

INTERLEUKIN-1 α AND INTERLEUKIN-1 β PRODUCTION IN PERIPHERAL WHOLE BLOOD FROM PATIENTS WITH UROLOGICAL CANCER

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The activities of interleukin-1 α (IL-1 α) and interleukin-1 β (IL-1 β) were investigated in peripheral whole blood from 30 patients with bladder cancer, 12 patients with renal cell carcinoma, 18 patients with prostatic cancer and 16 healthy subjects. Heparinized blood was cultured in the absence and presence of various concentrations of bacterial lipopolysaccharide (LPS). The culture supernatants were obtained and activities of IL-1 α and IL-1 β were determined by enzyme-linked immunosorbent assay (ELISA). In the absence of LPS stimulation, neither IL-1 α nor IL-1 β was spontaneously produced in blood cultures from patients with bladder cancer, renal cell carcinoma or prostatic cancer compared with control subjects. After stimulation with various concentrations of LPS, blood cultures from patients with bladder cancer, renal cell carcinoma, prostatic cancer, those from control subjects produced IL-1 α and IL-1 β in a dose-dependent manner, and IL-1 β was predominant in all supernatants. The activities of IL-1 α and IL-1 β showed no significant differences between the patients with bladder cancer, renal cell carcinoma or prostatic cancer and control subjects. This study suggested that the patients with bladder cancer, renal cell carcinoma and prostatic cancer did not spontaneously produce IL-1 α or IL-1 β , but that the ability to produce IL-1 α and IL-1 β in response to LPS stimulation was not significantly impaired.

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Key words : Urological cancer, IL-1 α and IL-1 β production, Peripheral whole blood, Enzyme-linked immunosorbent assay

INTRODUCTION

The abnormalities of immune response in the patients with urological cancer have been reported¹⁾. The demonstration of immune reactivity in such patients has lead to a considerable clinical significance²⁾. Interleukin-1 (IL-1), a macrophage/monocyte derived protein, has been shown to mediate a wide range of biological activities, including the stimulation of cell-mediated and humoral immune responses, pro-inflammatory effects, and catabolic effects on various tissues³⁾. Recent advances in recombinant DNA technology have made human IL-1 available in highly purified form. There are two biochemically different forms, IL-1 α and IL-1 β ⁴⁾, which appear to share similar biological activities⁵⁾.

In the literature, some investigators have described spontaneous IL-1 production in a considerable portion of leukemia patients^{6,7)} and abnormal IL-1 production in peripheral blood mononuclear cells from patients with malignant tumor following stimulation by bacterial lipopolysaccharide (LPS)^{8,9)}. However, relatively little is known about spontaneous

IL-1 production by solid tumors. Furthermore, most of the studies about LPS-stimulated IL-1 production in cancer patients have used bioassays which could not discriminate IL-1 α and IL-1 β activities and were easily interfered by various substances, such as IL-2, mitogens and growth factors in samples¹⁰⁾. To examine the immune responses of the patients with urological cancer, we used enzyme-linked immunoabsorbent assay (ELISA) to determine spontaneous production and LPS-stimulated IL-1 α and IL-1 β production in peripheral whole blood from patients with urological cancer in comparison with that from normal healthy subjects.

PATIENTS AND METHODS

Blood donors

Thirty patients with bladder cancer, 12 patients with renal cell carcinoma and 18 patients with prostatic cancer were included in this study. All diagnoses were histopathologically confirmed. Patients receiving radiation or chemotherapy were not included in this study. The age range of the patients with bladder cancer, renal cell carcinoma

and prostatic cancer was 37–88 years (mean 59 years), 51–70 years (mean 62 years) and 45–89 years (mean 69 years), respectively. Sixteen healthy subjects were included as the control subjects. The age range was 35–79 years (mean 55 years). None of the cancer patients or control subjects had any evidence of infection. Blood samples from patients were obtained before any treatment.

Peripheral whole-blood cultures

One ml of peripheral blood was drawn and placed in a heparinized (10 U/ml) tube. Within 2 hours of sampling, 0.1 ml heparinized blood were cultured in 24-well multi-cluster plates (Costar, Cambridge, MA) in 1.0 ml RPMI-1640 medium (Gibco, Grand Island, NY) at 37°C for 24 hours in the absence or presence of various concentrations (0.1, 0.3, 1, 3, and 5 μ g/ml) of 0.01 μ l LPS (*Escherichia coli* O55 : B55, Difco). The culture supernatants were then mixed with PBS/0.05% Thimerosal at the ratio of 1:2 and stored at –70°C until IL-1 α and IL-1 β assay.

