

PURIFIED PROTEIN DERIVATIVE (PPD) TUBERCULIN AS A BIOLOGICAL RESPONSE MODIFIER: I. SUPPRESSION OF TUMOR MARKERS BY INTRAVENOUS ADMINISTRATION OF PPD

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ABSTRACT

Forty-two patients with neoplastic diseases received intravenous administration of PPD (PPD-V), and tumor markers were monitored between January, 1986 and February, 1987. Tumor markers such as CEA, AFP, SCC, CA125, IAP and polyamines decreased significantly following the PPD-V therapy. In some cases, PPD-V therapy also reduced the progression of clinical manifestation of the disease. We could not detect serum IL-2 or TNF activity. IL-2 production and sensitivity of lymphocytes were also undetectable. Intravenous administration of PPD was concluded to be effective as an adjuvant therapy against human neoplastic disease. A reduction of tumor markers was observed in 19 of the 26 assessable patients (73%). We discussed the mechanism of suppression of tumor markers by PPD-V therapy.

Key words: BRM, PPD-V, suppression of tumor markers, adjuvant immunotherapy.

Abbreviations used in this paper: BRM, biological response modifier; PPD, purified protein derivative (of tuberculin); BCG, Bacillus Calmette-Guérin; IFN, interferon; IL-2, interleukin-2; NK, natural killer cell; NKAF, natural killer activating factor; CSF, colony stimulating factor; TNF, tumor necrotizing factor; AFP, alpha fetoprotein; CEA, carcinoembryonic antigen; CA125, carbohydrate antigen 125; IAP, immunoregulatory acidic protein; LDH, lactate dehydrogenase; SCC, squamous cell carcinoma antigen.

INTRODUCTION

In patients with neoplastic diseases, the immune response is suppressed with the advance of the disease.¹⁾ The aim of nonspecific immunotherapy is to improve immunopotency so that the host defense mechanism will be able to overcome the growth of malignant tumors. Recently, such substances as BCG, OK432 (Picibanyl), PSK (Krestin) and Lentinan, previously called immunopotentiators, have been studied as biological response modifiers (BRM) with the potential to induce cytokines (e.g., IFN, IL-2, CSF, TNF, NK and NKAF).²⁾ The antitumor effects of BCG, especially, have been studied for about thirty years. Since Old et al. reported that BCG inhibits the growth of experimental tumors in mice,³⁾ the antitumor effects of BCG have been extensively studied in many experimental systems of animal cancer, and the activation of the

reticulo-endothelial systems (RES) of the host has been suggested to be the mechanism of its antitumor effects.³⁻⁵⁾ The antitumor activities of BCG have also been reported to depend on their viability.⁶⁾ Around 1970, BCG was introduced into the treatment of human acute leukemia⁷⁾ and melanoma.⁸⁾ Thereafter, antitumor effects of BCG have been reported in many kinds of human cancers.⁹⁻¹⁷⁾ We have been studying nonspecific immunotherapy against animal or human cancer for many years. Recently, we established an intravenous method of PPD administration as a promising adjuvant therapy against cancer. In this paper we report some of the results of our clinical studies.

MATERIALS AND METHODS

Patients Twenty-two men and 20 women received intravenous administration of PPD (PPD-V) between January 1986 and February 1987. The mean age of the patients was 76.4 years, ranging from 45 to 93 years. Cancer or related diseases were definitively diagnosed in 36 patients and six patients were suspected to have cancer. Blood chemical analysis and complete blood cell count were done for all patients using the Toshiba Biochemical Autoanalyser (model TBA-480, Toshiba Medical Co., Ltd.) and the Sysmex Microcellcounter (model CC-180, Toa Medical Electronics Co., Ltd.) every one or two weeks during our study.

Administration of PPD Ten to one hundred micrograms of PPD was diluted with 20 to 100 ml of sterile saline and administered intravenously for more than 30 min. PPD was administered regularly once a week in almost all cases. All patients were monitored for their vital signs very carefully for more than 12 hr after injection. All patients received a tuberculin test every month throughout the study.

Monitoring of tumor markers Tumor markers such as CEA, AFP, CA125, SCC, IAP and polyamines were quantitatively measured at the Special Reference Laboratory Co., Ltd. (S.R.L.).¹⁸⁾

Assay for the lymphokines IL-2 and TNF were measured before or 3 hr, 5 hr and one week after the administration of PPD. Serum IL-2 activity, IL-2 production and sensitivity of lymphocytes were assayed at S.R.L. Serum TNF activity was examined at Asahi Kasei Kogyo Co., Ltd.

