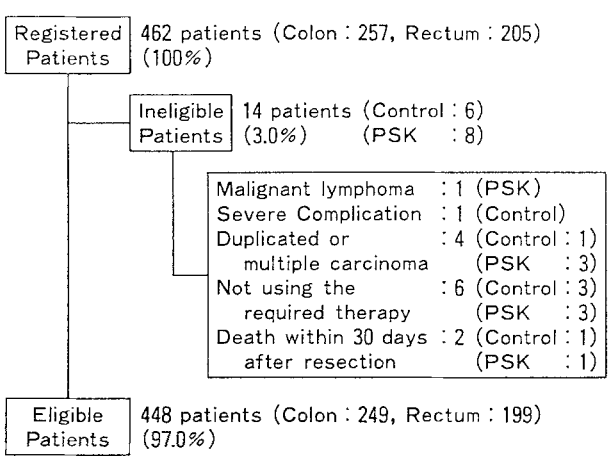

Figure 2. Details on registered patients.

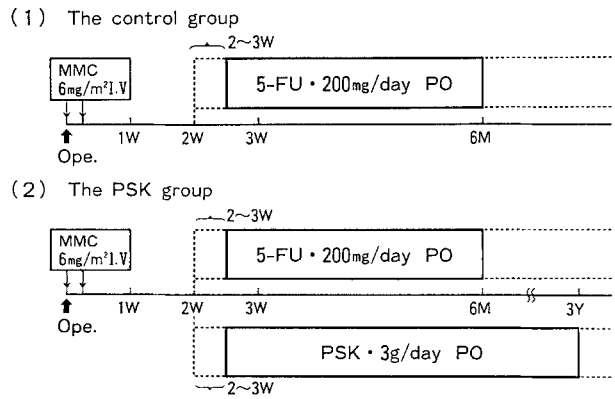

Figure 3. Treatments.

Of the 227 patients assigned to the control group, 200 (88.1 percent) received 5-FU for up to six months or until tumor recurrence after the initial surgery. Among the 27 patients for whom 5-FU therapy was abbreviated, the most common reasons for such abbreviation were side effects (13 patients) and postoperative complications (9 patients). The most frequent side effects were gastrointestinal disorders (Table 4).

In terms of the PSK® group, of the 221 patients, 191 (86.4 percent) were administered 5-FU for up to six months after the resection or until tumor recurrence, while 201 (91.0 percent) were administered PSK® for over six months after the resection. Among the 30 patients for whom 5-FU therapy was abbreviated or the 20 patients for whom PSK® therapy was abbreviated, the most common reasons for such abbreviation were almost the same as those reasons for the control group, but few side effects were caused by PSK®.

Statistical Analysis

Statistical analyses were carried out according to the procedures of SAS.[1] The disease-free curves and the survival curves were generated by the Kaplan-Meier method.[2] The patients who died without recurrence were treated as censored. The log-rank test (L-R)[3] was used for comparison of the two groups. All *P* values are two-sided. Differences in the characteristics of patients were analyzed using the chi-squared test.

RESULTS

At the end of February 1990, the median follow-up time for this study was four years (range, three to five years). At this time, 448 eligible patients were simultaneously investigated regarding tumor

Table 4.
Side Effects for Which Treatment was Abbreviated

Side Effects*	Control (n = 227)	PSK® (n = 221)
Gastrointestinal		
Nausea	4 (1.8%)	2 (0.9%)
Vomiting	1 (0.4%)	1 (0.5%)
Anorexia	2 (0.9%)	3 (1.4%)
Stomach discomfort	1 (0.4%)	1 (0.5%)
Diarrhea	1 (0.4%)	6 (2.7%)
Gastric ulcer	1 (0.4%)	
Hematologic		
Leukopenia	2 (0.9%)	7 (3.2%)
Thrombocytopenia		1 (0.5%)
Anemia	1 (0.4%)	1 (0.5%)
Others		
Abnormal hepatic function	2 (0.9%)	5 (2.3%)
Malaise	1 (0.4%)	1 (0.5%)
Oral dryness	1 (0.4%)	
Palpitation		1 (0.5%)
Eruption	1 (0.4%)	

* Including those caused by mitomycin C.

recurrences and deaths. It was not clear whether 24 patients survived or not, since they had not continued to visit their respective hospitals. Therefore, local government authorities were contacted to find out whether these 24 patients had survived, and, at the end of February 1990, it was clearly established who among these patients had survived.

