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Phase I Trial of Intravenously Administered Endotoxin (*Salmonella abortus equi*) in Cancer Patients[1]

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ABSTRACT

We report a phase I study in cancer patients being treated with i.v. bolus injections of highly purified lipopolysaccharide (LPS) *Salmonella abortus equi*. Twenty-four patients with disseminated cancer received escalating doses of LPS at 2-week intervals. Dose escalation was performed in six dose levels treating 3–6 patients at each level. Dose levels 1 and 2 consisted of 0.15 and 0.3 ng/kg, respectively. Further dose escalation up to 5.0 ng/kg was enabled by pretreatment with ibuprofen, which attenuated the constitutional side effects of LPS. The maximum tolerated dose was 4.0 ng/kg with dose-limiting toxicity being World Health Organization grade III hepatic toxicity. Hematological changes included transient decreases in WBCs affecting granulocytes, monocytes, and lymphocytes in a marked different pattern. Endogenous cytokine release occurred in an LPS dose-dependent manner as measured by tumor necrosis factor-α, interleukin-6, and macrophage colony-stimulating factor serum levels. Moderate antitumor activity in colorectal cancer was observed in the case of 2 patients. Phase II trials of LPS are currently in progress.

INTRODUCTION

In 1894 Coley (1, 2) first reported hemorrhagic necrosis of solid tumors in patients following bacterial infections. The interest in biological approaches to cancer therapy increased during the last two decades by an increasing body of knowledge about cytokines and their function in host defense against bacterial infection and also against tumors.

In 1943 Shear (3) *et al.* isolated the LPS[3] as the active agent in Coley's mixed bacterial vaccine. The failure of endotoxin to kill tumor cells in culture indicated that its antitumor activity must be mediated by host-dependent mechanisms.

TNF-α was first identified in mice primed with *Bacillus Calmette-Guérin* and subsequently challenged with LPS. Serum from these mice induced a hemorrhagic tumor necrosis when transferred into tumor-bearing animals (4).

Since 1985 TNF-α has been investigated in many clinical trials; however, its suitability in cancer therapy remains unclear (5–9). This is partly due to its toxicity, which severely limits the dose that can be given safely to humans.

In addition the lack of major therapeutic activity of TNF-α may be due to differences in the biological reactions induced by exogenously administered TNF-α in comparison to endogenously produced TNF-α. In the latter case TNF-α is acting within the physiological cytokine cascade, known to be induced by LPS (10, 11).

The clinical use of LPS also is limited by its toxic side effects. Constitutional side effects can be attenuated by a cyclooxygenase inhibitor (ibuprofen) without impairing the biological reactions as measured by cytokine release and cellular parameters (10, 11).

Since LPS dose escalation under ibuprofen protection has proven to be clinically safe and to produce high serum levels of TNF-α and IL-6 (11), the therapeutic potential of LPS should be evaluated systematically. Human clinical trials with LPS have recently been undertaken (11–13), but the optimal dose and schedule remain to be established. We report here the results of a phase I clinical trial of highly purified LPS in patients with advanced cancer. Study objectives were to determine the toxicity, maximal tolerated dose, and biological effects of LPS administered i.v. and to seek evidence of antitumor activity.

PATIENTS AND METHODS

Preparation of Endotoxin. Lipopolysaccharide from *Salmonella abortus equi* was isolated from bacteria by the phenol-water method (14) and purified further by the phenol-chloroform-petroleum ether extraction procedure (15). The resulting LPS was essentially free of protein (<0.08%) and free of nucleic acid. The preparation was electrodialyzed, converted to the uniform sodium salt (16), and lyophilized.

Patient Selection. All patients entering this trial were evaluated and treated at the university of Freiburg, Department of Hematology and Oncology, Freiburg, Federal Republic of Germany. Patients eligible for the study included adults older than 18 years with histopathologically confirmed metastatic cancer refractory to standard therapy or for whom no effective conventional treatment was available. The selected patients had a performance status ≥60% (Karnowsky scale) (17) and an estimated life expectancy of >3 months. Other selection requirements were adequate baseline physiological function including adequate hematological status (hemoglobin, 10 g/liter; WBC count, 4,000/mm^3; platelet count, 100,000/mm^3), hepatic function (serum bilirubin, <2 mg/dl), and renal function (creatinine, <1.5 mg/dl). Exclusion criteria included seizure disorders, central nervous system metastasis, requirement of anticoagulants, corticosteroids, or nonsteroidal anti-inflammatory drugs, and cardiac or pulmonary failure.

