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Intradermal administration of lipopolysaccharide in treatment of human cancer

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Abstract Lipopolysaccharide (LPS) has been recognized as a potent antitumor agent in animal tumor models; however, its use in human cancer therapy has been limited to only one trial, in which LPS from *Salmonella* was given intravenously. It was not very successful because of poor tumor response and was also toxic. We originally developed LPS prepared from *Pantoea agglomerans* (LPSp), and this was a well-purified, small-molecular-mass (5 kDa) agent. We chose intradermal rather than intravenous administration in the hope that the former would release LPS slowly into the bloodstream, and thus be less toxic while preserving antitumor activity. In our animal tumor models, intradermal administration was indeed less toxic and more beneficial for tumor regression than intravenous administration. We made a pilot study with intradermal administration of LPSp on the treatment of ten advanced cancer patients. Five of them had evaluable tumor, which had failed earlier to respond to conventional chemotherapy. Cyclophosphamide was also administered in this trial, in anticipation of its synergistic effect with LPSp. In this study LPSp was injected intradermally into each patient twice a week, starting with an initial dose of 0.4 ng/kg, and raising it to 600 or 1800 ng/kg. A 400-mg/m^2 dose of cyclophosphamide was given intravenously every 2 weeks. After completion of the dose escalation, the treatment was continued for at least 4 months, and it was found that 1800 ng/kg LPSp was well tolerated. A significant level of cytokines was observed in the sera for at least 8 h. These results indicate higher tolerable doses and remarkably more continuous induction of the cytokines than were reported in a previous study by others using intravenous administration. Three of the five evaluable tumors showed a significant response to our combined therapy. Intradermally administered, LPS was less toxic and elicited a tumor response in combination with cyclophosphamide; it can thus can be applied to cancer treatment even in humans.

Key words Lipopolysaccharide · Tumor necrosis factor · Immunotherapy · Cytokine · LPSp

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Introduction

About a century ago Coley (1881–1935) showed the therapeutic efficacy of a mixed bacterial vaccine, so-called Coley's toxin, to human cancer [18]. According to a report by Nauts, the 5-year survival rate without recurrence was 42% in 204 patients with inoperable advanced cancer who received only this vaccine [18]. Regardless of how one interprets this result, this information shows that a patient's immune system, when given appropriate immunostimulation, can attack its own cancer and cause complete regression of the tumor. In that period, however, there was no knowledge of the mechanism involved in tumor regression by Coley's toxin. Unfortunately, clinical interest in Coley's therapy diminished in preference to radiotherapy and chemotherapy in the course of their advent into cancer therapy [18]. It has not developed into an established biotherapy for cancer.

In 1943 Shear and Turner isolated lipopolysaccharide (LPS) as the active agent in Coley's toxin [16], and since that time the mechanism underlying the therapeutic efficacy of LPS for cancer has been studied using animal models. LPS is well recognized as a potent immune stimulator as well as a multicytokine inducer, and is known to have potent antitumor activity through a host-defense mechanism. Practically, LPS has been very clearly demonstrated to cause tumor regression in animal tumor models. It is viewed as promising for cancer immunotherapy even in humans, as previously shown in animals. However, its use in human cancer therapy has been limited to only one trial,

since LPS is rather difficult to prepare as a pure and stable product and is also toxic. The one trial was performed by Engelhardt et al. using LPS from *Salmonella*, which they injected intravenously into advanced cancer patients [4, 5]. This study showed that the maximum tolerated dose (MTD) was 8.0 ng/kg, and this dose resulted in only 1 case of partial response out of 24 [4]. This indicates that, although LPS can cause tumor regression even in human, its MTD is too low to be effective in human cancer therapy when it is administered by the intravenous route.

