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A randomized trial of chemoimmunotherapy of acute nonlymphocytic leukemia in adults using a protein-bound polysaccharide preparation

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Summary. *The effect of immunotherapy with a protein-bound polysaccharide preparation termed PSK on remission duration and survival of adults with acute nonlymphocytic leukemia (ANLL) was studied in a prospective randomized cooperative trial. After having achieved complete remission and receiving a consolidation therapy, 73 patients were randomized either to maintenance chemotherapy or to maintenance chemotherapy plus immunotherapy with PSK. Ultimately 36 patients in the chemotherapy group and 31 in the chemoimmunotherapy group were evaluable. Six months after the last entry, immunotherapy with PSK showed a borderline beneficial effect on remission duration ($P = 0.089$) and on duration of survival ($P = 0.062$). When the data were analyzed 12, 18, and 24 months after the last entry there were no significant differences in duration of remission and survival between the two groups. However, analysis of the data of patients who had maintained complete remission for more than 270 days revealed that immunotherapy had a suggestive beneficial effect ($P = 0.105$), prolonging the 50% remission period by 418 days (885 vs 467 days). Thus, immunotherapy with PSK seems to be active in the treatment of adult ANLL when used for maintenance therapy in combination with chemotherapy, especially in patients with a good prognosis.*

Introduction

Following the encouraging results of immunotherapy with Bacillus Calmette-Guérin (BCG) and allogeneic leukemia cells in acute lymphoblastic leukemia (ALL) reported by Mathé et al. [10], several groups have reported beneficial effects of immunotherapy in the treatment of acute nonlymphocytic leukemia (ANLL), mostly with BCG [3, 4, 16, 19, 27]. However, there has been considerable controversy with regard to the value of immunological manipulation for the treatment of acute leukemia, especially because most of the positive results of earlier studies were not recorded in controlled randomized trials [23].

We have conducted a controlled randomized trial of immunotherapy in adult ANLL to determine whether immunotherapy using a protein-bound polysaccharide termed PSK would prolong the duration of remission and survival in patients with ANLL in a multi-institutional cooperative study.

PSK is an aqueous extract of the mycelium of *Coriolus versicolor*, a mushroom belonging to the *Basidiomycetes* genus, and has been found to possess antitumor activity against several experimental tumors, including sarcoma 180, hepatoma AH-13 [24] and 3-methylcholanthrene-induced sarcoma [14], through host-mediated immune mechanisms. Combined use of antitumor drugs and PSK showed a synergistic effect against P388 leukemia in mice [13] and KMT-17 in rats [1], and combined administration of PSK and concanavalin A-bound L1210 vaccine induced synergistic resistance to L1210 leukemia [6]. PSK also suppressed the induction of intestinal tumors in rats that had been given dimethylhydrazine [21].

Patients and methods

Seventy-three previously untreated patients with either acute myeloblastic leukemia (AML) or acute monocytic leukemia (AMoL) (FAB classification M1, M2, M4, M5) and aged 15–60, who had achieved their first complete remissions at 10 major university and cancer center hospitals in Japan, entered this study (Table 1). To avoid factors that might affect the prognosis of acute leukemia, only adult patients with peroxidase-positive AML and AMoL and aged not more than 60

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Table 1. Patient characteristics

Characteristics	Chemotherapy + PSK	Chemotherapy	Total
No. of patients	35	38	73
Excluded	3	1	4
Dropped out	1	1	3
No. of evaluable patients	31	36	67
Age 15–19	3	2	5
20–29	9	5	14
30–39	6	15	21
40–49	9	6	15
50–59	4	8	12
(Mean)	(35.2 ± 11)	(37.8 ± 10)	
Male/Female	18/13	16/20	34/33
Type of Leukemia			
AML	25	32	57
AMoL	6	4	10
Remission induction therapy			
DCMP two-step	14	18	32
BHAC · DMP	17	18	35

There was no significant difference between the cohorts according to the *t*-test