All previous anticancer therapy had been discontinued for a minimum of 4 weeks before a patient entered the study. All patients underwent a complete medical history and physical examination. The following diagnostic studies were performed leukocyte, differential, and platelet counts; coagulation profile; biochemical screening profile (electrolytes, creatinine, uric acid, total protein, albumin, bilirubin, cholesterol, triglycerides, AST, ALT, alkaline phosphatase, lactate dehydrogenase, cholinesterase, and γ-glutamyl trans-peptidase); C-reactive protein and the tumor marker carcinoembryonic antigen; an electroencephalogram; chest X-ray; sonography; and, when necessary, special diagnostic studies.

The study was approved by the institutional review board and signed informed consent was obtained from all patients.

None of the patient data described in this phase I clinical trial have been previously published (11).

Study Design. The LPS was prepared as a sterile solution in phosphate-buffered saline in single-dose vials of 0.1 g/ml or 1.0 g/ml, diluted with 0.9% saline immediately before use, and administered to patients by biweekly bolus i.v. injections. A minimum of three patients was treated at each dose level. The first four patients enrolled in the phase

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[3] The abbreviations used are: LPS, lipopolysaccharide; TNF-α, tumor necrosis factor-α; IL, interleukin; BW, body weight; M-CSF, macrophage colony-stimulating factor; WHO, World Health Organization; MTD, maximal tolerated dose; AST, aspartate aminotransferase; ALT, alanine aminotransferase; NSCLC non-small cell lung cancer; N, normal (upper limit of normal range).

I study received 0.15 ng/kg of BW as the initial dose. The dose was escalated interindividually up to 5 ng/kg BW. In addition, patients who experienced severe chills and fever above 39°C following LPS injection (above 1 ng/kg BW) received two doses of 800 mg each of ibuprofen (Hoechst AG, Frankfurt, Federal Republic of Germany) orally, the first given 90 min before and the second together with the LPS injection.

All the patients were hospitalized for close observation during 24 h following LPS injection.

Vital signs were monitored before injection and at 30-min intervals for 6 h after endotoxin injection. Rectal and transcutaneous temperatures were measured continuously with a thermistor (Hellige AG, Freiburg, Federal Republic of Germany). Patients were weighed weekly.

Blood samples for complete blood cell counts and immunological parameters were obtained with an i.v. sampling catheter before LPS injection and at postinjection times of 1, 1.5, 2, 3, 4, 6, and 24 h.

The following immunological parameters were examined: serum TNF-α, IL-6, and M-CSF levels. For cytokine assays, the blood samples were centrifuged, and the serum was decanted within 10 min and stored at −70°C.

A serum chemistry profile, including renal and liver function tests, electrolyte, triglyceride, and cholesterol levels, and coagulation profile were obtained before and 1.5, 3, 6, and 24 h after LPS injection.

Tumor size was measured by physical examination or by appropriate roentgenographic examination at the end of 8 weeks. In the event of tumor regression or stable disease, patients continued to receive the treatment until disease progression was observed. The criteria for responses have been described previously (18).

Toxicities were assessed according the WHO grading criteria (18).

The MTD was defined as one dose level below the dose at which more than one third of the patients showed toxic effects in the liver according WHO grade III (AST/ALT > 5.1–10 × N) (8).

Tumor Necrosis Factor-α Assay. Human TNF-α was measured by an enzyme-linked immunosorbent assay (T Cell Sciences, Inc., Cambridge, MA). The sensitivity of this assay is approximately 10 pg TNF-α/ml. It is unaffected by the presence of denatured TNF-α, lymphotoxin (TNF-β), IL-1, or IL-2. Mean TNF-α serum levels in healthy volunteers were <10 pg/ml. One mg of recombinant TNF-α as a standard for this assay is equivalent to 2×10^7 units of activity as defined in an L929 cytotoxicity assay in the presence of actinomycin D.

Interleukin-6 Assay. Serum from patients was tested for IL-6 activity by a quantitative enzyme-linked immunosorbent assay (R&D Systems, Inc., Minneapolis, MN) following the instructions of the manufacturer. The assay showed no measurable cross-reactivity with other cytokines and is sensitive up to 10 pg IL-6/ml.

Macrophage Colony-stimulating Factor Assay. M-CSF was determined in serum samples sent to Cetus, Inc., Emeryville, CA. They use a radioimmunoassay that accurately measures M-CSF concentrations as low as 60 units/ml (1.2 ng/ml), as described previously by Shadle *et al.* (19).