Characteristics of the Patients

The control group and the PSK® group were well balanced regarding clinical and macroscopic characteristics, such as sex, age, tumor extent, and regional lymph node involvements (Table 5A). However, regarding the size of rectal tumors, the PSK® group consisted of patients who had larger tumors than those of the control group ($P < 0.05$, chi-squared test). These two groups were also well balanced in terms of microscopic characteristics, such as histologic differentiation, tumor extent, regional lymph node involvements, and Dukes' classification (Table 5B).

Tumor Recurrences

By the end of February 1990, 133 patients had had tumor recurrences (79 in the control group and 54 in the PSK® group) (Table 6A). Figure 4 shows the rates of initial tumor recurrence in relation to the patterns of tumor recurrence in each group. The rates of tumor recurrence of the PSK® group were lower than those of the control group regarding any patterns of recurrence.

By the end of February 1990, 100 patients had died because of tumor recurrence. Of these, 62 were in the control group and 38 were in the PSK® group (Table 6B).

Disease-Free Survival

All Patients. The three-year disease-free survival estimate for the patients in the PSK® group was 77.2 percent, and it was 67.7 percent for those in the control group according to the Kaplan-Meier method. Disease-free survival curves are plotted in Figure 5A. These curves show that treatment with adjuvant immunochemotherapy with PSK® produced a distinct advantage compared with the treatment with chemotherapy alone ($P = 0.0134$).

Colonic Cancer and Rectal Cancer. In the colonic cancer patients, the disease-free survival curve of the PSK® group was predominantly better than that of the control group, and the difference between the two groups was statistically significant ($P = 0.0467$) (Fig. 5B). Regarding the rectal cancer patients, the disease-free survival curve of the PSK® group was also better than that of the control group but the difference between the two groups was not statistically significant ($P = 0.1212$) (Fig. 5C).

Table 5A.
Clinical and Macroscopic Characteristics

Characteristics		Colon		Rectum		Total	
		Control	PSK®	Control	PSK®	Control	PSK®
Sex	Male	65	72	63	53	128	125
	Female	60	52	39	44	99	96
Age (yr)	Under 40	10	7	3	7	13	14
	40–49	21	20	20	17	41	37
	50–59	41	37	33	30	74	67
	60–69	30	37	33	34	63	71
	70 and over	23	23	13	9	36	32
Size of tumor (cm)	0–1.9	0	0	1	0	1	0
	2.0–3.9	23	20	17	5	40	25
	4.0–7.9	87	90	75	80	162	170
	8.0 and over	14	12	8	12	22	24
	Unknown	1	2	1	0	2	2
Extent of tumor*	T1	0	0	0	0	0	0
	T2	1	2	4	5	5	7
	T3	10	12	19	21	29	33
	T4	114	110	79	71	193	181
Regional lymph node*	N0	0	2	1	3	1	5
	N1 + N2	65	69	61	54	126	123
	N3	60	53	40	40	100	93

* TNM classification.

Table 5B.
Microscopic Characteristics

Characteristics		Colon		Rectum		Total	
		Control	PSK®	Control	PSK®	Control	PSK®
Histologic differentiation	Well	64	79	52	49	116	128
	Moderate	53	39	39	40	92	79
	Poor	3	2	2	4	5	6
	Others	5	4	9	3	14	7
	Unknown	0	0	0	1	0	1
Extent of tumor*	pT1	1	1	1	0	2	1
	pT2	7	11	16	14	23	25
	pT3	63	62	40	47	103	109
	pT4	54	50	45	35	99	85
	Unknown	0	0	0	1	0	1
Regional lymph nodes*	pN0	64	71	43	47	107	118
	pN1 + pN2	38	29	33	25	71	54
	pN3	23	24	26	25	49	49
Dukes'	A	39	42	32	34	71	76
	B	25	29	11	13	36	42
	C	61	53	59	50	120	103

* TNM classification; p = pathologic.