Determine activities of IL-1 α and IL-1 β

The culture supernatants were measured for their IL-1 α and IL-1 β activities with ELISA as previously described¹¹⁾. These assays used human IL-1 α and IL-1 β ELISA kits (Otsuka Pharmaceutical Co., Ltd.). Briefly, microplates were coated with anti-IL-1 α monoclonal antibody in 100 μ l/well of PBS, pH 7. Following overnight incubation at 4°C, the wells were blocked with 1% skim milk in PBS for at least 1 hour at room temperature and washed three times with PBS containing 0.05% Tween-20 (Tween-PBS). Samples or standard IL-1 α (100 μ l) in 0.1% bovine serum albumin-PBS were added to the wells and the plates were incubated at 4°C for 24 hours. The plates were then washed three times, 100 μ l of rabbit anti-IL-1 α antibodies were added to each well and incubated for 2 hours at 23°C. Subsequently, the plates were washed, supplemented with 100 μ l of POD-labeled goat anti-rabbit IgG and incubated at 23°C for 2 hours. Finally, 100 μ l of enzyme substrate (1 mg/ml O-phenylenediamine in 0.1 M sodium citrate buffer, pH 5) was added to each well and incubated at room temperature for 5 minutes. The reaction was stopped by addition of 100 μ l of 2 N H₂SO₄ to each well and the absorbance at 492 nm was determined using a Titertek Multiscan. This kit is specific for IL-1 α , and does not measure other cytokines. The assay of IL-1 β was also measured by IL-1 β ELISA kits (Otsuka Pharmaceutical Co., Ltd.). These assays could reliably detect as little as 0.3 ng/ml blood of IL-1 α and 0.6 ng/ml blood of IL-1 β .

Statistical analysis

All determinations were done in duplicate. The results were expressed as mean (ng/ml blood) $\pm$ SD. Statistical analysis was determined by Student's test and chi-square test. P values of <0.05 were

considered to indicate significance.

RESULTS

Kinetics of IL-1 α and IL-1 β production

The kinetics of IL-1 α and IL-1 β production in whole-blood cultures in the presence of 5 μ g/ml concentration of 0.01 μ l LPS was examined in one healthy individual. The culture supernatants were measured for their IL-1 α and IL-1 β activities with ELISA after 4, 8, 19, 24, 28, 48, 72, 96 and 120 hours' incubation. The results are shown in Fig. 1. IL-1 α and IL-1 β activities increased with time (bar A), reached a plateau in 19–72 hours (bar B) and gradually increased after 72 hours (bar C). The same experiments were repeated in whole-blood cultures under different concentrations with comparable results. Thus, for all subsequent experiments, IL-1 α and IL-1 β production was measured in the culture supernatants harvested after a 24-hour incubation period.

Spontaneous production of IL-1 α and IL-1 β in patients with urological cancer and control subjects

As shown in Table 1, in the absence of LPS stimulation, small amounts of IL-1 α were detected in 2 out of 30 (6.6%) patients with bladder cancer (0.75 $\pm$ 0.11 ng/ml blood) and 2 out of 18 (11%) patients with prostatic cancer (0.76 $\pm$ 0.14 ng/ml blood), respectively. IL-1 α was not detected in the patients with renal cell carcinoma and the control subjects. In contrast, small amounts of IL-1 β were detected in 5 out of 30 (16.6%) patients with bladder cancer (3.11

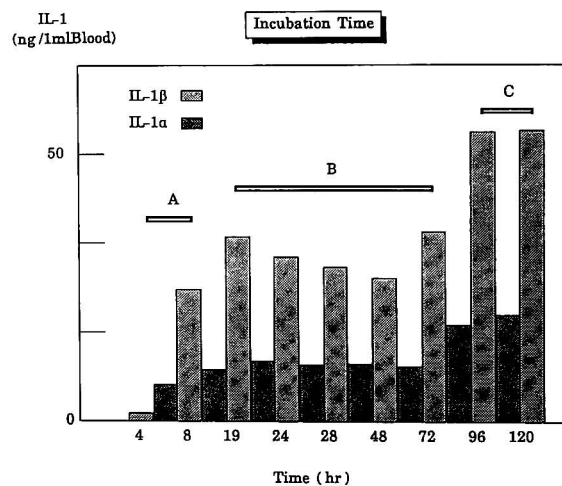


Fig. 1. The kinetics of IL-1 α and IL-1 β production in whole-blood cultures under the stimulation of 5 μ g/ml concentration of 0.01 μ l LPS in one healthy individual. The culture supernatants were measured for their IL-1 α and IL-1 β activities with ELISA after different incubation times. IL-1 α and IL-1 β activities increased with time (bar A), reached a plateau in 19–72 hours (bar B) and gradually increased after 72 hours (bar C).

