Biological activity of human urine IL-1 inhibitor

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[Summary]

Properties of IL-1 inhibitor present in the urine of febrile patients were examined. The molecular weight of IL-1 inhibitor was estimated to be about 21 KD by high pressure liquid chromatography (HPLC). IL-1 inhibitor was inactivated by incubation at 56°C for 30 min or treatment with acid at pH 3.0, but its activity was not affected at pH 5-11. It was also unaffected by treatment with neuraminidase.

IL-1 inhibitor suppressed not only thymocyte proliferation induced by IL-1 but also in vitro plaque forming cell (PFC) formation and the development of fever after intraperitoneal administration of IL-1 in mice. These data suggest that IL-1 inhibitor acts not only in vitro but also in vivo.

Key words: interleukin-1 (IL-1), IL-1 inhibitor

【概要】

発熱患者の尿中に存在する IL-1 インヒビターの性状を検討した。high pressure liquid chromatography (HPLC) により、分子量は約 21,000 と見積もり、IL-1 インヒビターは、56°C で 30 分間の熱処理および
IL-1 インヒビターは、IL-1 刺激によるマウス胸腺細胞の増殖を抑制するだけでなく、IL-1 刺激により誘導される in vitro 抗体産生細胞の出現を抑制した。また IL-1 インヒビターは、IL-1 のマウスへの投与により誘導される発熱を抑制した。この成績は、IL-1 インヒビターは in vitro だけでなく in vivo においても作用することを示している。

I. Introduction

IL-1 is a cytokine produced primarily by macrophages. It is known to play an important role as a mediator of the body's defense against invading foreign bodies. In the immune response, IL-1 stimulates, an activity defined as lymphocyte activating factor (LAF) and enhances immune responses. It also affects non-immunocompetent cells. For example, it is involved in inflammation and tissue repair that follows by affecting cells related to connective tissue such as fibroblasts, synovial cells, and chondrocytes. IL-1 also has effects on the central nervous system: it stimulates hypothalamus, inducing fever via prostaglandin E₂ (PGE₂) and affects the hypophysis, promoting the secretion of ACTH. IL-1 also stimulates synthesis of acute phase proteins by hepatocytes, and accelerates breakdown of muscles cell proteins.

A number of recent reports suggested that IL-1 was released also by cells other than macrophages such as epidermal cells, and neutrophiles. The facts that IL-1 affects a variety of tissues and that it is derived from different cell types are consistent with its role as a major mediator of inflammation and immune responses. One important issue is how the actions of IL-1 are regulated. Recent studies revealed the presence of IL-1 inhibitor in the urine of febrile patients, but properties of IL-1 inhibitor have yet to be elucidated. Moreover, although inhibition of IL-1-induced thymocyte proliferation by IL-1 inhibitor has been investigated extensively, much remains to be clarified about other biological activities of IL-1. In this study, we examined the properties of IL-1 inhibitor and evaluated its effects on enhancement of in vitro plaque forming cell (PFC) formation and elevation of body temperature.

II. Materials and methods

1. Animals

Female C3 H/HeJ mice between the ages of 4 and 8 weeks and male Wistar strain rats between the ages of 6 and 7 weeks were used.

2. Urine and its gel filtration

Urine of patients with various diseases presenting fever of 38°C or higher was collected before initiation of treatments with antibiotics, non-glucocorticoid anti-inflammatory drugs, or steroids. The urine samples were centrifuged at 3,000 rpm for 10 min, and the supernatant was dialyzed against phosphate buffered saline (PBS), then against RPMI 1640, and sterilized by filtration through a 0.45 μm membrane filter.

For purification of IL-1 inhibitor or determination of molecular weight of IL-1 inhibitor, urine containing IL-1 inhibitor was concentrated about 200 times by ultrafiltration using an UF membrane filter (m.w. 5,000 cut off). Then, this concentrate was subjected to gel filtration using a 2.5×80 cm Sephacryl S-200 column or high pressure liquid chromatography (HPLC) using 50 mM Tris, 100 mM NaCl, pH 7.5 as the eluent. The IL-1 inhibitory activity of each fraction was determined by means of proliferation of C3 H/HeJ mice thymocytes, and fractions with IL-1 inhibitory activity were used as partially purified IL-1 inhibitor.