Survival

All Patients. Survival curves for all eligible patients in this study are plotted in Figure 6A. The three-year survival estimate was 85.8 percent for the PSK® group and 79.2 percent for the control group. Thus, treatment with adjuvant immunochemotherapy was obviously more advantageous than treatment with chemotherapy alone (P = 0.0130).

Table 6A.
Number of Recurrences

Location		Year					Total
		0–1	1–2	2–3	3–4	4–5	
Colon	Control	16	13	4	1	1	35
	PSK®	8	7	4	2	1	22
Rectum	Control	16	21	3	3	1	44
	PSK®	13	13	5	1	0	32
Total	Control	32	34	7	4	2	79
	PSK®	21	20	9	3	1	54

Table 6B.
Number of Deaths from Recurrences

Location		Year					Total
		0–1	1–2	2–3	3–4	4–5	
Colon	Control	3	14	5	5	2	29
	PSK®	1	9	3	1	2	16
Rectum	Control	5	11	10	6	1	33
	PSK®	3	8	7	3	1	22
Total	Control	8	25	15	11	3	62
	PSK®	4	17	10	4	3	38

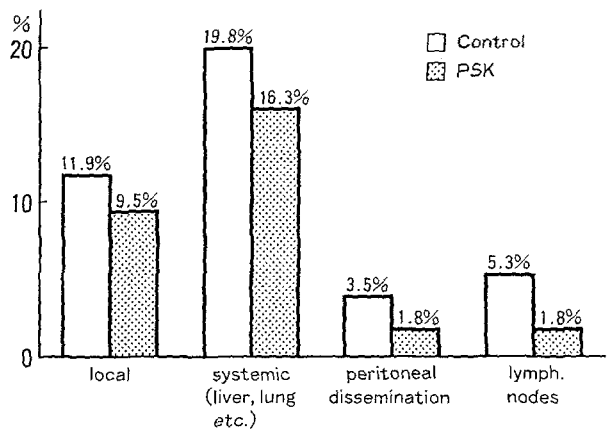

Figure 4. Rates of tumor recurrence.

Colonic Cancer and Rectal Cancer. In the colonic cancer patients, the survival curve of the PSK® group was better than that of the control group, and the difference between the two groups was statistically significant ($P = 0.0430$) (Fig. 6B). On the other hand, in the case of the rectal cancer patients, although the survival curve was also better than that of the control group, the difference between the two groups was not significant ($P = 0.1290$) (Fig. 6C).

DISCUSSION

Intravenous 5-FU has been widely attempted for use as the adjuvant chemotherapy for colorectal cancer in the United States and Western Europe. However, there has been no evidence of significant effect.[4] On the other hand, in Japan, oral preparation of 5-FU has been recognized as the standard treatment of adjuvant chemotherapy for gastrointestinal cancer,[5] but this treatment has also shown no obvious benefit with regard to colorectal cancer. Therefore, adjuvant immunochemotherapy consisting of combinations of chemotherapy and various nonspecific immunomodulators has been applied.[6–8] In the United States and Western Europe, levamisole, an old antiparasitic drug, is available with 5-FU for investigational use as an adjuvant