Table 1. Spontaneous elaboration of IL-1 α and IL-1 β in patients with urological cancer and control subjects

Subjects (n)	Spontaneous IL-1 α elaboration		Spontaneous IL-1 β elaboration	
	No. of positive patients (%)	Activity of IL-1 α (ng/ml blood $\pm$ SD)	No. of positive patients (%)	Activity of IL-1 β (ng/ml blood $\pm$ SD)
Bladder cancer (30)	2 (6.6%)	0.75 $\pm$ 0.11	5 (16%)	3.11 $\pm$ 1.93
Renal cell carcinoma (12)	0	0	2 (16%)	1.17 $\pm$ 0.68
Prostatic cancer (18)	2 (11%)	0.76 $\pm$ 0.14	0	0
Controls (16)	0	0	1 (6.2%)	3.17

Table 2. IL-1 α production in patients with urological cancer and control subjects*

Subjects (n)	Concentrations of LPS added (μ g/ml)				
	0.1	0.3	1	3	5
Bladder cancer (30)	0.98 $\pm$ 0.81	1.24 $\pm$ 0.94	1.76 $\pm$ 1.31	2.52 $\pm$ 1.72	3.16 $\pm$ 2.2
Renal cell carcinoma (12)	0.85 $\pm$ 0.40	1.25 $\pm$ 0.57	1.82 $\pm$ 0.97	2.47 $\pm$ 1.13	3.02 $\pm$ 1.38
Prostatic cancer (18)	1.83 $\pm$ 1.29	2.01 $\pm$ 1.14	2.77 $\pm$ 1.46	4.14 $\pm$ 2.24	5.36 $\pm$ 3.24
Controls (16)	1.15 $\pm$ 0.70	1.43 $\pm$ 0.78	1.94 $\pm$ 0.94	2.64 $\pm$ 1.25	3.44 $\pm$ 1.56

* IL-1 α production was stimulated with various concentrations of LPS, and activities of IL-1 α were determined by ELISA as described in the text. Each value is expressed as mean (ng/ml blood) $\pm$ SD.

Table 3. IL-1 β production in patients with urological cancer and control subjects*

Subjects (n)	Concentrations of LPS added (μ g/ml)				
	0.1	0.3	1	3	5
Bladder cancer (30)	17.3 $\pm$ 13.0	20.4 $\pm$ 14.3	27.4 $\pm$ 16.4	34.4 $\pm$ 18.0	41.7 $\pm$ 23.6
Renal cell carcinoma (12)	14.5 $\pm$ 7.7	19.0 $\pm$ 11.3	26.3 $\pm$ 8.9	35.6 $\pm$ 14.3	40.1 $\pm$ 13.5
Prostatic cancer (18)	17.6 $\pm$ 10.5	22.9 $\pm$ 14.5	30.2 $\pm$ 7.8	42.9 $\pm$ 14.1	46.0 $\pm$ 16.6
Controls (16)	14.0 $\pm$ 7.1	18.8 $\pm$ 7.8	26.3 $\pm$ 9.0	34.3 $\pm$ 10.5	39.1 $\pm$ 11.4

* IL-1 β production was stimulated with various concentrations of LPS, and activities of IL-1 β were determined by ELISA as described in the text. Each value is expressed as mean (ng/ml blood) $\pm$ SD.

$\pm$ 1.93 ng/ml blood), and 2 out of 12 (16%) patients with renal cell carcinoma (1.17 $\pm$ 0.68 ng/ml blood), and 1 out of 16 (6.2%) control subjects (3.17 ng/ml blood), respectively. IL-1 β was not detected in the patients with prostatic cancer. The positive rates and activities of IL-1 α and IL-1 β in patients with bladder cancer, renal cell carcinoma or prostatic cancer showed no significant differences compared to the control subjects. These findings demonstrated that neither IL-1 α nor IL-1 β was spontaneously produced in patients with bladder cancer, renal cell carcinoma or prostatic cancer compared with control subjects.