3. Treatment of IL-1 inhibitor (heat, neuraminidase, pH)

For treatment of IL-1 inhibitor with heat or neuraminidase, urine containing IL-1 inhibitor was incubated at 56°C for 30 min or incubated with 0.1 U/ml neuraminidase at 37°C for 15 min. For treat-
ment of IL-1 inhibitor with acids and alkalis, urine
containing IL-1 inhibitor was dialyzed against
RPMI 1640 adjusted to pH 3.0, 5.0, 7.0, 9.0, and
11.0 for 2 hr, then against RPMI 1640, pH 7.4, and
sterilized by filtration through 0.45 μm membrane
filter.

4. IL-1
Exdate cells collected from the peritoneal cavity
of Wistar strain rats after an injection of 3% thio-
glycollate were suspended in RPMI medium contain-
ing 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml
streptomycin, 10⁻⁸ M 2-mercaptoethanol, and
10% fetal bovine serum (FBS) (complete RPMI) to
a cell concentration of 10⁶/ml. This cell suspen-
sion was incubated in a culture flask at 37°C in 5% CO₂
for 4 hr, and non-adherent cells were removed.
The same volume of complete RPMI containing 20
μg/ml lipopolysaccharide (LPS) was added, and
the cells were incubated again at 37°C in 5% CO₂
for 48 hr. After incubation, the supernatant was
centrifuged at 10,000 G to remove cell components,
dialyzed against RPMI 1640, and filtered through a
0.45 μm membrane filter. It was used as rat IL-1.

IL-1 derived from P 388 D₁ cell line was obtained
as follows. The mouse macrophage cell line P 388
D₁ was suspended in complete RPMI containing 1
μg/ml of phorbol myristic acetate at a cell
concentration of 2 × 10⁶/ml, and cultured at 37°C in
5% CO₂ for 5 days. The culture supernatant was
centrifuged at 10,000 G for 10 min, dialyzed against
RPMI 1640, and filtered through a 0.45 μm mem-
brane filter.

5. Mouse thymocyte assay
The IL-1 activity was measured by C3H/HeJ
thymocyte proliferation. Thymuses were obtained
from mice after cervical dislocation, minced, filter-
ed through a 100 μm stainless mesh, washed 3 times
with Hanks’ balanced salt solution (HBSS), and
suspended in complete RPMI at a cell concentra-
tion of 10⁶/ml. Thymocytes were cultured for 72 hr at
10⁶ cells/well in flat-bottomed 96-well Nunc plates
in the presence or absence of 1~2 μg/ml of phyto-
hemagglutinin (PHA-P, Wellcome, Partford,
England), 50 μl of 16 hold diluted rat IL-1, and various
dilutions of urine or partially purified IL-1 inhibitor.
The final volume in each well was 0.2 ml. The
cultures were done in triplicate and were pulsed
with 0.5 μCi per well of ³H-thymidine for the last
8 hr of the 72 hr incubation. The cells were harvest-
ed and were processed for scintillation counting.
The data are expressed as counts per minute (cpm).

The percent inhibition of thymocyte proliferation
was calculated by the following formula:

\[
% \text{ inhibition} = 1 - \left( \frac{\text{cpm} \ ³H\text{-thymidine in the presence of IL-1+inhibitor}}{\text{cpm} \ ³H\text{-thymidine in the presence of IL-1+media}} \right) \times 100
\]

As shown in Fig. 1, Probit-transformed % inhibi-
tions were plotted against log dilution according to
probit analysis\(^{(1)}\). The magnification of dilution of
Fig. 2 Quantification of IL-1 inhibitor activity in the urine of febrile and afebrile stages of patients

Urine was dialyzed, sterilized, and tested for inhibitory activity in the presence of rat IL-1 diluted at 1:16. The inhibitory activity of each sample was calculated using the formula described in the materials and methods section.

urine that showed 50% inhibition was calculated and arbitrarily assigned as units.