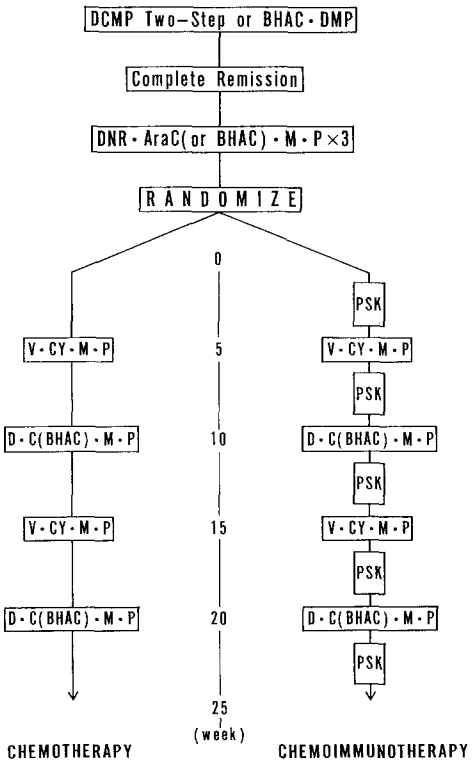

Fig. 1. Study design

were included in this study. They attained complete remission with either DCMP two-step therapy [26] or BHAC-DMP therapy [7]. DCMP two-step therapy consisted of a 10- to 14-day course of cytosine arabinoside (Ara C, 80 mg/m^2/day, 2-h IV infusion), 6-mercaptopurine (6-MP, 70 mg/m^2/day PO) and prednisolone (PSL, 20 mg/m^2/day, PO) and daunorubicin (DNR, 25 mg/m^2/day IV), which was given on days 1 and 2 and then every 2–4 days if required. The dose of Ara C and the frequency of DNR were adjusted to give peripheral white blood cell counts and nucleated cell counts in bone marrows of less than 1,500/mm^3 and 15,000/mm^3, respectively, at the end of the 10- to 14-day treatment period. Then, if leukemic blasts remained in the bone marrows 3–7 days after discontinuation of the above therapy, the second step of therapy was administered with relatively low doses of Ara C (40 mg/m^2/day), 6-MP (40 mg/m^2/day), and PSL (20 mg/m^2/day) for 3–7 days. In BHAC-DMP therapy, the treatment schedule was almost the same as DCMP two-step therapy except that Ara C was substituted by behenoyl cytosine arabinoside (BHAC, 170 mg/m^2/day, 2-h IV infusion) and the second step was not included in the protocol. After having achieved complete remission, the patients received three additional courses of consolidation therapy with the same drug combination. Ara C (80 mg/m^2/day) or BHAC (170 mg/m^2/day), 6-MP (70 mg/m^2/day), and PSL (20 mg/m^2/day) were given for 7 days, and DNR (25 mg/m^2/day) was given on the first and last days.

After the completion of consolidation therapy, the patients were registered with the randomization office located at Nagoya University School of Medicine by telephone. They were randomized to either maintenance chemotherapy or maintenance chemotherapy plus immunotherapy by a block-randomization method assigned for each hospital (Fig. 1). Maintenance chemotherapy consisted of two regimens and these were given alternately every 5th week for a period of 2 years. One regimen was a 5-day course of the same DC (or BHAC) MP combination therapy as was used for consolidation therapy, and the other consisted of vincristine (1.5 mg/m^2/week × 2), cyclophosphamide (600 mg/m^2/week × 2), 6MP (70 mg/m^2/day × 2 week), and PSL (20 mg/m^2/day × 2 week). Immunotherapy consisted in PSK (Krestin, Kureha Chemical Industry Co. Ltd, Tokyo) 3 g/patient/day PO, which was given daily except during maintenance chemotherapy. No placebo was given to the group receiving chemotherapy alone. The patients in both chemotherapy and chemoimmunotherapy groups were followed at out-patient clinics at 1- to 2-week

intervals until relapse. After the maintenance chemotherapy was terminated 2 years later, they were seen at 2- to 4-week intervals. PSK was continued as long as the patients were in remission. The regimens for re-induction and maintenance therapy following relapse were not specified.