LPS prepared from *Pantoea agglomerans* (LPSp) was originally developed in our institute. LPSp is a well-purified, small-molecular-mass (5 kDa) LPS which has been standardized and stabilized as an investigational medical drug [8, 13, 17]. For the treatment of cancer patients, we chose intradermal rather than intravenous administration of LPSp in the hope that the former would release LPS slowly into the bloodstream, and thus be less toxic, and preserve antitumor activity. In our animal tumor models, intradermal administration was indeed less toxic and more beneficial for tumor regression than intravenous administration. We examined the antitumor effect of LPSp on various murine syngeneic tumors. Well-established, poorly immunogenic tumors such as MH134 hepatoma and Lewis lung carcinoma were found to regress completely without regrowth when LPSp was administered by the intradermal route in combination with cyclophosphamide [2]. Administration of LPSp alone or cyclophosphamide alone showed suppression of the tumor growth, but neither caused complete regression [2]. Intravenous administration of LPSp showed less antitumor effect than intradermal administration, and did not cause complete regression, even in combination with cyclophosphamide. the median lethal dose for intradermal administration was observed to be five times higher than that for intravenous administration [9].

In a pilot study on the treatment of advanced cancer patients with intradermal administration of LPSp in combination with cyclophosphamide, we evaluated the toxicity and biological effect, and sought evidence of antitumor activity of this combined therapy. We report here that this treatment induced continuous release of cytokines and caused tumor response with less toxicity.

Materials and methods

Preparation of LPS

Lipopolysaccharide was isolated from *Pantoea agglomerans* by the hot phenol method of Westphal in our institute [13]. LPSp was dissolved in saline and prepared in different concentrations.

Selection of patients

Patients eligible for this study were those with histologically confirmed advanced cancer on whom conventional therapies were not expected to have an effect, with 0–3 points of performance status and adequate baseline physiological function, including hematological status, renal function and hepatic function. The exclusion criteria included infectious disease or fever, and cardiac or pulmonary failure. All previous conventional therapies had been discontinued for more than 4 weeks. Signed informed consent was obtained from all ten individuals.

Study design

All the patients were hospitalized for observation while they underwent this therapy. A 200-µl sample of LPSp solution was carefully injected intradermally in an upper limb twice a week; the initial dose was 0.4 ng/kg, since this dose had no adverse effect even when administered by the intravenous route, according to Engelhardt et al. [4, 5]. It was planned to raise the dose to 600 ng in the first five patients and then, after evaluation of the MTD, to raise it in the next five patients to 1800 ng/kg. A 400-mg/m^2 dose of cyclophosphamide was given intravenously every 2 weeks, anticipating its synergistic effect with LPSp, as had been observed earlier in a mouse experiment [1, 2]. When the tumor regressed or was stable, the treatment with these doses was continued for at least a further 4 months. Indomethacin (50 mg), which is a cyclooxygenase inhibitor, was given 30 min before LPSp injection. This was shown to be beneficial for reducing the degree of fever, while not suppressing the release of cytokines such as tumor necrosis factor (TNF) [4, 5]. Vital signs were monitored every 30 min until at least 6 h after injection. The patients were evaluated weekly for complete blood count, coagulation profile, liver function and renal function. An electronic cardiogram and chest roentgenogram were performed after completion of the dose escalation.

Measurement of serum cytokines levels

At the completion of the dose escalation to 600 ng/kg, sera were obtained just before and 1, 2, 4 and 8 h after LPSp injection, and stored at −80 °C until analysis. At the completion of the dose escalation to 1800 ng/kg in cases 6–10, the sera were obtained just before and 2 h after injection of LPSp. The level of TNF, interleukin-6 (IL-6) and granulocyte-colony-stimulating factor (G-CSF) was measured by enzyme-linked immunosorbent assay with commercially available kits (Medgenix Diagnostics, Brussels, Belgium; Endogen Inc., Massachusetts, USA; Amersham, Buckinghamshire, England). The sensitivities of the assay for TNF, IL-6 and G-CSF were 5 pg/ml, 4 pg/ml and 10 pg/ml respectively.

Evaluation of tumor response

Tumor responses were evaluated by physical examination, appropriate roentogenic studies and ultrasound, and assay of serum tumor markers if they were available, at appropriate intervals.