Reagents PPD was purchased from Nippon BCG Seizo Co.

RESULTS

Typical cases of PPD-V therapy Table 1 shows the diagnosis, histological evidence and total PPD dose of the 42 patients. The suppression of tumor markers by PPD-V therapy was evaluated in 26 patients who showed an abnormal level of tumor markers and who were treated regularly with PPD-V therapy once a week for more than five weeks or who received a total dose of more than 360 μ g. Nine representative cases are described below. *Case 5:* The patient was found to be suffering from gastric cancer (IIc + III) during the treatment of a stroke. Since March 1986, the patient has received PPD-V therapy. We could not find any significant morphological change in the endoscopic examinations nor any sign of metastasis. Serum level of CEA decreased after the beginning of the PPD-V therapy and became normal after September 1986 (Fig. 1-A).

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Table 1. Summary of patients who received PPD-V therapy

No.	Age	Sex	Disease	Histo- tology	Total dose of PPD(μ g)	Term of PPD-V therapy (weeks)	Remarks
1	56	M	Gastric cancer (BorIV)	Ad	160	6	ER
2	61	F	Gastric cancer (IIc)	Ad	560	21	NA
3	83	F	Colon cancer (suspected)		160	5	NA
4	77	M	Gastric cancer (IIc)	Ad	160	3	NA
5	69	M	Gastric cancer (IIc + III)	Ad	4,460	49	ER
6	79	M	Chronic myelocytic leukemia		760	14	NC
7	68	F	Liver cancer (suspected)		960	15	ER
8	81	M	Colon cancer (POHON1S1)	Ad	250	4	NA
9	77	M	Gastric cancer (IIc)	Ad	160	3	ER
10	63	M	Lung cancer (T3N2M1)		260	4	NA
11	80	M	Prostatic cancer		700	11	NA
12	84	F	Uterine cancer (cervix)	Sq	3,960	41	ER
13	78	F	Gastric cancer (BorI)	Ad	1,070	17	PR
14	84	M	Gastric cancer (IIa)	Ad	1,120	18	PR
15	81	M	Lung cancer (T3N0M1)	La	10	1	NA
16	81	F	Colon cancer		10	1	NA
17	75	M	Lung cancer (T3N2M1)	Ad	1,710	20	NC
18	85	F	Lung cancer (T3N1M1)	Sm	2,000	22	NC
19	82	F	Gastric cancer (IIc + III)	Ad	2,260	24	NC
20	76	F	Breast cancer (stageII)		2,760	20	ER
21	79	M	Malignant lymphoma		2,320	27	ER
22	45	F	Brain tumor (suspected)		560	7	NA
23	83	F	Uterine cancer (body)		60	2	NA
24	81	F	Lung cancer (T2N1MX)		960	11	NC
25	73	F	Pseudomyxoma peritonei(ovary)		2,160	23	ER
26	83	F	Gastric cancer (BorII)	Ad	460	6	NC
27	89	F	Myelofibrosis		660	8	ER
28	78	M	Prostatic cancer		160	3	NA
29	89	M	Liver cancer (suspected)		260	4	NA
30	88	M	Lung cancer (T2N1MX)	Sm	660	8	ER
31	63	M	Gastric cancer	Ad	760	8	PR
32	67	F	Myelodysplastic syndrome		960	11	ER
33	88	F	SMT of stomach (suspected)		260	4	NA
34	82	M	Metastatic liver cancer		760	9	ER
35	65	M	Chronic myelocytic leukemia		560	7	ER
36	87	M	Gastric cancer (BorII)	Ad	560	7	NC
37	61	F	Lung cancer (M1)	Ad	210	5	ER
38	73	M	Gastric cancer (BorIII)	Sr	10	1	NA
39	93	F	Unknown		10	1	NA
40	67	F	Rectal cancer		360	5	ER
41	64	M	Rectal & esophageal cancer		250	5	PR
42	91	M	Prostatic cancer		10	1	NA

Ad : Adenocarcinoma
 Sq : Squamous cell carcinoma
 La : Large cell carcinoma
 Sm : Small cell carcinoma
 Sr : Signet ring cell carcinoma
 SMT : Submucosal tumor

ER : Effective response
 PR : Partial response
 NC : Not controlled
 NA : Not assessable