Actuarial remission and survival were calculated according to the Kaplan-Meier product-limit method. The generalized Wilcoxon test and the Cox-Mantel test were used to compare duration of remission and survival. Treatment cohorts were compared by the chi-square test with Yates' correction. Remission duration was calculated from the date of complete remission to relapse. Survival was computed from the date of diagnosis to death since the remission induction therapy was started within a week of diagnosis in all cases.

Results

During the 3-year period from 1 October 1978 to 30 September 1981 a total of 73 adult patients with ANLL (AML + AMoL) who had achieved complete remission entered the study. Of these, 35 were randomized to chemoimmunotherapy and 38 to chemotherapy (Table 1). Two patients in the chemoimmunotherapy group and one in the chemotherapy group were excluded from the evaluation because they were found to have received different remission induction therapies including aclacinomycin A and adriamycin. One patient in the chemoimmunotherapy group was excluded because she had been registered almost 1 year after achieving complete remission. One patient from each group was regarded as a drop-out case, because each was unable to receive maintenance chemotherapy due to severe hepatitis. Therefore, 31 patients in the chemoimmunotherapy group and 36 in the chemotherapy group were analyzed for evaluation. There were 25 cases of AML and 6 of AMoL in the former group and 32 cases of AML and 4 of AMoL in the latter. Peroxidase staining of leukemia cells was positive except for one patient from each group, for whom staining was not performed. As remission induction therapy, 14 patients received DCMP two-step and 17 BHAC-DMP in the chemoimmunotherapy group, and 18 received DCMP two-step and 18 BHAC-DMP in the chemotherapy group. There were 18 male and 13 female patients in the former group, and 16 male and 20 female patients in the latter, with average ages of 35.2 and 37.8 years, respectively. There was no statistically significant difference between the above cohorts of both groups when compared by the chi-square test.

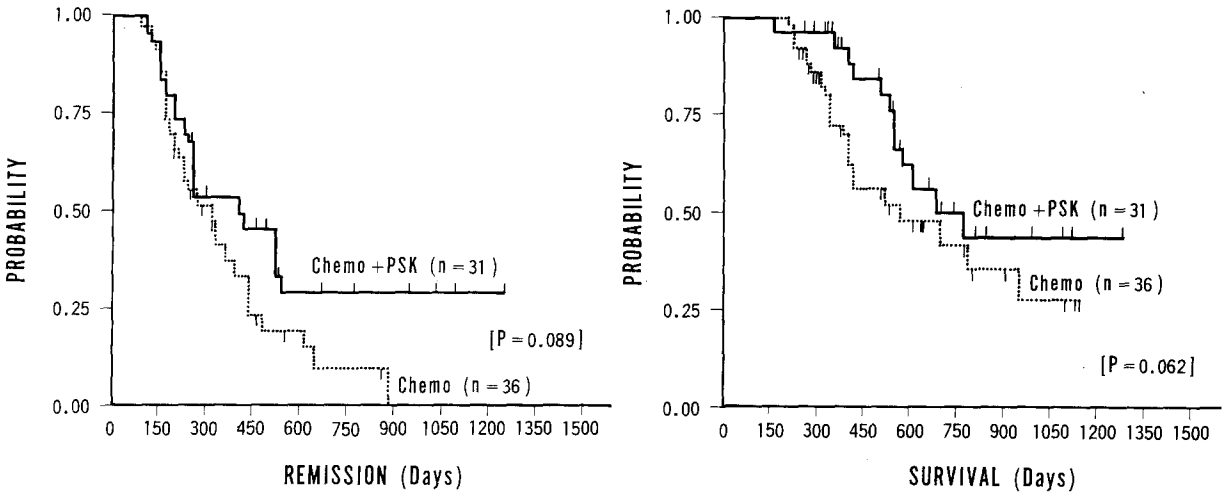

Fig. 2. Remission duration (*left*) and survival length (*right*) analyzed 6 months after the last entry