Table 1 *Patient Characteristics*

Total no. of patients	24
Males	19
Females	5
Age (yr)	
Median	56
Range	33–67
Performance status (Karnowsky)	
100%	14
90%	6
70%	4
Diagnosis	
Colorectal cancer	10
NSCLC	5
Renal cell cancer	2
Pancreatic cancer	2
Sarcoma	2
Anal cancer	1
Gallbladder cancer	1
Tracheal cancer	1
Previous therapy	
None	2
Chemotherapy	6
Radiotherapy	10
Surgery	19

Table 2 *Side Effects of LPS*

	Side effects (no. of patients)					
Dose level	1	2	3[a]	4	5	6
Dose of LPS (ng/kg)	0.15	0.3	1.0	2.0	4.0	5.0
No. of patients	4	4	3	3	6	4
Fever[b]						
WHO grade 0			2	1	1	
WHO grade I	3	1	1	2	4	1
WHO grade II	1	3			1	3
Chills	1	3				
Fatigue			1	1	2	4
Headache					1	2
Nausea						1
Myalgia					1	3
Hypotension					2	
Dizziness					1	2
Dyspneae					1	1
Hepatic toxicity[c]						
WHO grade 0	4	4	3	2	2	
WHO grade I				1	1	
WHO grade II					3	1
WHO grade III						3
Renal toxicity[d]						
WHO grade 0	4	4	3	3	6	3
WHO grade I						1

[a] From dose levels 3 to 4 patients received ibuprofen, 1600 mg, p.o., before LPS injection.
[b] WHO grade 0, none; WHO grade I, ≤38°C; WHO grade II, 38–40°C.
[c] AST/ALT: WHO grade 0, 0–≤1.25 × N; WHO grade I, 1.26–2.5 × N; WHO grade II, 2.6–5.0 × N; WHO grade III, 5.1–10 × N.
[d] Creatinine: WHO grade 0, 0–≤1.25 × N; WHO grade I, 1.26–2.5 × N.

RESULTS

Patient Population

Between January 1989 and March 1990 a total of 24 patients with disseminated cancer were entered into the study. Their characteristics are shown in Table 1. There were 19 men and 5 women ranging in age from 33 to 67 years, with a median age of 56 years. Ten patients had colorectal cancer, 5 had NSCLC, 2 had renal cell carcinoma, 2 had pancreatic cancer, 2 had sarcoma, and 1 each had anal, gallbladder, and tracheal cancer. The patients with pancreatic cancer had received no prior therapy, whereas all other patients had been treated with radiation therapy, chemotherapy, and/or surgery which was terminated at least 4 weeks prior to the onset of the study treatment.

Toxicity

The most common clinical toxicities associated with LPS are detailed in Table 2. Fever and chills occurred in all patients 1–2 h after LPS injection throughout all dose levels. These constitutional side effects were reduced but not entirely prevented by oral pretreatment with ibuprofen (1,600 mg).

Fatigue persisting up to 2 days after treatment was also a frequent complaint but only at dose levels 4–6. Headache only occurred in 1 of 4 and 2 of 4 patients at the highest dose levels of 4.0 and 5.0 ng/kg BW, respectively. One patient at dose level 6 had nausea for 1 h at the time of peak temperature. Four patients experienced myalgia 1–2 h after LPS injection. Two patients complained of transient dizziness.

Two patients with NSCLC developed hypotension with a systolic blood pressure of 90 mm Hg, which could be corrected by human albumin administration. Additionally, these two patients had dyspnea without a decrease in oxygen pressure in blood specimens and no signs of pulmonary edema. These episodes lasted for about 30 min starting 90 min after LPS injection.

One patient with NSCLC complained of severe transient

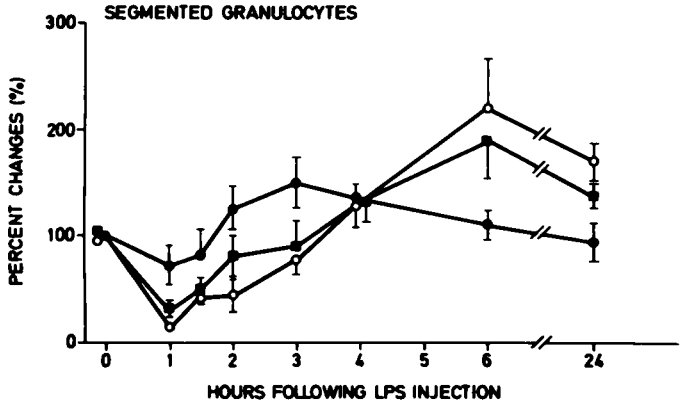

Fig. 1. Segmented granulocyte counts following LPS injection to cancer patients at escalating doses. *Point*, mean percentage change compared to pretreatment level; *bar*, ±SEM; subdivided into three LPS dose groups: dose level 1 and 2 (n = 8 patients), dose levels 3 and 4 (n = 6), and dose levels 5 and 6 (n = 10).