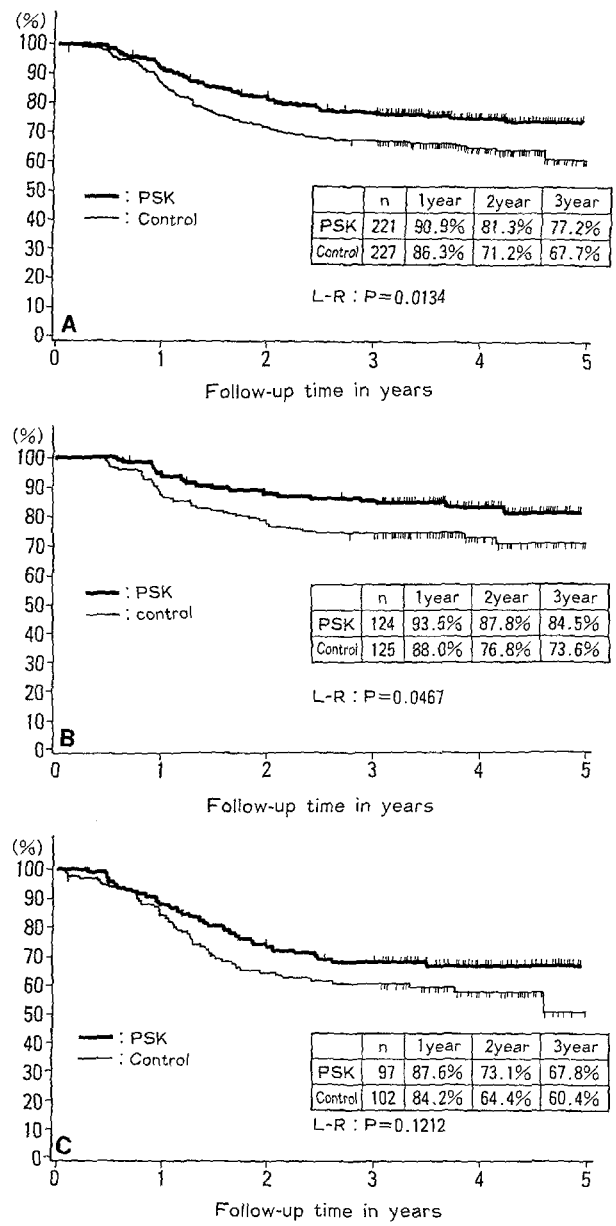

Figure 5. A. Disease-free survival (all patients). B. Disease-free survival (colon). C. Disease-free survival (rectum).