6. Plaque forming cells (PFC) induced by IL-1 in vitro

Ninety-five µl of 2.5×10⁶/ml of spleen cells from C3 H/HeJ mice were cultured for 5 days in flat-bottomed, 96-well NUNC plates with 5 µl of 30% sheep red blood cell (SRBC) in the presence or absence of rat IL-1 and partially purified IL-1 inhibitor.

Fig. 3 Quantification of IL-1 inhibitor activity in the urine of normal controls, afebrile patients and febrile patients

After incubation, cells in each well were harvested, washed twice with HBSS containing 2.5% FBS, and suspended in complete RPMI 1640 to a concentration of 2.5×10⁶ cells/ml. One hundred microliters of this cell suspension was mixed with 50 µl of 30% SRBC, 50 µl rabbit complement, and 50 µl RPMI 1640, and 100 µl of the mixture was incubated in Cunningham chamber at 37°C for 60 min. The number of PFCs observed in the chamber was counted.

7. Measurement of body temperature in mice

The rectal temperature of C3 H/HeJ mice was determined using a thermometer.

III. Results

1. IL-1 inhibitor activity in the urine of febrile patients

The urinary IL-1 inhibitor activity was determined in 8 patients (2 with pneumonia, 4 with lung
cancer, 1 with pulmonary tuberculosis, and 1 with infection of the urinary tract) during the active stage of the diseases, when clinical symptoms such as fever were conspicuous, and during remission, when clinical symptoms had disappeared and laboratory data had been normalized (Fig. 2). The IL-1 inhibitor activity was elevated during the active phase but was reduced or undetected during remission.

The IL-1 inhibitor activity was determined also in normal individuals, afebrile patients and febrile patients (Fig. 3). IL-1 inhibitor activity was not detected in most of healthy individuals and afebrile patients, but low IL-1 inhibitor activity was detected in some healthy individuals and some afebrile patients. High IL-1 inhibitor activity was detected in febrile patients.

Urine of febrile patients containing IL-1 inhibitor was concentrated and fractionated by gel filtration using HPLC after calibration with molecular weight markers. The IL-1 inhibitor activity was determined in each fraction according to the degree of inhibition of thymocyte proliferation in the presence of IL-1, and the molecular weight of IL-1 inhibitor was estimated. The IL-1 inhibitor activity was observed in fractions of 21 KD separated with HPLC (Fig. 4).

Fig. 4  IL-1 inhibitor activity of urine fractionated by molecular sieve chromatography on HPLC

Febrile urine was dialyzed, concentrated (200-fold) by ultrafiltration and 3 ml was applied to the column. Effluent fractions were dialyzed, sterilized, and tested for inhibitory activity on thymocyte proliferation in the presence of rat IL-1. Percent inhibition was calculated according to the formula shown in the text.
2. Effects of heat, neuraminidase treatment, and pH on the IL-1 inhibitor activity

The IL-1 inhibitor activity on IL-1 induced thymocyte proliferation was lost after incubation at 56°C for 30 min (Table 1), but it was not lost after treatment with 0.1 U/ml neuraminidase for 1 hr (data not shown). No changes were observed in the inhibitor activity at pH 5.0~11.0, but the activity was lost after treatment at pH 3 (data not shown).

3. Effects of IL-1 inhibitor on in vitro plaque forming cell count in the presence of IL-1

When mouse spleen cells were cultured with SRBC in the presence of IL-1 for 4~6 days, PFC formation was enhanced, and the peak PFC count was observed 5 days after the beginning of culture (data not shown). When IL-1 inhibitor was added to this system, it suppressed in vitro PFC formation in the presence of IL-1 (Table 2).

4. Effects of IL-1 inhibitor on pyrogenic action of IL-1

Whether IL-1 inhibitor suppresses elevation of

<table>
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<th>Table 2 Effect of IL-1 inhibitor on IL-1 induced PFC in vitro</th>
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<td>Rat IL-1 (μl/well)</td>
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*: Urine contained IL-1 inhibitor
**: After spleen cells were cultured with SRBC for 5 days, cells were harvested, mixed with SRBC and rabbit complements, put into Cunningham chamber, incubated for 60 min at 37°C and number of PFC was counted.
***: This represents the mean and standard deviation (S.D.) of triplicate cultures.
****: Statistical significance (p<0.001) was noted as compared with the culture without urine contained IL-1 inhibitor by student's T test.