Results

Patients studied

Ten patients were studied (Table 1), ranging in age from 22 to 66 years with 51 years as the median. Clinical diagnoses were uterine cervical cancer in three patients, ovarian cancer in six and malignant brain meningioma in one. Prior therapy other than surgery consisted of chemotherapy in six patients, radiotherapy in two, chemotherapy and radiotherapy in two. Five (cases 1, 2, 4, 6 and 9) out of the ten patients had evaluable tumor at the start of treatment, and in all these five, the tumor had been shown to progress during the prior chemotherapy. In four out of these

Table 1 Characteristics of the patients in this study. Ten patients were studied. Prior therapy other than surgery consisted of chemotherapy in six patients, radiotherapy in two, chemotherapy and radiotherapy in two. Five out of the ten patients had evaluable tumor at the start of treatment, and in all these five the tumor had been shown to progress during the prior therapy. *S* surgery, *C* chemotherapy, *R* irradiation

Case	Age (years)	Diagnosis	Histology	Maximum dose (ng/kg)	Prior therapy	Evaluable tumor
1	59	Ovarian cancer	Clear-cell carcinoma	8	S, C	Yes
2	43	Cervical cancer	Adenocarcinoma	600	S, C, R	Yes
3	44	Cervical cancer	Squamous-cell carcinoma	600	S, R	No
4	61	Ovarian cancer	Endometrioid adenocarcinoma	600	S, C	Yes
5	57	Ovarian cancer	Mucinous cystadenocarcinoma	600	S, C	No
6	50	Ovarian cancer	Clear-cell carcinoma	1800	S, C	Yes
7	52	Ovarian cancer	Undifferentiated carcinoma	1800	S, C	No
8	66	Ovarian cancer	Endometrioid adenocarcinoma	1800	S, C	No
9	22	Brain tumor	Malignant meningioma	1800	S, C, R	Yes
10	53	Cervical cancer	Squamous-cell carcinoma	1800	S, R	No

Table 2 Dose and toxicity profile for intradermal administration of lipopolysaccharide from *P. agglomerans* (LPSp) to patients. LPSp was injected intradermally into ten patients, starting with an initial dose of 0.4 ng/kg. It was planned to raise the dose of 600 ng in the first five patients and then, after evaluation of the maximum tolerated dose, to raise it in the next five patients to 1800 ng/kg. Nine of the ten patients tolerated the administration of LPSp at 600 ng/kg or 1800 ng/kg well, and showed minimum side-effects, including fever, fatigue and mild nausea. One patient had a fever even at a dose of 0.4 ng/kg, probably because of inflammatory carcinomatous peritonitis of her advanced ovarian carcinoma, so the dose was raised to no more than 8.0 ng/kg. The numbers of patients are shown in parentheses

	Dose of LPS (ng/kg)																
	0.4 (10)	1.0 (10)	2.0 (10)	4.0 (10)	8.0 (10)	20.0 (9)	40.0 (9)	100.0 (9)	200.0 (9)	400.0 (9)	600.0 (9)	800.0 (5)	1000 (5)	1200 (5)	1400 (5)	1600 (5)	1800 (5)
Fever																	
WHO grade I	1	1	1	1	1	–	–	1	1	2	2	3	3	4	1	1	–
WHO grade II	–	–	–	–	–	–	–	–	–	1	1	–	–	–	3	3	4
WHO grade III	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Fatigue	–	–	–	–	–	–	–	1	1	1	2	2	3	3	4	3	4
Nausea	–	–	–	–	–	–	–	–	–	–	–	–	1	1	1	2	3
Hypotension	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Dyspnea	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Hepatic toxicity	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Renal toxicity	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

five (cases 1, 2, 4 and 6), prior chemotherapy included cyclophosphamide and cisplatin as a conventional regimen for ovarian and cervical cancer.