LPS-stimulated IL-1 α and IL-1 β production in patients with urological cancer and control subjects

To ascertain whether the difference in the activities of IL-1 α and IL-1 β was related to the concentration of LPS in patients with bladder cancer, renal cell carcinoma, prostatic cancer and control subjects, various concentrations of LPS were used. Tables 2 and 3 show the activities of IL-1 α and IL-1 β stimulated by various concentrations of LPS. As shown in Fig. 2 and 3, LPS stimulated IL-1 α and IL-1 β activities in a dose-dependent manner in culture

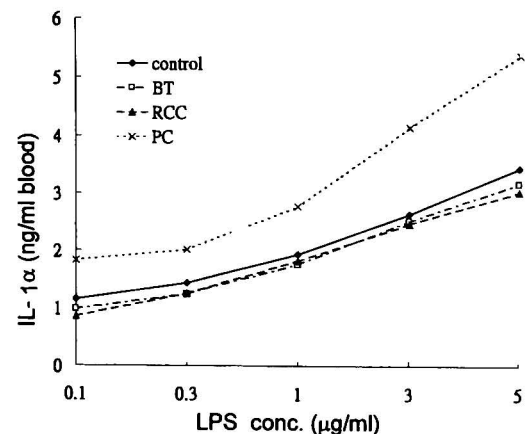


Fig. 2. IL-1 α production stimulated by various concentrations of LPS in peripheral whole-blood cultures from patients with urological cancer and control subjects. Abbreviations: BT=bladder cancer, RCC=renal cell carcinoma, PC=prostatic cancer.

supernatants from patients with bladder cancer, renal cell carcinoma, prostatic cancer and control subjects. The activity of IL-1 β exceeded that of IL-1 α in

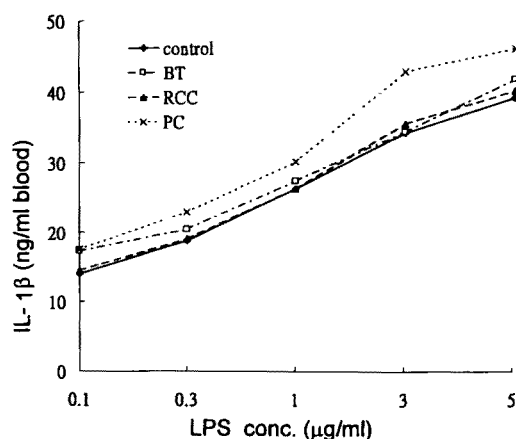


Fig. 3. IL-1 β production stimulated by various concentrations of LPS in peripheral whole-blood cultures from patients with urological cancer and control subjects. Abbreviations: BT=bladder cancer, RCC=renal cell carcinoma, PC=prostatic cancer.

patients with bladder cancer, renal cell carcinoma, prostatic cancer and control subjects, indicating that most of IL-1 in culture supernatants was IL-1 β .

LPS-stimulated IL-1 α production in patients with bladder cancer and renal cell carcinoma tended to be lower than that in control subjects, but that in patients with prostatic cancer tended to be higher. In contrast, IL-1 β production in patients with bladder cancer, renal cell carcinoma and prostatic cancer tended to be higher than that in control subjects.

The activities of IL-1 α and IL-1 β showed no significant differences between the patients with bladder cancer, renal cell carcinoma, or prostatic cancer and the control subjects.

DISCUSSION

IL-1 is a macrophage/monocyte-derived cytokine which is capable of enhancing human monocyte-macrophage-induced tumor cytotoxicity and to exert direct antiproliferative effects on certain tumor cells⁵⁾ However, contradictory findings have indicated that IL-1 acts as an autocrine growth factor for leukemic cells. Spontaneous IL-1 elaboration in the serum was found in a considerable portion of leukemia patients^{6,7)}