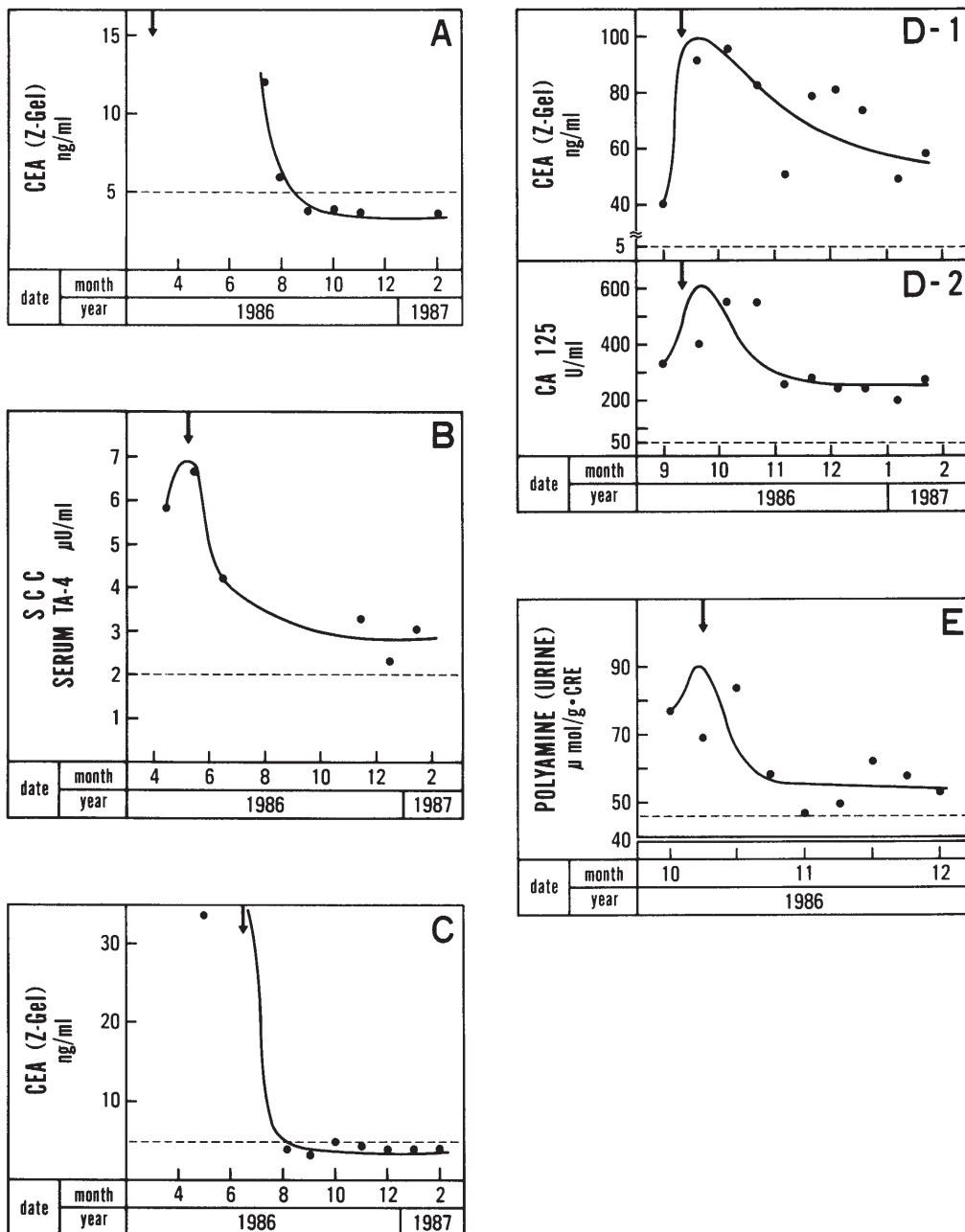


Fig. 1. Reduction of serum tumor markers after intravenous administration of PPD. PPD-V therapy was initiated on date indicated (↓) and conducted regularly once a week. A: Serum CEA level in case 5; B: Serum SCC level in case 12; C: Serum CEA level in case 20; D-1: Serum CEA level in case 25; D-2: Serum CA125 level in case 25; E: Urinary polyamine level in case 30. Discontinuous line shows upper normal value of tumor markers.