Toxicity

Nine of the ten patients tolerated the administration of LPSp at 600 ng/kg or 1800 ng/kg well, and showed minimum side-effects. One patient had a fever even at the dose of 0.4 ng/kg, probably because of inflammatory carcinomatous peritonitis of her advanced ovarian carcinoma, so the dose was raised to no more than 8.0 ng/kg. The incidence of clinically adverse effects is shown in Table 2. Fever and chills was seen in three out of nine patients (33%) at a dose of 600 ng/kg and in four out of five patients (80%) at 1800 ng/kg. The fever, regardless of its WHO grade, could be inhibited by additional administration of 25 mg or 50 mg indomethacin. All fevers returned to normal within 12 h in all patients. Mild fatigue was observed in most of the patients with fever, and three out of five (60%) had mild nausea at 1800 ng/kg. No other toxicities including hepatic, renal toxicity, hypotension or dyspnea were observed.

Cytokine levels in serum

When dose escalation to 600 ng/kg LPS was completed, the serum levels of TNF, IL-6 and G-CSF were determined by enzyme-linked immunosorbent assay (Table 3). Baseline TNF levels were below 15 ng/kg in all patients. In eight out of nine patients, a significant level of TNF was induced, ranging between 200 pg/ml and 2800 pg/ml. The levels in sera peaked 1 h or 2 h after the injection and at 8 h still remained at a significant amount. The average peak level of TNF was 680 pg/ml, and the other cytokines, G-CSF and IL-6, were observed to be significantly induced following

Table 3 Serum level of tumor necrosis factor (*TNF*), interleukin-6 (*IL-6*) and granulocyte-colony-stimulating factor (*G-CSF*) in nine patients after intradermal administration of LPSp. At the completion of the dose escalation to 600 ng/kg, the sera were obtained just before and 1, 2, 4 and 8 h after LPSp injection. At the completion of the dose escalation of 1800 ng/kg in cases 6–10, the sera were obtained just before and 2 h after injection of LPSp. The levels of TNF, IL-6 and G-CSF were measured by enzyme-linked immunosorbent assay. The sensitivities of the assay for TNF, IL-6 and G-CSF are 5 pg/ml, 4 pg/ml and 10 pg/ml respectively. Baseline TNF levels were below 15 ng/kg in all patients. In eight out of nine patients, a significant level of TNF was induced, ranging between 200 pg/ml and 2800 pg/ml. The levels in sera peaked 1 h or 2 h after the injection and at 8 h still remained significant. The other cytokines, G-CSF and IL-6, were observed to be significantly induced, following the release of TNF, and also to remain for at least 8 h. *ND* not detected

Case	Dose ng/kg	TNF					IL-6					G-CSF				
		0 h	1 h	2 h	4 h	8 h	0 h	1 h	2 h	4 h	8 h	0 h	1 h	2 h	4 h	8 h
2	600	15	460	220	64	52	45	29	186	ND	ND	ND	ND	251	557	236
3	600	9	780	460	146	102	40	75	37	20	36	ND	ND	786	438	150
4	600	15	1027	2800	1227	326	32	165	2020	1560	296	ND	ND	801	425	103
5	600	15	28	202	42	32	ND	ND	10	12	6	ND	ND	62	44	46
6	600	13	102	320	80	40	20	22	707	112	102	ND	11	483	126	86
	1800	13	–	720	–	–	22	–	1020	–	–	ND	–	762	–	–
7	600	10	38	210	50	30	ND	ND	100	18	16	ND	ND	56	24	20
	1800	15	–	520	–	–	ND	–	326	–	–	ND	–	216	–	–
8	600	10	17	18	14	10	ND	ND	25	6	8	ND	ND	ND	ND	ND
	1800	10	–	36	–	–	ND	–	28	–	–	ND	–	ND	ND	ND
9	600	12	182	1800	620	180	11	132	2010	425	282	ND	ND	236	52	50
	1800	10	–	1960	–	–	ND	–	2570	–	–	ND	–	326	–	–
10	600	10	325	120	190	102	ND	106	256	244	182	ND	ND	216	78	36
	1800	8	–	318	–	–	ND	–	362	–	–	ND	–	268	–	–

the release of TNF, and also remained for at least 8 h. When 1800 ng/kg LPSp was administered in cases 6–10, the serum level of the cytokines was determined. The cytokines were induced significantly 2 h after injection in four out of five patients, and the level was higher than that observed in the 600-ng/kg administration.