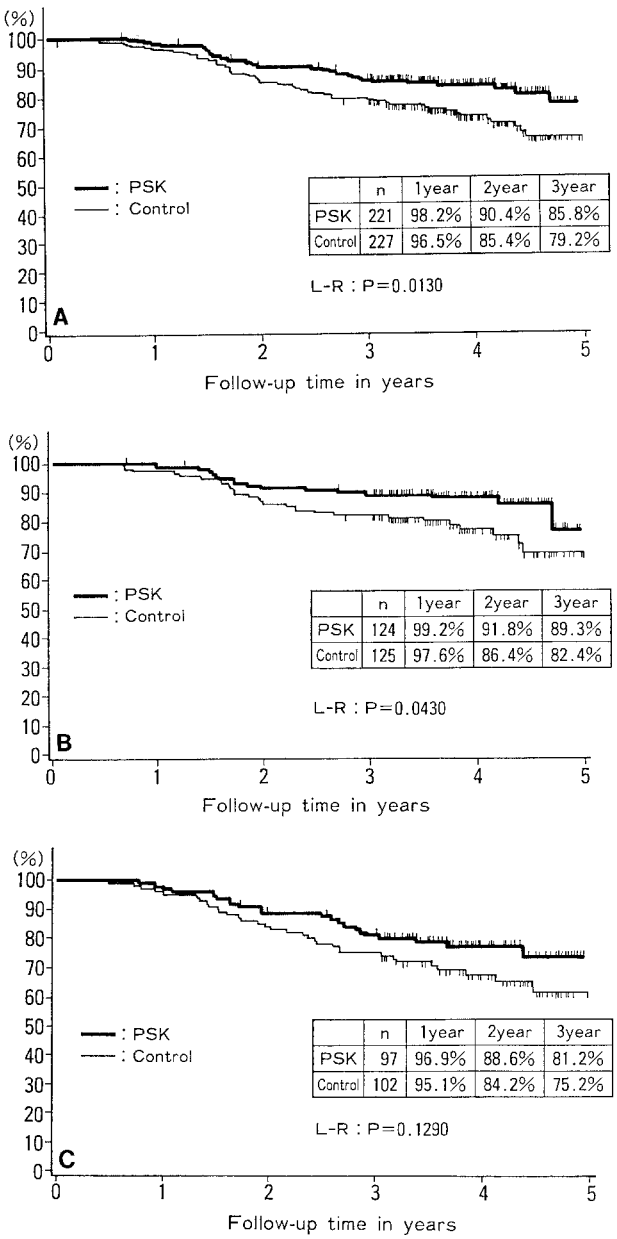

Figure 6. A. Survival (all patients). B. Survival (colon). C. Survival (rectum).

immunochemotherapy for resectable colon cancer.[9, 10] Moertel *et al.*[11] concluded that adjuvant therapy with levamisole and fluorouracil should be the standard treatment for Stage C colon carcinoma. They speculated that levamisole acts as an immunorestorative agent in patients who are immunosuppressed by both recent surgery and subsequent chemotherapy and that this effect is exerted through T cell activation, augmentation of macrophage activity, and an increase in chemotactic response of morphonuclear cells and monocytes.

PSK® is an immunomodulator developed in Japan. It has been sold on the market for more than 10 years, since it is an oral preparation and has almost no side effects that present a clinical problem.

Various suppressed immune responses of tumor-bearing animals were restored to normal levels by administration of PSK® in the tumor models tested.[12] Kamisato *et al.*[13] reported that PSK® produced marked morphologic and biochemical changes in macrophages when added into the cultures of mouse peritoneal macrophages.

On the other hand, Hosokawa *et al.*[14] found that PSK® inhibited the growth of recurrent or metastatic tumor cells in autochthonous C57BL/6 mice after the surgical removal of 3-methylcholanthrene-induced primary tumors. Mickey *et al.*[15] also investigated growth alteration effects of PSK® in an experimental prostatic cancer model and concluded that immunomodulation with PSK® may enhance the antineoplastic effects of chemotherapeutic agents. To clarify the potential immunomodulating activities of PSK®, Hirose *et al.*[16] examined the direct effect of PSK® on cytokine gene expression and production in human peripheral blood mononuclear cells and found that PSK® was a potent inducer of gene expression for the interleukins IL-1α, IL-1β, IL-6, and IL-8, for tumor necrosis factor, and for monocyte chemotactic and activating factor. Kikuchi *et al.*[17] also examined the effects of PSK® on IL-2 production by peripheral lymphocytes of patients with advanced ovarian carcinoma during chemotherapy and found that, when PSK® treatment was initiated after completion of combination chemotherapy, the OKT4/OKT8 cell ratio and IL-2 production were significantly higher than those of the control groups.

In the present cooperative study, the median follow-up time was four years (range, three to five years), and, in an examination of all colonic and rectal cancer patients, the PSK® group showed significantly better results than the control group with regard to both the disease-free survival and survival periods.

It has been reported that tumor recurrence of colorectal cancer is the most frequent within one to two years after the operation. In the present follow-up study, tumor recurrence occurred within one year in 53 cases and from one to two years in 54 cases, two to three years in 16 cases, three to four years in 7 cases, and four to five years in 3 cases. There was a marked decrease in tumor re-

currences from two years after the operation. Therefore, the pattern of the disease-free survival curve of the patients in this multicenter cooperative study appeared to be basically fixed. The ratio of recurrences in the control group to those in the PSK® group was about the same as that of the number of deaths, so the current significance of the survival period should continue in the future because the current disease-free survival curve is strongly reflected in the future survival curve. There were more than 450 patients in the present study, and only 14 (3.0 percent) ineligible patients were excluded from the analysis because of the use of a central registration system. It was suggested that adjuvant immunochemotherapy using PSK® is beneficial for curatively resected patients with colorectal cancer.

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