In contrast to extensive studies in leukemia patients, little is known about the spontaneous IL-1 elaboration in the patients with solid tumors. In an *in vitro* study, human bladder cancer cell line (T24) was reported to release IL-1 α and IL-1 β in the conditioned medium¹²⁾ In this study, in all unstimulated culture supernatants the activity of IL-1 α was only measurable in 2 out of 30 patients with bladder cancer and 2 out of 18 patients with prostatic cancer. The activity of IL-1 β was measurable in 5 out of 30 patients with bladder cancer and 2 out of 12

patients with renal cell carcinoma. We took particular care to exclude the possibility that IL-1 α and IL-1 β elaboration were caused by contamination. Our findings suggested that the peripheral whole-blood cultures from patients with urological cancer did not spontaneously produce IL-1 α or IL-1 β significantly in comparison with control subjects. Several clinical and animal studies have suggested that the capacity of peripheral blood monocytes to synthesize IL-1 is altered during periods of active tumor growth^{13,14)} However, most of these studies primarily used bioassays to determine the amount of IL-1 production and could not discriminate the two forms of IL-1, IL-1 α and IL-1 β . In order to determine the immune status of patients with urological cancer, we examined the effects of various concentrations of LPS on IL-1 α and IL-1 β production in whole-blood cultures. LPS is a potent stimulator of IL-1 synthesis in human monocytes¹⁵⁾. Some reports have also suggested B lymphocyte and large granular lymphocyte secrete IL-1 under the LPS stimulation^{16,17)} Recently, the measurement of IL-1 production in whole-blood cultures has also developed^{18,19)} The advantages of whole-blood cultures include the small amount of blood required and the better reflection of the *in vivo* situation that occurs after monocyte isolation. Both IL-1 α and IL-1 β seem to be synthesized as larger precursors that are processed and secreted as the biological active form after stimulation of LPS²⁰⁾.

In this study, we used ELISA to determine LPS-stimulated IL-1 α and IL-1 β production in whole-blood cultures from patients with urological cancer. The LPS-stimulated IL-1 α production in patients with bladder cancer and renal cell carcinoma tended to be lower than control subjects and IL-1 β production in patients with bladder cancer, renal cell carcinoma and prostatic cancer tended to be higher than control subjects, but the differences showed no significance between each cancer patient group and control subjects. It is speculated that blood cells of patients with urological cancer show no apparent impaired ability in the process of synthesis or secretion of IL-1 α and IL-1 β compared to control subjects. The measurement of LPS-stimulated IL-1 α and IL-1 β production seems to be of little clinical value.

We also found that the production of IL-1 α and IL-1 β from patients with urological cancer and control subjects was highly dependent upon the concentration of LPS added to the culture medium. IL-1 β was predominant in culture supernatants after LPS stimulation. This result is in agreement with the report of Lonnemann²¹⁾, who found IL-1 β predominant in extracellular fluid.

In conclusion, there was no significant spontaneous production of IL-1 α and IL-1 β in patients with

urological cancer. After stimulation with LPS, the blood cultures of patients with urological cancer and control subjects produced IL-1 α and IL-1 β in a dose-dependent manner. IL-1 β was predominant in culture supernatants after LPS stimulation. The patients with urological cancer showed no significant impairment in ability to produce IL-1 α and IL-1 β in response to LPS stimulation.

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和文抄録

泌尿器科癌患者末梢血における Interleukin- 1α , Interleukin- 1β 産生能の検討

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泌尿器科癌患者における病態把握, 免疫学的解析のために, 末梢血による IL- 1α , IL- 1β 産生能を検討した. 対象は60例の泌尿器科癌患者. その内訳は膀胱癌30例, 腎癌12例, 前立腺癌18例であった. また, 対照群は健常者16例. 測定方法はヘパリン採血した全血0.1 ml ずつを, 6段階 (0, 0.1, 0.3, 1, 3, 5 $\mu\text{g}/\text{ml}$) の濃度のリポポリサッカライド添加無血清培地にて24時間培養し, その上清を IL- 1α , IL- 1β に対するモノクローナル抗体を用いた ELISA 法にて測定した.

リポポリサッカライドを含まない条件での全血1 ml あたりの IL- 1α , IL- 1β 産生量は, 膀胱癌患者,

腎癌患者, 前立腺癌患者では健常者と比較して有意差を認めなかった. 次に5段階濃度のリポポリサッカライドを用いた場合, 膀胱癌患者, 腎癌患者, 前立腺癌患者, 健常者における IL- 1α , IL- 1β 産生量は dose response を示した. また, IL- 1β の産生量は IL- 1α より高値であった. しかし, 膀胱癌患者, 腎癌患者, 前立腺癌患者と対照群の間での IL- 1α , IL- 1β 産生量は有意差を認めなかった. 以上の結果より, 泌尿器科癌患者における IL- 1α , IL- 1β 産生細胞の機能は障害されていないことが示唆された.

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