Schedule of LPS administration

After the 4 months of treatment, the 600 ng/kg or 1800 ng/kg LPSp was adminstered to several patients in this study at various intervals. The influence of the interval between administrations was determined by assaying toxicity and

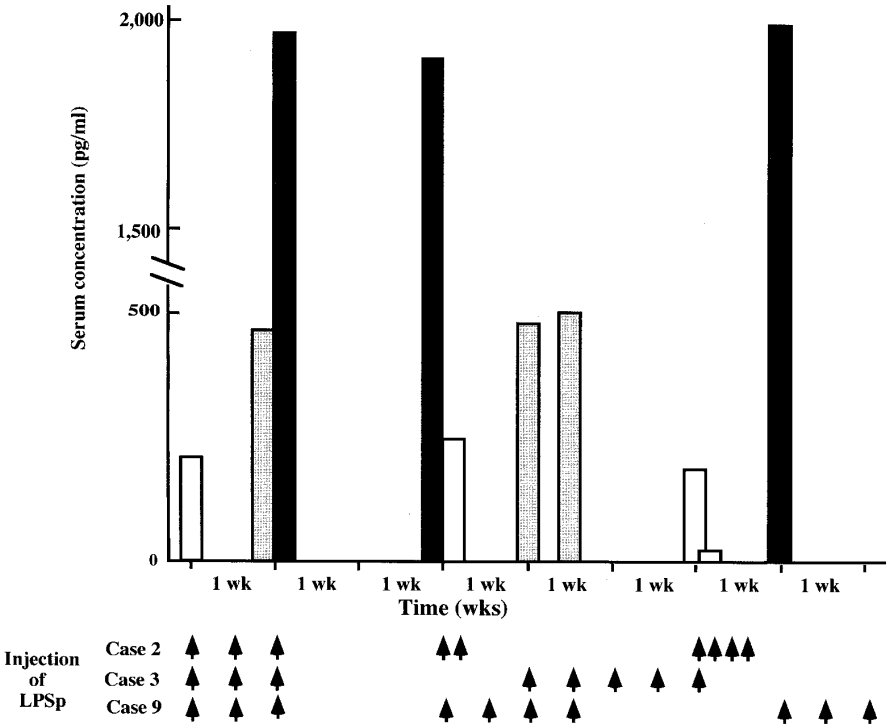

Fig. 1 The serum level of tumor necrosis factor (TNF) in cases 2 (❑), 3 (▩) and 9 (■) after intradermal administration of lipopolysaccharide from *P. agglomerans* (*LPSp*) at various intervals. After the 4 months of treatment, the LPSp was administered at various intervals to patients 2, 3 and 9. The time of injection is indicated in each patient. (▲). Serum samples were obtained 2 h after LPSp injectin on several occasions. The serum level of TNF is shown. As in case 2, daily consecutive injections resulted in remarkable attenuation of TNF response. However, there was no difference in induction of TNFα between repeated injections twice a week and those at longer intervals