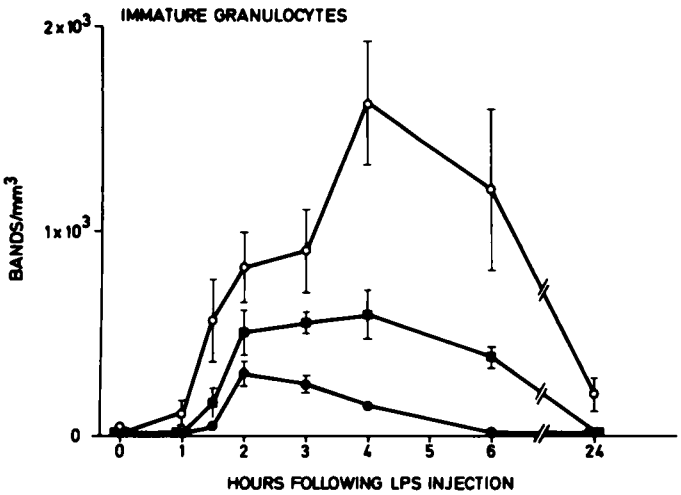

Fig. 2. Immature granulocyte counts following LPS injection to cancer patients at escalating doses. *Point*, mean number of bands/mm^3; *bar*, ±SEM; subdivided into three LPS dose groups as given in legend to Fig. 1.

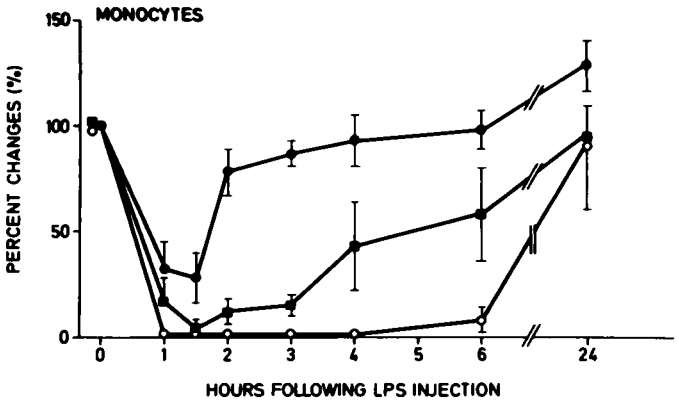

Fig. 3. Monocyte counts following LPS injection to cancer patients at escalating doses. *Point*, mean percentage change compared to pretreatment level; *bar*, ± SEM; subdivided into 3 LPS dose groups as given in legend to Fig. 1.

back pain. Administration of pethidin (Dolatin) i.v. afforded prompt relief.

Hepatic toxicity was seen in a dose-dependent manner, starting at LPS dose level 4 (WHO grade I in 1 of 3 patients) and increasing to WHO grade III in 3 of 4 patients at dose level 6.

AST and ALT serum activities were elevated 3–6 h after LPS injection and normalized 24 h later. The increase in hepatic transaminase levels was paralleled by an increase in alkaline phosphatase levels.

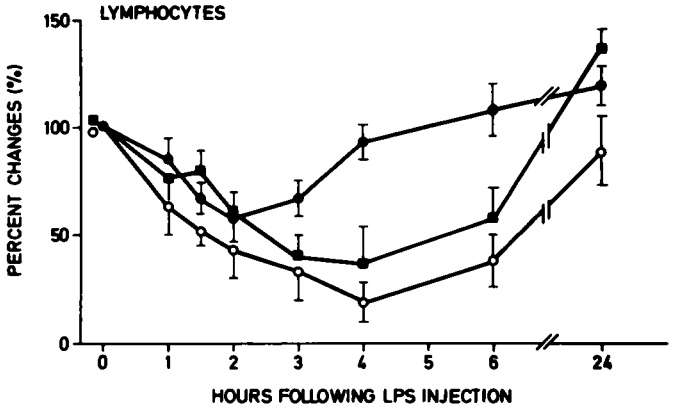

Fig. 4. Lymphocyte counts following LPS injection to cancer patients at escalating doses. *Point*, mean percentage change compared to pretreatment level; *bar*, ±SEM; subdivided into 3 LPS dose groups as given in legend to Fig. 1.

Renal toxicity grade I was observed at dose level 6 in one patient who had been nephrectomized because of renal cell cancer. His creatinine increased up to 1.5 mg/dl 6 h after LPS injection and returned to normal 18 h later.

MTD

WHO grade III hepatic toxicity was seen in 3 of 4 patients treated at dose level 6 (5.0 ng/kg BW). According to the definition given, above the MTD of the LPS used in this study is 4.0 ng/kg BW.

