<table>
<thead>
<tr>
<th>Title</th>
<th>Interleukin-1 alpha and interleukin-1 beta production in peripheral whole blood from patients with urological cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Author(s)</td>
<td>KUO, junne-Yih; OHMOTO, Yasukazu; YOSHIDA, Osamu</td>
</tr>
<tr>
<td>Citation</td>
<td>泌尿器科紀要 (1998), 44(6): 397-402</td>
</tr>
<tr>
<td>Issue Date</td>
<td>1998-06</td>
</tr>
<tr>
<td>URL</td>
<td><a href="http://hdl.handle.net/2433/116199">http://hdl.handle.net/2433/116199</a></td>
</tr>
<tr>
<td>Right</td>
<td>Type</td>
</tr>
</tbody>
</table>
| Textversion | publisher |京都大学
INTERLEUKIN-1α AND INTERLEUKIN-1β PRODUCTION
IN PERIPHERAL WHOLE BLOOD FROM PATIENTS
WITH UROLOGICAL CANCER

Junne-Yih Kuo
From the Department of Urology, National Yang-Ming University and Division of Urology,
Department of Surgery, Veterans General Hospital-Taipei, Taiwan, Republic of China

Yasukazu Ohmoto
From the Laboratories of Cellular Technology, Otsuka Pharmaceutical Co. Ltd., Tokushima

Osamu Yoshida
From the Department of Urology, Faculty of Medicine, Kyoto University, Kyoto

The activities of interleukin-1α (IL-1α) and interleukin-1β (IL-1β) 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-1α and IL-1β were determined by enzyme-linked immunosorbent assay (ELISA). In the absence of LPS stimulation, neither IL-1α nor IL-1β 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-1α and IL-1β in a dose-dependent manner, and IL-1β was predominant in all supernatants. The activities of IL-1α and IL-1β 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-1α or IL-1β, but that the ability to produce IL-1α and IL-1β in response to LPS stimulation was not significantly impaired.

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

INTRODUCTION

The abnormalities of immune response in the patients with urological cancer have been reported. The demonstration of immune reactivity in such patients has lead to a considerable clinical significance. 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 tissues. Recent advances in recombinant DNA technology have made human IL-1 available in highly purified form. There are two biochemically different forms, IL-1α and IL-1β, which appear to share similar biological activities.

In the literature, some investigators have described spontaneous IL-1 production in a considerable portion of leukemia patients and abnormal IL-1 production in peripheral blood mononuclear cells from patients with malignant tumor following stimulation by bacterial lipopolysaccharide (LPS). 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-1α and IL-1β activities and were easily interfered by various substances, such as IL-2, mitogens and growth factors in samples. To examine the immune responses of the patients with urological cancer, we used enzyme-linked immunosorbent assay (ELISA) to determine spontaneous production and LPS-stimulated IL-1α and IL-1β 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 µg/ml) of
0.01 µl 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-1α and IL-1β assay.

Determine activities of IL-1α and IL-1β
The culture supernatants were measured for their
IL-1α and IL-1β activities with ELISA as previously
described. These assays used human IL-1α and
IL-1β ELISA kits (Otsuka Pharmaceutical Co.,
Ltd.). Briefly, microplates were coated with anti-
IL-1α monoclonal antibody in 100 µl/well of PBS, pH
7. Following overnight incubation at 4°C, the wells
were blocked with 1% skim milk in PBS at least 1
hour at room temperature and washed three times
with PBS containing 0.05% Tween-20 (TWEEN-PBS).
Samples or standard IL-1α (100 µl) 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 µl of rabbit
anti-IL-1α antibodies were added to each well and
incubated for 2 hours at 23°C. Subsequently, the
plates were washed, supplemented with 100 µl of
POD-labeled goat anti-rabbit IgG and incubated at
23°C for 2 hours. Finally, 100 µl 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 µl 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-1α, and does not measure other
cytokines. The assay of IL-1β was also measured by
IL-1β ELISA kits (Otsuka Pharmaceutical Co.,
Ltd.). These assays could reliably detect as little as
0.3 ng/ml blood of IL-1α and 0.6 ng/ml blood of IL-
1β.

Statistical analysis
All determinations were done in duplicate. The
results were expressed as mean (ng/ml blood) ± 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-1α and IL-1β production
The kinetics of IL-1α and IL-1β production in
whole-blood cultures in the presence of 5 µg/ml
concentration of 0.01 µl LPS was examined in one
healthy individual. The culture supernatants were
measured for their IL-1α and IL-1β activities with
ELISA after 4, 8, 19, 24, 28, 48, 72, 96 and 120 hours'
incubation. The results are shown in Fig. 1. IL-1α
and IL-1β 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-1α and IL-1β production was measured in
the culture supernatants harvested after a 24-hour
incubation period.