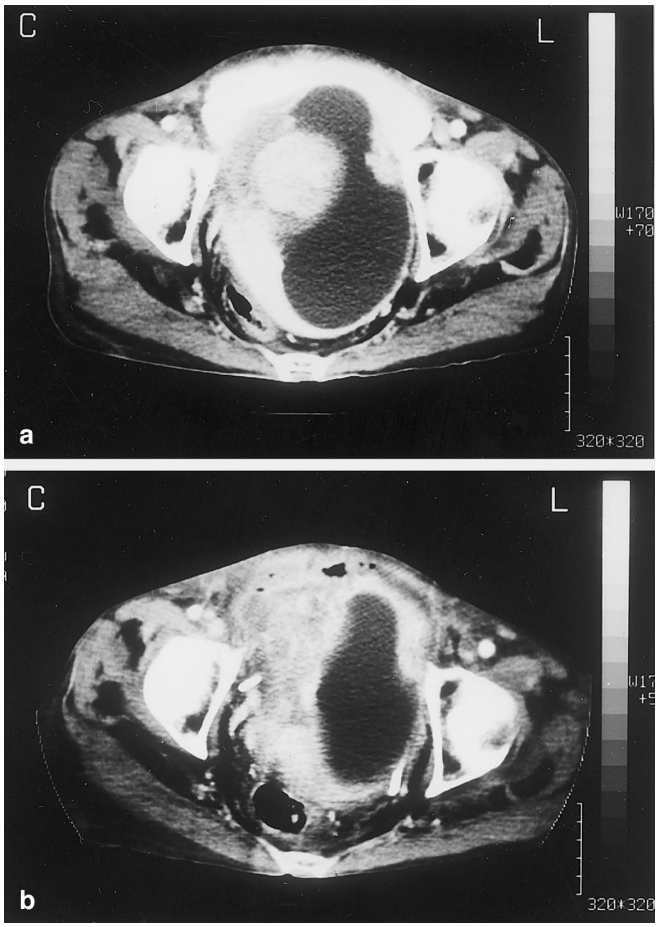

Fig. 2a, b Computed tomography (CT) scan film of the pelvis of patient 4 before (**a**) and after (**b**) treatment. The patient had a giant tumor of ovarian cancer in the pelvis. After treatment the tumor decreased 30% in size (**b**)

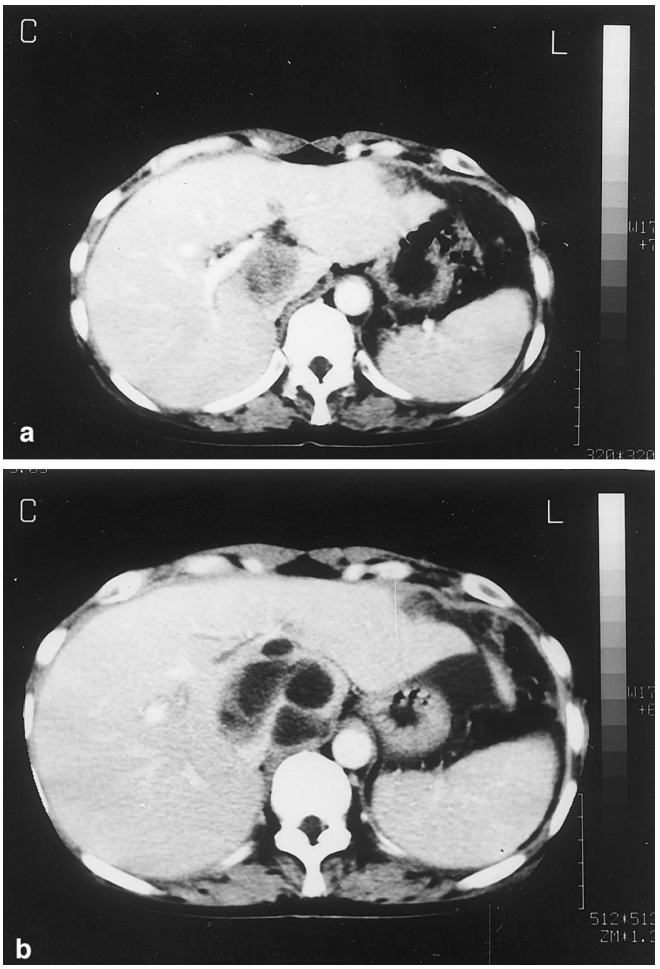

Fig. 3a, b CT scan of the liver of patient 6 before (**a**) and after (**b**) treatment. This patient with ovarian cancer had multiple metastatic tumors in the liver (a). After treatment the density of the tumor remarkably decreased in CT scan (b). The lysis of these tumors was obvious

cytokine release in these patients, but no toxicity higher than that observed during the earlier 4 months of treatment was seen. Serum samples were obtained 2 h after LPSp injection on several occasions, and the level of TNFα was determined (Fig. 1). As shown in case 2, consecutive daily injections resulted in a remarkable attenuation of the TNFα response; however, there was no difference in induction of TNFα between injections repeated twice a week and those at longer intervals.