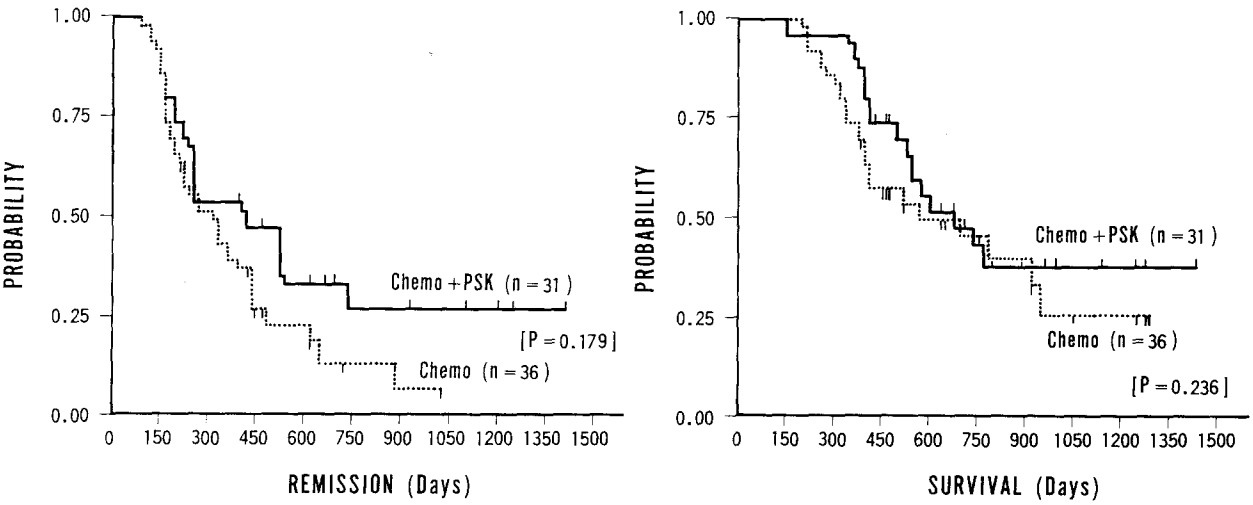

Fig. 3. Remission duration (*left*) and survival length (*right*) analyzed 12 months after the last entry

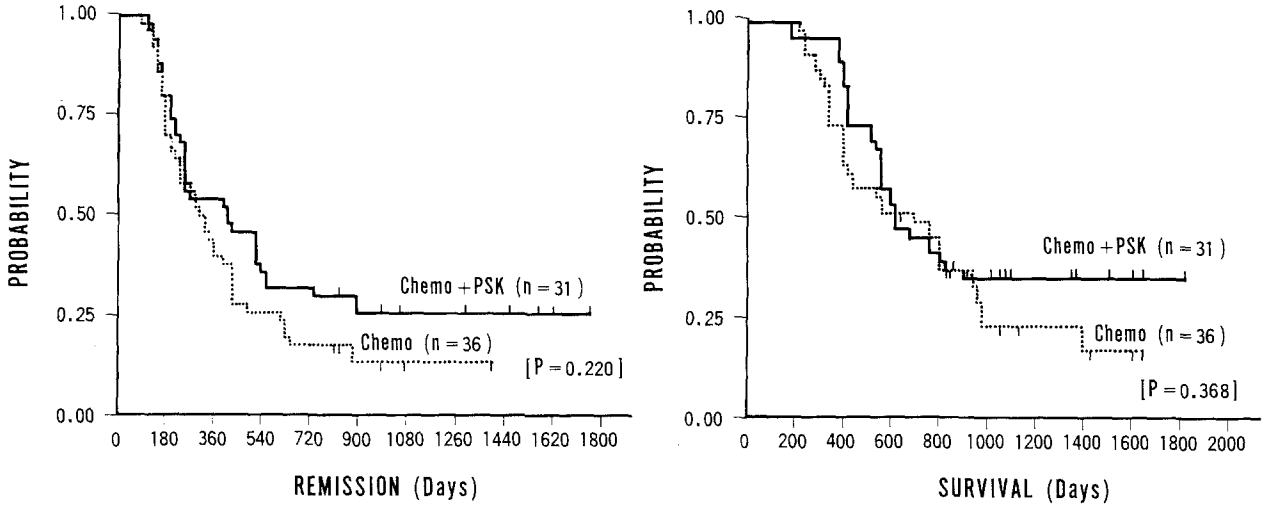

Fig. 4. Remission duration (*left*) and survival length (*right*) analyzed 24 months after the last entry