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<th>Table 3 Change of temperature after injection of IL-1 inhibitor into C3H/Hej mice i.p.</th>
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<td>Time after injection of IL-1 (hr)</td>
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<td>Saline (1.0 ml) (control)</td>
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<td>IL-1 (1.0 ml)**</td>
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<td>IL-1 (0.5 ml)</td>
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<td>Urine (0.5 ml)****</td>
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<td>Urine (0.5 ml)</td>
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<td>Media (0.5 ml)</td>
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*: This represents the mean and S.D. of degree Celsius changed after injection of urine which contain IL-1 inhibitor.
**: P388D1-derived IL-1 (see text).
***: Statistical significance (p<0.05) was noted as compared with control by student's T test.
****: Ten-fold concentrated urine which contain IL-1 inhibitor.
body temperature induced by IL-1 was examined. When IL-1 (1.0 or 0.5 ml) derived from P 388 D1 cell line was injected intraperitoneally in C 3 H/He mice, increases in body temperature were noted after 1 and 2 hours (Table 3). However, the body temperature did not rise when IL-1 inhibitor was administered simultaneously with IL-1, with no significant difference as compared with the animals treated with saline.

IV. Discussion

Our results showed that IL-1 inhibitor is detected in small amounts in the urine of some normal individuals, and that it increases during febrile episodes and decreases during afebrile periods in patients with various diseases (Fig. 2 and 3). The effects of drugs on the IL-1 activity can be excluded, because only the urine collected before initiation of treatments with drugs such as antibiotics, non-glucocorticoid anti-inflammatory drugs or steroids were used for the analysis in this study.

We estimated the molecular weight of IL-1 inhibitor in the urine of febrile patients at 21,000, being in agreement with the previous report10). Epstein-Barr (EB) virus transformed B lymphoid cell line have been shown to produce a 95,000 molecular weight IL-1 inhibitor12). There are also a report of immunosuppressive material called uromodulin in the urine of pregnant women13), a report of 3,000~5,000 and 99,000 molecular weights IL-1 inhibitors produced from macrophages14), and a report of a 45,000~70,000 molecular weight IL-1 inhibitor derived from neutrophils15). In view of the differences in the molecular weight, these IL-1 inhibitors are considered to be different substances. The relationship between urinary inhibitors and cell-derived inhibitors is unknown.

We found that the activity of IL-1 inhibitor in the urine of febrile patients was lost after heating at 56°C for 30 min or treatment at pH 3, but was not affected by treatment with neuraminidase. Therefore, the sialylated carbohydrate structure appears to be unrelated to this IL-1 inhibitor activity.

The auxiliary ability of macrophages to produce antibodies is reported to be substituted by IL-115). Antibody production by nude mouse spleen cells and spleen cells depleted T-cells is also reportedly enhanced by IL-116). These observations suggest that IL-1 also directly activates B-cells.

To examine whether the promotive effect of IL-1 on antibody production is suppressed by IL-1 inhibitor, we primed cells with SRBC and induced PFC formation with IL-1 in the presence or absence of IL-1 inhibitor. In this experiment, stimulative effect of IL-1 on PFC formation was reduced by the addition of IL-1 inhibitor (Table 1). This finding suggests the possibility that IL-1 inhibitor blocks the action of IL-1 on antibody production in vitro.

IL-1, secreted from cells such as macrophages during infection and inflammation, is considered to stimulate production of prostaglandin E2 (PGE2), which causes elevation of body temperature17). Higher body temperature with associated activation of immunocompetent cells is considered to be advantageous for the defense of the body. Fever, however, means increased load to the body, and excessive rise in body temperature may be harmful to the body. When IL-1 inhibitor was administered with IL-1 to C 3 H/He mice, the elevation of body temperature by IL-1 was suppressed (Table 2). This finding, suggesting that IL-1 inhibitor suppresses biological activities of IL-1 in vivo as well as in vitro, may provide additional evidence that IL-1 inhibitor is involved in the feedback mechanism of the body that regulated the actions of IL-1.

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References

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