Hematological Effects

The changes in segmented granulocyte, immature granulocyte (bands), monocyte, lymphocyte, and platelet counts following different dosages of LPS are shown in Fig. 1-5. One h after LPS injection a dose-dependent decrease in total WBC counts occurred. This decline is related to a marked decrease in segmented granulocytes and monocytes. Three to 6 h after LPS injection a dose-dependent increase in granulocytes up to 200% of normal values occurred. The granulocytosis persisted until 6 h after LPS injection but by 24 h had returned to pretreatment levels (Fig. 1).

Fig. 2 depicts the response in bands showing a direct relationship with the LPS dose administered. A marked increase in bands was observed 1 h after injection at the dose levels 3–6, which peaked 4 h after LPS injection with maximum values of 1600/mm^3. By 24 h immature granulocytes had returned to pretreatment levels.

The decrease of monocyte counts (Fig. 3) was also dose dependent and at the highest dose levels 5 and 6 the monocytes nearly completely disappeared for 3 h.

Lymphocytes decreased more gradually, reaching lowest values 4 h after LPS injection with minimal values of 40 and 20% at dose levels 3 and 4 and 5 and 6, respectively (Fig. 4). Both lymphocyte and monocyte counts returned to preinjection levels 24 h after LPS injection.

Platelets levels decreased only slightly. The average decline (77%) was more pronounced at the higher dose levels 5 and 6 (Fig. 5). There was no decrease in platelet counts below the normal range in any patient.

There were no changes in the coagulation parameters pro-

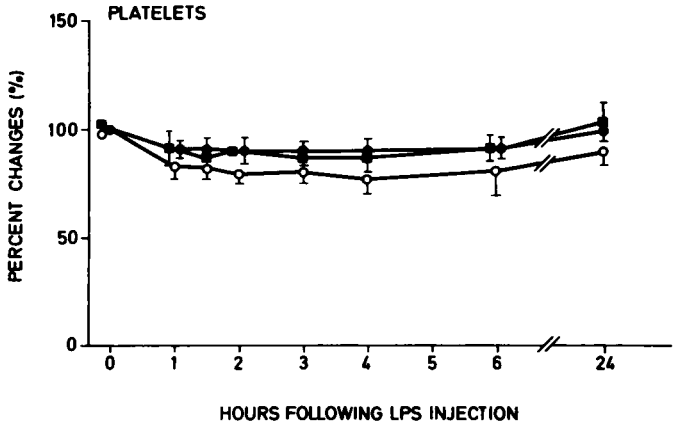

Fig. 5. Platelet counts following LPS injection to cancer patients at escalating doses. *Point*, mean percentage change compared to pretreatment level; *bar*, ± SEM; subdivided into 3 LPS dose groups as given in legend to Fig. 1.

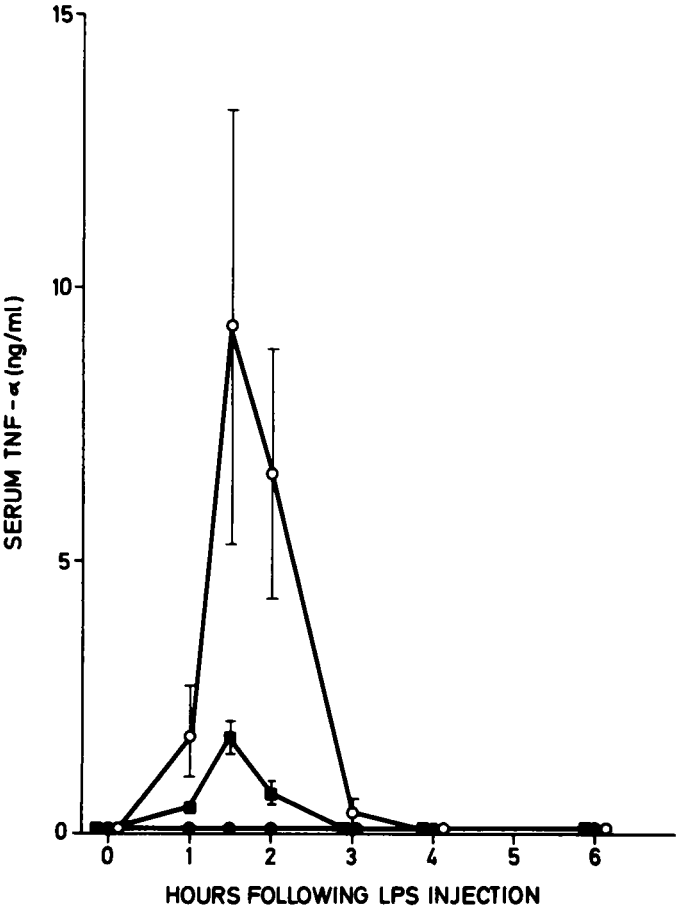

Fig. 6. Serum levels of TNF-α following i.v. bolus injection of escalating doses of LPS to cancer patients. *Point*, mean; *bar*, ±SEM; subdivided into 3 LPS dose groups as given in legend to Fig. 1.

thrombin time, partial thromoplastin time, and fibrinogen concentration.