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Case 7: The patient was suspected to be suffering from hepatoma by computer tomography (CT) and abdominal ultrasonography (US) in the course of the treatment of hepatitis-B-negative liver cirrhosis. Serum level of AFP began to decrease after the PPD-V therapy and became normal within a month (Table 2). *Case 12:* Squamous cell carcinoma of the cervix uteri (stage II) was diagnosed in this patient by gynecological and cytological examination. The patient was too old to receive surgical resection of the cancer so she underwent PPD-V therapy. Serum level of SCC decreased after the PPD-V therapy, and there has been no increase of SCC up to February 1987 (Fig. 1-B). There has also been no evidence of progression of the cancer or of metastasis to date. *Case 20:* The patient was found to have a tumor (18×20×10 mm) in the left mammary gland and mammary carcinoma (stage III) was diagnosed by surgical examination. Serum level of CEA decreased soon after the treatment with PPD-V therapy (Fig. 1-C). Weekly administration of PPD is being continued. The serum level of CEA has been normal and the size of the tumor has also shown no change. *Case 25:* Peritoneal pseudomyxoma of the ovary was diagnosed and the patient received surgical resection of the tumor followed by chemotherapy at a different hospital. Immediately after admission to our hospital, she was treated with PPD-V therapy. Both serum level of CEA (Fig. 1-D-1) and CA125 (Fig. 1-D-2) decreased after the therapy but not to the normal level. *Case 30:* A small cell carcinoma of the lung (stage III) was diagnosed and the patient was admitted to our hospital in October 1986. PPD-V therapy was administered soon after admission. The polyamine level in the urine was monitored as a marker, and began to decrease rapidly after the therapy was started (Fig. 1-E). *Case 34:* Metastatic liver cancer of unknown origin was diagnosed in this patient by CT, US and RI scintiphotography. The patient has been administered PPD-V therapy since December 1986. The serum levels of CEA and LDH began to decrease respectively, soon after the start of therapy (Fig. 2). The AFP level has been normal throughout the course of the disease. *Case 37:* The patient was found to be suffering from pulmonary adenocarcinoma (stage Ia) in the cancer screening of Gamagori City in November 1984. In February 1985 the patient received surgical treatment followed by chemotherapy. Thereafter, the patient has been continuously treated with PSK and intradermal administration of BCG. However, the serum level of CEA began to increase gradually up to

Table 2. AFP before and after administration of PPD in case 7

PPD-V therapy	Date	AFP(ng/ml)
Before	85/11/9	370.0
	86/2/13	1,100.0
	3/13	1,300.0
After*	4/11	71.0
	4/25	29.0
	5/14	12.0
	5/29	7.8
	6/12	4.9
	6/25	36.0

* PPD-V therapy was begun on March 20, 1986.