Tumor response

Five out of ten patients were evaluable for tumor response. In one, the dose was raised to no more than 8.0 ng/kg. In four out of five patients (cases 2, 4, 6 and 9), tumor response could be evaluated after 4 months of treatment with 600 ng/kg or 1800 ng/kg. In three of these four, a favorable change in the tumor was noted. Minor response with significant decrease of tumor markers was seen in two patients (cases 4 and 9), one of whom had a giant tumor of ovarian cancer in the pelvis and the tumor decreased 30% in size (Fig. 2) after the treatment; the other patient had malignant meningioma with extracranial invasion. The invasive tumor at the neck decreased in size, which was judged to be a minor response. In one patient with ovarian cancer with multiple metastatic tumors in the liver (case 6), the lysis of these tumors was clearly demonstrated by computed tomography scan (Fig. 3). In one patient (case 2), the tumor remained stable during the 4 months of treatment.

Discussion

In an earlier mouse experiment, we had found that anti-tumor activity of intradermally administered LPSp was augmented by repeated injections at shorter intervals and combination with cyclophosphamide [2]. As a feasible

schedule of clinical application, expecting a more favorable therapeutic effect, we selected frequently repeated administration of LPSp, i.e. twice a week. In such a schedule of repeated injection, however, development of LPS tolerance must be taken into consideration. Previous reports have shown that this was greatly influenced by several factors. It was enhanced by application of larger amounts of LPS, shorter intervals between the injections and a higher content of protein in the agent [5, 7]. One earlier report showed that during intravenous administration of 4.0 ng/kg LPS to humans, monitored by release of TNFα, tolerance was developed with repeated weekly injections, but was less with bi-weekly injections [5]. Another report showed that repeated injections of 30 ng of LPS every 2 days did not lead to tolerance to induction of leukocytosis of LPS, but to tolerance to pyrogenic properties [7]. In our administration schedule, using intradermal injection of less than the MTD of LPSp, it was difficult to predict to what degree tolerance was developed. In this pilot study, the development of tolerance was not evaluated in detail. However, we have some results that suggest that tolerance of LPSp was not greatly developed in this study. To several patients of our study, after the 4-month treatment, the LPSp was administered at intervals of more than 2 weeks. However, we observed that there was not much increase of toxicity or release of cytokines even at these intervals. Further controlled study is necessary to determine what dosage and intervals result in tolerance of LPSp by intradermal administration.

This study demonstrated that, when administered by the intradermal route twice a week, 1800 ng/kg LPSp was much less toxic than when given intravenously and this dose was apparently still below the MTD. By the intravenous route, the MTD of LPS administered repeatedly at weekly intervals was reported to be 8.0 ng/kg, and that of first challenge was determined to be 4.0 ng/kg [4]. The MTD in this trial is much higher than that for intravenous injection. The bacterial origin of LPS used in our study was different from that used by Engelhardt et al., which might have allowed a higher MTD for the LPSp. The biological activities of LPSp were extensively studied in our institute using various animal models [8, 13], and compared to those of LPS of other bacterial origins. These studies revealed that, when given intravenously, there was not much difference in toxicities between LPSp and the other LPS of higher molecular mass prepared from *Escherichia coli*. It follows that the much higher MTD in our study cannot well be explained by the difference in bacterial origin; it might instead be due to the route of administration, apart from the bacterial origin or administration schedule.