Serum Concentrations of Cytokines

Tumor Necrosis Factor-α. Baseline serum TNF-α levels were <0.01 ng/ml in all patients. The increments in TNF-α serum levels observed after treatment with LPS were dose dependent. Circulating TNF-α was detectable by 1 h and peaked 1.5 h after LPS injection. Peak concentrations of 18 ng/ml were obtained at LPS dose level 6 (Fig. 6). TNF-α serum concentrations then returned to pretreatment levels 3–4 h after LPS injection.

Five patients with NSCLC treated at LPS dose levels 5 and 6 had significantly higher TNF-α peak serum levels, ranging from 14 to 18 ng/ml compared to peak serum levels ranging from 3 to 10 ng/ml in the other five patients treated at the same dose level but having other tumors (data not shown).

Interleukin-6. Pretreatment serum concentrations of IL-6 were <0.01 ng/ml with the exception of one patient having far advanced renal cell cancer, who had pretreatment serum levels of 0.08–0.15 ng/ml. Similarly to TNF-α, circulating IL-6 was detectable by 1 h after LPS injection, but peak levels were obtained 0.5 h after TNF-α peak levels (Fig. 7). The maximum increase and broadness of the peak both showed a direct correlation to the LPS dose (Fig. 7).

Macrophage Colony-stimulating Factor. Serum for measurement of M-CSF levels was obtained from four patients being treated with 4.0 ng/kg LPS (dose level 5). Basal values of M-CSF were between 6 and 16 ng/ml. The increase in serum M-CSF was detectable as early as 1.5 h after LPS injection. It reached maximum at 3 h and subsequently declined to pretreatment levels (Fig. 8).

Schedule of LPS Application

At the MTD dose level (4.0 ng/kg) weekly injections of LPS resulted in a marked attenuation of the TNF-α response be-

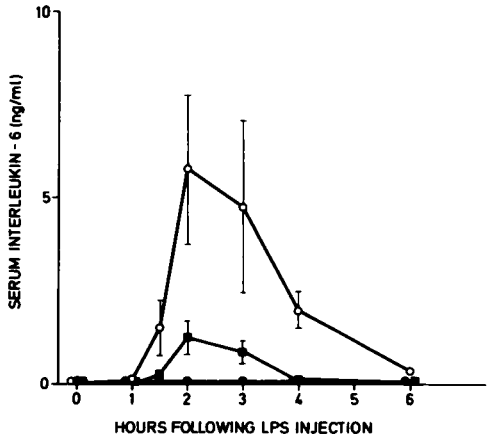

Fig. 7. Serum levels of IL-6 following i.v. bolus injection of escalating doses of LPS to cancer patients. *Point*, mean; *bar*, ±SEM; subdivided into 3 LPS dose groups as given in legend to Fig. 1.

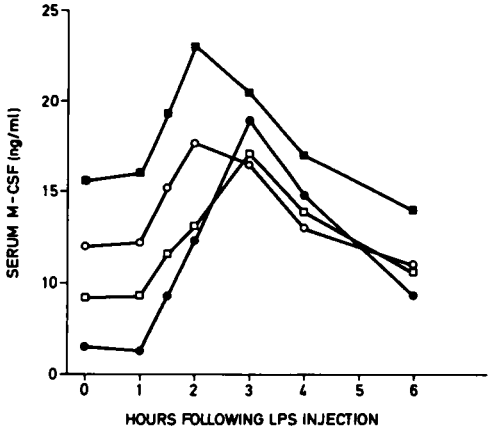

Fig. 8. Serum levels of M-CSF following LPS injection to cancer patients. Serum levels of four individual patients being treated with 4.0 ng/kg LPS (dose level 5).