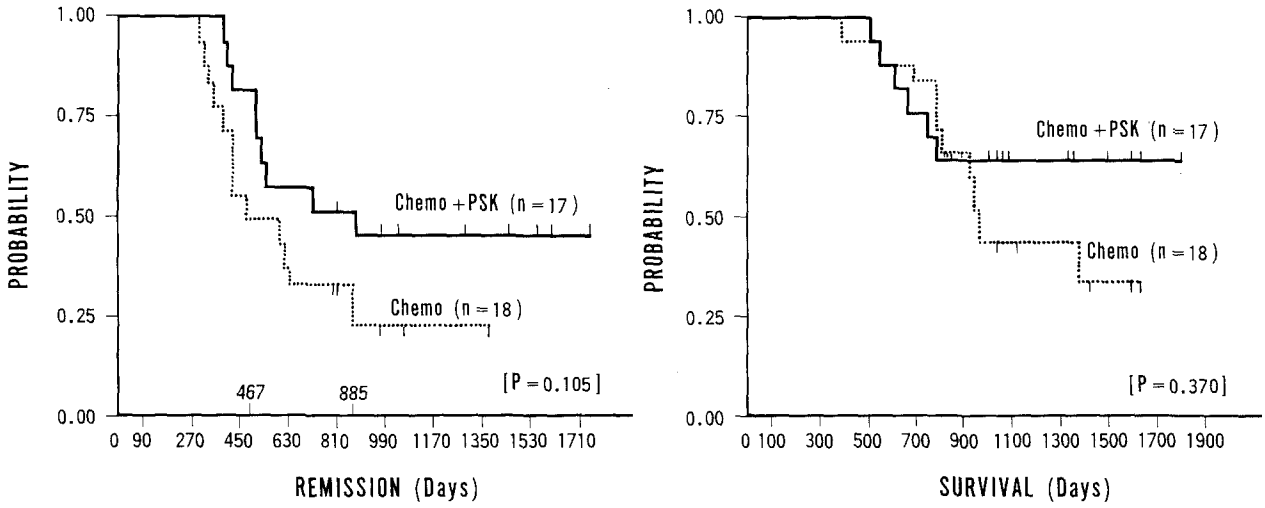

Fig. 5. Remission duration (*left*) and survival length (*right*) analyzed 24 months after the last entry in patients who maintained complete remission for more than 270 days

Remission and survival curves were compared by the generalized Wilcoxon test and the Cox-Mantel test at 6, 12, 18, and 24 months after enrollment was terminated on 30 September 1981. Figure 2 shows the remission and survival curves at 6 months after the last entry. Immunotherapy with PSK showed a borderline beneficial effect on remission duration ($P = 0.089$ by the Cox-Mantel test) and survival length ($P = 0.062$ by the generalized Wilcoxon test). When the data were analyzed 12, 18, and 24 months after the last entry, the immunotherapy showed no significant beneficial effects on either remission or survival (Figs. 3 and 4). However, when data for 17 patients in the chemoimmunotherapy group and 18 patients in the chemotherapy group who had maintained remission for more than 270 days were analyzed at 24 months after the last entry there were considerable differences indicating a favorable effect of immunotherapy in the 50% remission rate (885 days vs 467 days) and in the 50% survival rate (not reached vs 969 days). The overall remission and survival curves showed a suggestive difference in remission duration ($P = 0.105$ by the generalized Wilcoxon test) (Fig. 5).

There were no untoward side-effects attributable to PSK, such as GI trouble or abnormalities in liver and kidney function tests.

Discussion

There has been a substantial improvement in remission induction therapy over the past few years and intensive treatment has resulted in complete remission rates of 70%–85% in adult ANLL [2, 7, 18, 20, 26]. However, improvement of remission duration has proved to be more difficult, and the best means for prolongation of remission has become a major research object in adult ANLL. In the hope of prolonging remission by enhancing the immunological defense mechanism against leukemia cells, several investigators have tried immunotherapy, and promising results have been reported following the use of BCG or its derivatives, alone or in combination with irradiated tumor cells [3, 4, 16, 19, 27]. The effect of immunotherapy in ANLL, however, has been controversial, since most of the positive results have been obtained in uncontrolled nonrandomized trials [23].