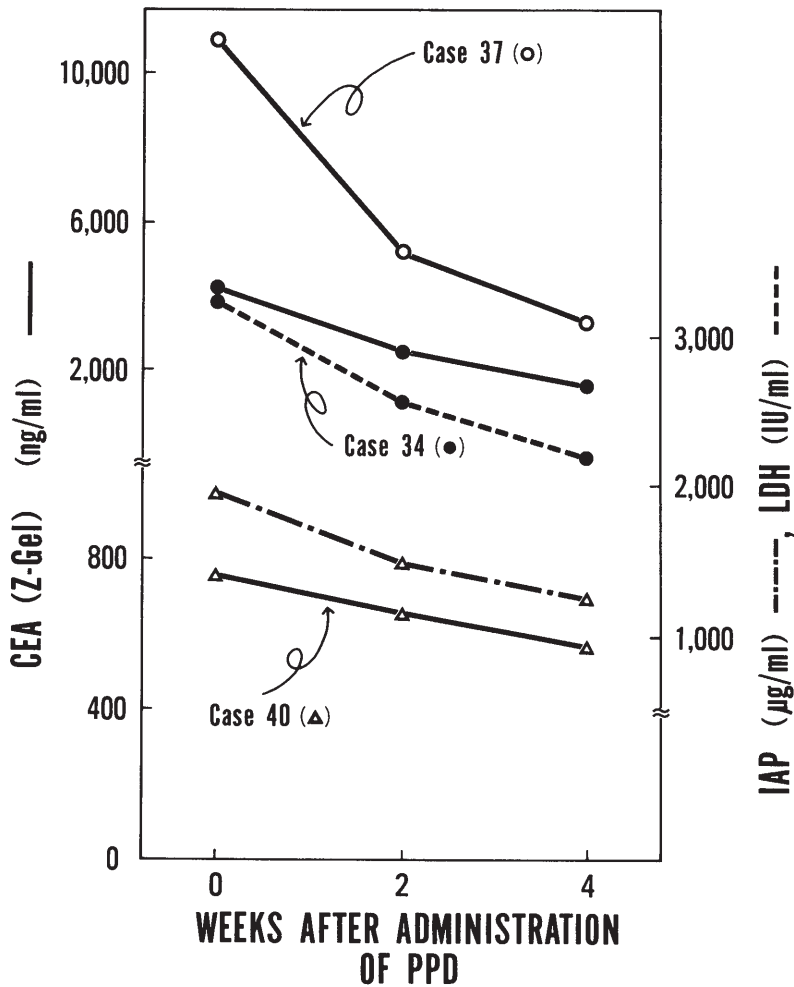


Fig. 2. Reduction of CEA (—), IAP (— · —) and LDH (— · —) in case 34 (●), 37 (○) and 40 (△). CEA, IAP and LDH in the serum were assayed before and after intravenous administration of PPD. Patients were regularly treated with PPD-V therapy once a week.

5,600 ng/ml in the summer of 1986. At the same time, osteolytic plaque was found in the left rib bone and the patient also complained of severe pain in the sacral bone. Metastases to the bone, liver and brain were also found by CT and RI scintiphotography. The patient was treated with radiation therapy of about 4,500 rad, but the serum level of CEA increased further to 11,000 ng/ml. Finally, PPD-V therapy was started and the serum level of CEA was monitored. The patient was much relieved from painful complications with the gradual decrease of CEA (Fig. 2). This patient had a temporary high fever after the intravenous administration of PPD. *Case 40:* Adenocarcinoma of the rectum was diagnosed in this patient who then underwent surgical resection of the tumor in November 1981. Re-operation was conducted in February 1983, followed by chemotherapy in the former hospital. However, the patient was found to have multiple metastases of the cancer and was admitted to our hospital in January 1987. Soon

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after admission, the patient received PPD-V therapy. Serum levels of CEA and IAP began to decrease gradually (Fig. 2).

The successful treatment of malignant neoplastic disease of the blood (case 32 and case 35) will be described elsewhere (in preparation).

The effect of PPD-V therapy was evaluated in 26 patients who received PPD-V therapy for more than five weeks or who received a total dose of more than 360 μg . As shown in Table 3, reduction of tumor markers was found in 19 patients (73%). There were 15 effective responders (58%) and 4 partial responders (15%) on the basis of our criteria (see the footnote in Table 3). No change in the reaction of tuberculin test has been demonstrated so far. However, further studies are required to determine whether or not delayed-type hypersensitivity (DTH) is enhanced by intravenous administration of PPD.

Complications of the PPD-V therapy Two of the 42 patients had a high fever of about 39°C. However, the fever decreased gradually without treatment within 6 hr. Fever did not correlate with the dose of PPD. One patient complained of a slight headache. No patient had complication of liver, renal or hematopoietic dysfunction. Anaphylactic shock was also not experienced during the total of 430 intravenous administrations of PPD.

Absence of IL-2 and TNF We could detect neither a significant increase in the serum level of IL-2 activity nor production of IL-2 or sensitivity of peripheral lymphocytes in 37 patients who received PPD-V therapy. An increase in the serum level of TNF could also not be detected in five patients after the administration of PPD.

Table 3. Suppression of tumor markers by PPD-V therapy*

	Number of cases		
	Alive	Dead	Total
Effective Response (ER)	8	7	15 (58%)
Partial Response (PR)	0	4	4 (15%)
Not Controlled (NC)	0	7	7 (27%)
Total	8 (31%)	18 (69%)	26

*The cases in which patients received less than 360 μg of PPD or were treated for less than five weeks were omitted from this table (13 cases). Three cases in which patients did not show an abnormal level of tumor markers were also omitted.

ER : Cases in which the level of tumor markers became normal or continuously decreased for more than one month.

PR : Cases in which the level of tumor markers decreased for less than one month.

NC : Cases in which no significant change or increase of tumor markers was found.