The cytokines were significantly released after intradermal administration of 600 ng/kg LPSp. Earlier studies have shown that administration of LPS induces release of cytokines such as TNF and IL-6 [4, 5, 6, 12]. Michie et al. measured serum TNF levels in 13 healthy volunteers after intravenous administration of LPS from *E. coli* [12], and showed that 4.0 ng/kg elicited about 240 pg/ml TNF at the peak level. Engelhardt et al. reported that the peak level of TNF in sera was roughly 9000 pg/ml or 1500 pg/ml when the MTD or 1.0–2.0 ng/kg LPS respectively was administered [5]. In these studies the concentration of these cytokines returned to pretreatment levels within only 3–4 h after injection [4, 5, 12]. Our results showed that the peak level of cytokines was lower, but was much more continuous. The peak level of TNF after 600 ng/kg LPSp injection ranged between 200 pg/ml and 2800 pg/ml and a significant level of TNF was maintained for 8 h or more. These doses of LPSp might induce tumor regression and be close to optimal biological doses, judging from the results that the peak TNF level that appeared in sera following intradermal administration of LPSp was about 5000 pg/ml in mice showing complete tumor regression (unpublished observations). Intradermally administered LPSp was absorbed very slowly and stayed in the dermis at the site of injection for more than 48 h [9]. It may thus have a priming and triggering effect on dendritic cells and Langerhans cells in the dermis, and these activated cells and LPSp may continuously enter the bloodstream and reach the local tumor site, resulting in the continuous release of TNF [9, 17]. These effects might be the cause of the continuous release of the serum cytokines observed in this study. In the mouse experiment we evaluated TNF induction following intradermal administration of LPSp, and found that TNF was continuously induced in the tumor site as well as in sera. A significant amount of TNF was still present in the tumor site even 24 h after intradermal administration (unpublished observation). The patients showed marked individual differences of cytokine release in response to LPSp. There seemed to be a tendency for a greater amount of cytokines to be induced by LPSp in patients with a large evaluable tumor. No clear relation was observed between the amount of cytokines induced and the tumor response obtained, since the number of patients in this study was low. In our trial TNF, IL-6 and G-CSF were determined as cytokines released in the sera, since they could be measured using the serum samples. Other members of the cytokine network, such as interferon γ (IFNγ) and IL-12, might be expected to be induced as well.

Several previous studies have demonstrated that induction of endogenous TNF caused tumor regression even in human patients. We developed an anticancer therapy by inducing endogenous TNF with administration of interferon γ and OK432 (a *Streptococcus* preparation) [10]. Although still limited, in 1985 (when we reported it) some efficacy was seen in cancer patients [10]. In the present study with intradermal administration of LPSp combined with cyclophosphamide, we obtained tumor responses in three out of five patients with less toxic doses. We chose to treat the patients with the combination of LPSp and cyclophosphamide rather than LPSp therapy alone since cyclophosphamide was observed in our animal experiments to augment the effect of LPSp synergistically [2]. Several other investigators have also reported that the antitumor effect of endogenous TNF induced by LPS was enhanced by combination with cyclophosphamide [1, 14]. They showed that its ability to enhance the antitumor effect was related to the elimination of some of the immunosuppressive mechanisms that negatively regulated LPS-induced effects. The five

patients had failed to respond to prior chemotherapy, suggesting that the tumor response was not due to cyclophosphamide alone, but to its combination with LPSp and CPM. Continuous release of cytokines, but at a lower peak level, might be beneficial in achieving an antitumor effect with less toxicity. We report here the results from only 4 months of treatment; in some patients, the treatment was continued, and the evaluation of toxicity and outcome of these long-term treatments is now in progress.

LPS has recently been shown to be a potent stimulator of macrophages, releasing not only TNF but also IL-12 [3]. IL-12 has been noted for its central role in causing cell-mediated immunity [11]. TNF was reported to cause acute hemorrhagic necrosis of the tumor and to be a co-stimulator of IL-12 by accelerating IFNγ production from natural killer cells and T cells [20]. Recombinant products of TNF, IFNγ and IL-12 are now available and clinical application of these cytokines has been attempted. It was found, however, that the efficacy of TNF or IFNγ, when administered exogenously as single agents, was limited by severe toxicity [15, 19]; the efficacy of IL-12 is not yet known as clinical trials are now in progress. Tumor cell killing is mediated by the cooperative action of various immunological cells and cytokines. From this point of view, LPS therapy may be more beneficial for tumor eradication in vivo, than a single-cytokine therapy.

We have found that LPS, when intradermally administered, is less toxic, elicits a tumor response in combination with cyclophosphamide and thus can be applied to cancer treatment even in humans. Our emphasis here is thus on the intradermal route for LPS administration. Further escalation of the doses and combined use with other biological response modifiers may offer even more hope.

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