In the present study, we have tested the efficacy of a protein-bound polysaccharide preparation, PSK, for adult ANLL in a multi-institutional controlled randomized trial. After the leukemic tumor burden had been reduced by remission induction therapy and further by three courses of consolidation therapy, PSK was given PO daily except during maintenance chemotherapy. PSK was shown to possess antitumor activity against several experimental tumors, exerted through a host-mediated immune mechanism [14, 24]. It has been used clinically and is now commercially available in Japan. Although many reports have described the efficacy of this nonspecific immunostimulator against several human malignancies when used in combination with chemotherapy or radiotherapy, only a few papers have reported the effect of PSK in randomized trials. Hattori et al. [5] randomized gastrectomized patients with stomach cancer three groups, PSK, tegafur, and PSK plus tegafur, after the administration of a large dose of mitomycin C. They found that the survival rate of patients who received PSK plus tegafur showed the most favorable results at 1 year in patients with stage IV disease, at 2 years in stage III, and at 3 years in all patients. However, no statistical analysis was provided. Nagao et al. [11] randomized 28 adult patients with acute leukemia in remission to chemotherapy or chemotherapy plus PSK as maintenance therapy, and found that the chemoimmunotherapy group had a longer duration of both remission and survival. However, in their study both ANLL and ALL were involved, and therefore remission induction and maintenance therapies were different; moreover, no statistical analysis was done to compare the two groups.

Interim analysis of the present study at 6 months after the last entry inidicated that immunotherapy with PSK improved duration of remission and survival in adult ANLL with borderline significance. However, analysis at 12, 18, and 24 months after the last entry showed no significant benefits of immunotherapy, although the duration of remission and survival tended to be longer in the immunotherapy group. At 24 months after the last entry, however, when the difference was analyzed only in patients who had maintained remission for more than 270 days, immunotherapy with PSK was found to have improved remission duration with suggestive significance ($P = 0.105$), and the 50% remission period was 418 days loner in the chemoimmunotherapy group. This indicated that immunotherapy with PSK was beneficial to the patients who could maintain hematological remission for a considerable period. Since several studies have revealed that one, probably the most important, of the prognostic factors affecting remission duration of ANLL patients is the intensity of remission induction therapy [6, 8, 17], the fact that PSK showed a beneficial effect in the patients with better prognosis may indicate that the immunotherapy has exerted its effect in cases where the tumor cell burden in patients was very small.

The mechanisms of action of PSK in ANLL are not entirely known. In animals, PSK is active in enhancing both humoral and cellular immunity. It potentiated the production of hemolytic plaque-forming cells of spleen and serum hemagglutinin against a low dose of sheep red blood cells given to mice [15], restored the depressed antibody-forming capacity of tumor-bearing mice [12], and enhanced the delayed hypersensitivity response of rats to KMT-17 tumor cells [1]. In man, there have been only a few reports of studies of the immunological effect of PSK. It was reported that the inhibitory effects of sera from patients with gastric cancer on PHA response of normal lymphocytes were reduced by daily administration of PSK for more than 2 months [25]. Thus, PSK might restore the suppressed immunity in patients with leukemia and possibly enhance the immune response to leukemia cells.

PSK showed a beneficial effect mainly on remission duration in the present study. This is easily conceivable, since the study was cancelled upon relapse of leukemia and re-induction chemotherapy regimens were left to the discretion of each institution. Some investigators reported beneficial effects of BCG immunotherapy in survival length rather than remission duration [9, 28]. However, if immunotherapy exerts an effect against tumor at all, remission duration should at least be prolonged in the case of acute leukemia.

PSK is far less toxic than other immunopotentiators. The LD_{50} following PO administration of PSK in both mice and rats was more than 20,000 mg/kg [24]. In the present study, no untoward side-effects attributable to PSK were observed. Thus, immunotherapy with PSK seems to be appropriate for the treatment of adult ANLL when it is used in combination with chemotherapy, especially in patients whose residual tumor cells are presumably minimal.

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