Spontaneous production of IL-1α and IL-1β in
patients with urological cancer and control subjects
As shown in Table 1, in the absence of LPS
stimulation, small amounts of IL-1α were detected in
2 out of 30 (6.6%) patients with bladder cancer (0.75
±0.11 ng/ml blood) and 2 out of 18 (11%) patients
with prostatic cancer (0.76±0.14 ng/ml blood),
respectively. IL-1α was not detected in the patients
with renal cell carcinoma and the control subjects.
In contrast, small amounts of IL-1β were detected in
5 out of 30 (16.6%) patients with bladder cancer (3.11
ng/ml blood) and 2 out of 18 (11%) patients with prostatic cancer (0.76±0.14 ng/ml blood). The
results were expressed as mean (ng/ml blood) ± SD.
Statistical analysis was determined by Student's test
and chi-square test. P values of <0.05 were
considered to indicate significance.

Fig. 1. The kinetics of IL-1α and IL-1β
production in whole-blood cultures under the stimulation of 5 µg/ml
concentration of 0.01 µl LPS in one
healthy individual. The culture supernatants were measured for their IL-1α
and IL-1β activities with ELISA after
different incubation times. IL-1α and
IL-1β 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-1α and IL-1β in patients with urological cancer and control subjects

<table>
<thead>
<tr>
<th>Subjects (n)</th>
<th>Spontaneous IL-1α elaboration</th>
<th>Spontaneous IL-1β elaboration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of positive patients (%)</td>
<td>Activity of IL-1α (ng/ml blood±SD)</td>
</tr>
<tr>
<td>Bladder cancer (30)</td>
<td>2 (6.6%)</td>
<td>0.75±0.11</td>
</tr>
<tr>
<td>Renal cell carcinoma (12)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Prostatic cancer (18)</td>
<td>2 (11%)</td>
<td>0.76±0.14</td>
</tr>
<tr>
<td>Controls (16)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Subjects (n)</th>
<th>Concentrations of LPS added (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.1</td>
</tr>
<tr>
<td>Bladder cancer (30)</td>
<td>0.98±0.81</td>
</tr>
<tr>
<td>Renal cell carcinoma (12)</td>
<td>0.85±0.40</td>
</tr>
<tr>
<td>Prostatic cancer (18)</td>
<td>1.83±1.29</td>
</tr>
<tr>
<td>Controls (16)</td>
<td>1.15±0.70</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Subjects (n)</th>
<th>Concentrations of LPS added (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.1</td>
</tr>
<tr>
<td>Bladder cancer (30)</td>
<td>17.3±13.0</td>
</tr>
<tr>
<td>Renal cell carcinoma (12)</td>
<td>14.5±7.7</td>
</tr>
<tr>
<td>Prostatic cancer (18)</td>
<td>17.6±10.5</td>
</tr>
<tr>
<td>Controls (16)</td>
<td>14.0±7.1</td>
</tr>
</tbody>
</table>

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

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

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

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

LPS-stimulated IL-1α 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-1β 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-1α and IL-1β 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 patients6,7).

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-1α and IL-1β in the conditioned medium12). In this study, in all unstimulated culture supernatants the activity of IL-1α 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-1β 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-1α and IL-1β elaboration were caused by contamination. Our findings suggested that the peripheral whole-blood cultures from patients with urological cancer did not spontaneously produce IL-1α or IL-1β 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 growth13,14). 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-1α and IL-1β. In order to determine the immune status of patients with urological cancer, we examined the effects of various concentrations of LPS on IL-1α and IL-1β 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 secret IL-1 under the LPS stimulation16,17). Recently, the measurement of IL-1 production in whole-blood cultures has also developed18,19). 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-1α and IL-1β 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-1α and IL-1β production in whole-blood cultures from patients with urological cancer. The LPS-stimulated IL-1α production in patients with bladder cancer and renal cell carcinoma tended to be lower than control subjects and IL-1β production in patients with bladder cancer, renal cell carcinoma and prostatic cancer tended to be higher than 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-1α and IL-1β compared to control subjects. The measurement of LPS-stimulated IL-1α and IL-1β production seems to be of little clinical value.

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

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

ACKNOWLEDGMENTS

The authors wish to express their gratitude to Miss Higuchi for expert technical assistance and our colleagues for allowing us to collect blood from their patients.

REFERENCES

5) Oppenheim JJ, Kovacs EJ, Matsushima K, et al.: There is more than one interleukin 1. Immunol Today 7: 45-56, 1986

(Received on October 17, 1997)
(Accepted on April 8, 1998)
泌尿器科癌患者末梢血における Interleukin-1α，Interleukin-1β 産生能の検討

国立陽明大学医学部泌尿器科学科，台北榮民總醫院外科部泌尿外科（主任：陳 光國教授）
郭 俊 逸
大塚製藥株式会社細胞工学研究所
大 本 安一
京都大学医学部泌尿器科学教室（主任：吉田 修教授）
吉 田 修

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，对照群は健常者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 μg/ml)の濃度のリポリサッカリ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。

リポリサッカリ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段階濃度のリポリサッカリドを用いた場合，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 産生細胞の機能は悪化されていないことが示唆された。

（泌尿紀要 44：397-402，1998）