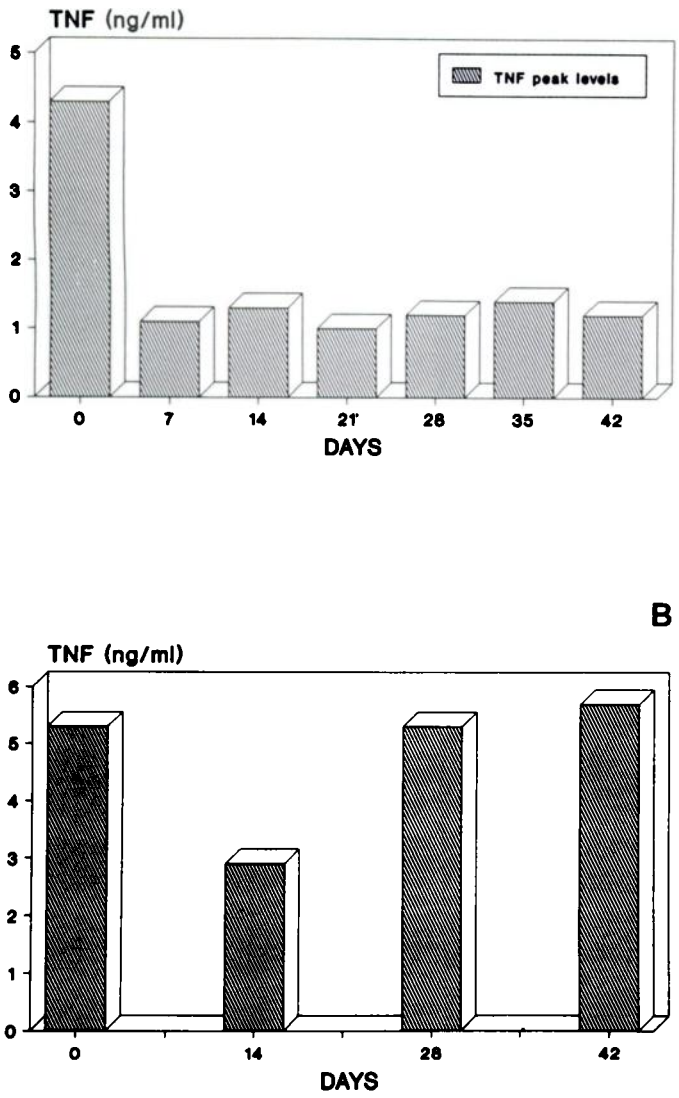

Fig. 9. Changes of maximal TNF-α serum levels in patients treated at different time intervals. *A*, mean TNF-α peak serum levels following weekly injections of 4.0 ng/kg LPS (dose level 5) to 3 patients (SEM < 10%); *B*, mean TNF-α peak serum levels following injections of 4.0 ng/kg LPS (dose level 5) to 3 patients at 2-week intervals (SEM < 10%).

tween the first and the second challenge, thereafter showing a plateau (Fig. 9*A*).

LPS application at 2-week intervals resulted in a less pronounced diminution of the TNF-α reaction between the first and second injection, showing a stepwise restoration of the initial magnitude of TNF-α production (Fig. 9*B*).

Tumor Responses

Tumor responses were evaluated 8 weeks after treatment corresponding to four courses of treatment. Only 18 patients completed four treatment courses and were therefore evaluable for response according to convention. Two partial responses were observed in patients with colorectal carcinoma, lasting for at least 3 and 4 months, respectively.

One patient, a 62-year-old woman, exhibited a partial response consisting of a 60% decrease in the size of a paraaortal mass on abdominal computed tomographic examination following protocol treatment with 8 courses at the LPS dose of 4.0 ng/kg. Another patient, a 55-year-old woman with metastatic colorectal carcinoma, exhibited a partial response following treatment with 12 cycles at an LPS dose of 4.0 ng/kg. This partial response consisted of a 70% decrease in the size of nodular pulmonary parenchymal metastases and was assessed by chest X-ray examinations in two directions. Stable disease was seen in 9 patients: 4 of these had colorectal carcinoma of 2, 2, 6, and 6+ months duration; diagnosis and duration of stable disease in the other patients were as follows: NSCLC, 3 and 2 months; renal cell cancer, 5 and 8+ months; and tracheal cancer 4+ months. Seven patients did not respond to the treatment.

DISCUSSION

We performed a phase I trial of highly purified LPS *S. abortus equi* in patients with advanced malignancies. The major aims of the present study were to ascertain the biological effects, toxicity, the MTD, and optimal schedule of LPS. Qualitatively, the toxicities induced by LPS injection were similar to those seen when TNF-α was administered as a single agent (5–9). Constitutional symptoms such as fever, chills, fatigue, headache, and myalgias were noted in the majority of patients in this study. Fever and chills were unacceptably severe at LPS dose level 3 (1.0 ng/kg) and greater.

Further escalation of the LPS dose up to 5.0 ng/kg was enabled by pretreatment with the cyclooxygenase inhibitor ibuprofen, which has been reported by us and others to attenuate the constitutional side effects of LPS in humans (10, 11). With respect to the antitumor efficacy of LPS we would like to point out that the cytokine release is not compromised by ibuprofen pretreatment (10, 11). Furthermore, ibuprofen does not inhibit the cytotoxic effects of TNF-α on tumor cells *in vitro* (20).