DISCUSSION

It seems likely that a decrease in tumor markers does not always correlate with the strength of the antitumor effects of the therapy. Tumor markers, however, do decrease in accordance with the regression of the cancer by chemotherapy in many cases, as previously reported.¹⁹⁻²⁴⁾ Furthermore, it is well known that surgical resection of the tumor results in a decrease of tumor markers.²⁵⁾ Therefore, we assumed that intravenous administration of PPD (PPD-V) had antitumor effects based upon the data described in the results. As shown in Table 3, reduction of tumor markers was found in more than 70% of 26 patients, according to our criteria. This therapy is not only effective as an adjuvant therapy against cancer for surgical treatment, radiation and chemotherapy but also more effective than other nonspecific immunotherapies using BRMs such as BCG, PSK, OK432 and Lentinan. The reason is that PPD-V therapy has a wide range of beneficial effects regardless of the difference in the stages of illness or in the histological and organic properties as shown in Table 1. In our experimental system of L1210 leukemia cell and CDF1 mouse, we also obtained results supporting the antitumor effects of PPD-V therapy (unpublished data). Note here that the pain due to the growth of the cancer decreased apparently as a result of this therapy. To confirm the antitumor effects of PPD-V therapy, we must investigate the survival rate of the patients receiving this therapy, further studies are now under way.

An interesting aspect of this work was the finding that PPD-V therapy was much more effective than BCG therapy as an antitumor therapy. The antitumor effects of BCG for human neoplastic disease have been extensively studied for more than 10 years; some studies have reported that BCG has an antitumor effect⁷⁻¹⁷⁾ but others have reported it to be ineffective.^{26,27)} In our case 37, the cancer was not reduced in spite of the large doses of BCG and PSK administered after treatment with surgical operation followed by chemotherapy. The serum level of CEA increased from 420 to 11,000 ng/ml within a year in spite of radiation therapy (see results), but decreased shortly after the beginning of PPD-V therapy. This clearly indicates that in case 37, intravenous administration of PPD was effective although intradermal administration of BCG was not.

In several cases in Table 1, PPD-V therapy was conducted by using other drugs such as mitomycin C, UFT or OK432. We could not determine, however, whether the decrease of tumor markers was enhanced by combination therapy. Further studies are required to decide the best combination to decrease the tumor markers.

Acute or chronic toxicity was tested in 16 rabbits and 100 mice. They were each given weekly 100 to 200 $\mu\text{g}/\text{kg}$ of PPD by intravenous administration for more than three months. Neither abnormality in their general status nor any significant changes in their blood cell count, blood chemical analysis and histological examination could be found throughout our study when compared with those examinations in untreated animals (in preparation). No complications of dysfunction of the liver, kidney or hematopoietic system were encountered with this therapy in any of the 42 patients during this study. Some patients had a slight fever but it was not so severe that PPD-V therapy had to be discontinued. The fevers complicated with the therapy did not continue for more than 6 hr. We never experienced anaphylactic shock in the total 430 intravenous administrations of PPD. Neither could experimental anaphylactic shock be demonstrated by weekly intravenous or intramuscular administration of PPD (100 to 200 $\mu\text{g}/\text{kg}$) to rabbits or mice. However, we must not exclude anaphylactic shock from the possible side effects of PPD-V therapy. Further experiments will be required to clarify this problem. Passive cutaneous anaphylaxis (PCA) in rabbits could not be observed when antisera against PPD obtained from rabbits treated with intravenous administration of PPD were used (in preparation). This result

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should be concomitant with the result that anaphylactic shock could not be demonstrated in the present study.

The mechanism of the suppression of tumor markers by PPD-V therapy is yet unknown. We could find neither IL-2 nor TNF activity in blood specimens, nor could we detect IL-2 production *in vitro* cultures of peripheral lymphocytes. However, we can not rule out the possibility that cytokines play a role in the mechanism of this phenomenon induced by intravenous administration of PPD. In this connection, recent studies by Kato et al. that report that intravenous administration of PPD followed by treatment with OK432 via the same route induces endogenous TNF in patients with renal cancer^{28,29)} are of interest. We also found that the population of T lymphocytes increases when immunocompromised patients are treated with PPD-V therapy (in preparation). Furthermore, our preliminary experiment on PPD-V therapy suggested that the *Leu3a* subset of T lymphocytes is increased when compared to the *Leu2a* subset although neither lymphocyte NK activity nor lymphocyte blastogenesis was enhanced (data not shown). Finally, we assume that PPD activates T lymphocytes immediately after the intravenous administration of PPD. Sensitized T lymphocytes, thus modified, might release some lymphokines which work against target cells. To clarify these points, cytological analysis (flow cytometry) of the subset of T lymphocytes and functional assay for lymphokines are now under way.

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