Dose-limiting toxicity was WHO grade III hepatic toxicity, indicated by an increase in serum activities of AST/ALT (Table 2) and less pronounced alkaline phosphatase; no bilirubin increase was observed. According to this pattern of changes in biochemical parameters, hepatic toxicity seems to be predominantly due to hepatocyte damage rather than to cholestasis. This is consistent with the hepatic toxicity pattern of TNF-α as described by others (8, 9).

The only patient developing transient elevation of serum creatinine (up to 1.5 ng/dl) had been nephrectomized because of renal cell cancer. The other side effects such as hepatic toxicity and hematological reactions were not more pronounced in this patient than the other patients treated at the same dose level.

Constitutional symptoms were controlled by ibuprofen pretreatment up to dose level 4 and reappeared less pronounced at dose levels 5 and 6 without becoming dose limiting again.

Hypotension, reported to be one of the major dose-limiting toxicities in TNF-α trials (7), was observed only in two NSCLC patients treated at dose level 5. These two patients developed further symptoms of dyspnea and restlessness, and one of them additionally complained of severe back pain, necessitating pethitin injection.

In these two patients we observed maximal TNF-α serum levels of 17 and 18 ng/ml which were in the range of TNF-α serum levels of patients treated with i.v. applied recombinant human TNF-α (7). In three other patients with NSCLC the TNF-α peak serum levels were of the same magnitude (14–18 ng/ml), whereas the other five patients treated at the same dose levels, but with other malignancies, produced TNF-α peak levels of 3–10 ng/ml. Differences in the capacity of monocytes to

produce TNF-α *ex vivo* between healthy individuals and tumor patients have been described (21), as have changes in TNF-α serum levels during the course of malignant disease in children (22).

Our observations suggest a tumor type-specific intensity of the LPS-induced TNF-α production. Such a correlation would be supported by the findings of Bartholeyns *et al.* (23), who reported marked differences in the sensitivity of tumor-bearing mice to the lethal effects of LPS according to the tumor type.

The MTD of LPS strongly depends on the application schedule. As published earlier (11), the LPS dose in cancer patients can be escalated up to 10 ng/kg BW when increasing LPS doses are applied to the same patient at weekly intervals. This is due to the development of LPS tolerance resulting from repeated injections. In addition, the greater amounts of LPS applied and shorter intervals between the repeated injections enhance the development of tolerance. Therefore, the maximal tolerable "first challenge dose," as determined in this trial, is only 4.0 ng/kg BW. Monitored by the serum TNF-α peak concentrations the MTD of 4.0 ng/kg BW, repeated weekly, revealed a marked reduction of the maximal TNF-α response between the first and the second application, thereafter showing a plateau. LPS administration at 2-week intervals resulted in a less pronounced attenuation of the maximal TNF-α release reaching the original amount within 4 weeks. With respect to the release of TNF-α, biweekly intervals of LPS application seemed to be optimal in terms of prevention of tolerance.

The magnitude and the time course of the changes in differential WBC counts (Figs. 1–5) proved to be LPS dose dependent and did not show any differences compared to published data from healthy volunteers (24–27) and cancer patients (7).

The rapid decrease in WBCs occurring 1 h after LPS injection is thought to be due to leukocyte adhesion to the vascular endothelium and to leukocyte emigration (28–30). *In vitro* studies have demonstrated LPS- and cytokine-induced expression and activation of adhesion molecules on leukocytes and endothelial cells (30–35). The *in vivo* relevance of these findings is under investigation. The subsequent increase in leukocyte counts may be explained by at least two mechanisms: demargination and increased cell release from the bone marrow.

The relevance of increased bone marrow activity is indicated by the sharp increase in bands (Fig. 2). This may be caused by an enhanced colony-stimulating activity, found by Moore *et al.* (13) in human postendotoxin sera (13), and, with respect to monocytes, by elevated M-CSF serum levels reported here.

As reported previously, the release of endogenous TNF-α and IL-6 in cancer patients is dependent on the LPS dose applied (11). While there is a marked interindividual variation in the magnitude of cytokine secretion (Figs. 6 and 7), the kinetics of TNF-α and IL-6 release, however, exhibit a distinct and reproducible pattern. This also holds true for M-CSF kinetics which peak 60 min later than TNF-α (Fig. 8). The sequence described suggests a major role of TNF-α in subsequent M-CSF and IL-6 release *in vivo*.

Although we are reporting a phase I trial, antitumor activity has been monitored. The observation of two partial responses and four occurrences of stable disease in patients with colorectal cancer may be due to the fact that this is the largest group in our patients treated. It does not necessarily preclude other tumor types to be even more sensitive to LPS.

The data generated in this study forms the basis for further investigations of i.v. LPS administration as a single agent and in combination with other biological response modifers in cancer patients.

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