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INDUCTION OF HISTIDINE AND ORNITHINE DECARBOXYLASE ACTIVITIES IN MOUSE TISSUES BY RECOMBINANT INTERLEUKIN-1 AND TUMOR NECROSIS FACTOR

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Abstract—The injection of recombinant interleukin-1 (IL-1) into mice induced histidine decarboxylase (HDC) activity in the bone marrow, spleen, lung and liver and ornithine decarboxylase (ODC) activity in the spleen and liver. The ability of IL-1 to induce these responses was the most potent of the various cytokines tested. The induction of these responses by IL-1 seemed to be more rapid than that produced by a lipopolysaccharide. The potency of IL-1α to induce both HDC and ODC activities was similar to that of IL-1β, and their combination did not potentiate the induction of these responses. In contrast, although the ability of recombinant tumor necrosis factor-α (TNFα) to induce these responses was less potent than that of IL-1α or IL-1β, the combination of TNFα and IL-1β produced higher HDC and ODC activities in some tissues tested than those induced by the combination of IL-1α and IL-1β. These results suggest that the syntheses of histamine and putrescine are regulated by IL-1 and/or TNFα in inflammatory or immune responses. Through these experiments, it was noticed that, in spite of a marked induction of HDC activity in the bone marrow, there was no detectable induction of ODC activity in this tissue. The meaning of HDC induction in the bone marrow is discussed.

Histamine formation is catalyzed by histidine decarboxylase (HDC†). HDC activity is induced markedly in tissues rich in macrophages, such as spleen, lung and liver, after the injection of various inflammatory or mitogenic substances into mice [1–3]. The most potent inducer of HDC activity tested thus far is a lipopolysaccharide (LPS), a potent stimulator of macrophages [2]. LPS can induce HDC activity in nude mice (T cell deficient) [2] and W/W^v mice (mast cell deficient) (unpublished data). C3H/HeJ mice, the macrophages of which have a low responsiveness to LPS, are much less responsive to LPS during HDC induction [2]. These observations suggest that macrophages may be important in the HDC induction by LPS or other mitogens [2]. LPS is known to stimulate macrophages to produce interleukin-1 (IL-1) and tumor necrosis factor-α (TNFα) [4, 5]. Recently, we found factors capable of inducing HDC activity in the culture medium of a macrophage cell line, P388D1. Although the purification of the factors was not completed, the molecular weight and isoelectric point of one of the factors were identical to those of IL-1 [6].

Ornithine is converted to putrescine by ornithine decarboxylase (ODC). ODC is a rate-limiting enzyme in the synthesis of polyamines which have been implicated as regulators of cellular metabolism, proliferation, differentiation and functions [7]. The mitogenic substances and the IL-1-like factors described above also induce ODC activity in the spleen and liver [1, 8, 9]. In this study, therefore, it was examined whether recombinant IL-1 and recombinant TNFα could induce HDC and ODC activities.

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† Abbreviations: HDC, histidine decarboxylase; ODC, ornithine decarboxylase; IL-1, interleukin-1; IL-2, interleukin-2; IL-3, interleukin-3; TNF, tumor necrosis factor; LPS, lipopolysaccharide; IFN, interferon; GM-CSF, granulocyte-macrophage-colony-stimulating factor; and G-CSF, granulocyte-colony-stimulating factor.

MATERIALS AND METHODS

Materials. Recombinant human interleukin-1α (IL-1α) and recombinant human tumor necrosis factor-α (TNFα) were provided by the Dainippon Pharmaceutical Co. (Osaka, Japan) and recombinant human IL-1β was from the Ohtsuka Pharmaceutical Co. (Tokushima, Japan). The specific activities of IL-1α and IL-1β were 2×10^7 units/mg protein. One unit of IL-1α and IL-1β is defined as the amount of the factors that stimulate half-maximal [^{3}H]thymidine incorporation into mouse thymocytes under the presence of concanavalin A. The specific activity of TNFα was 3×10^6 units/mg protein. One unit of TNFα is the amount that produces half-maximal cytotoxicity against L-M cells. The content of endotoxin in the preparations of IL-1α, IL-1β and TNFα was less than 0.02, 0.1 and 0.04 ng/mg protein respectively (assayed by the Limulus Test Kit). Recombinant human interleukin-2 (IL-2) (2.5×10^6 units/mg protein) was provided by the Shionogi Pharmaceutical Co. (Osaka, Japan). One unit equals the amount of IL-2 required to produce half-maximal incorporation of [^{3}H]thymidine into CTLL-2 cells. Recombinant mouse interferon-γ (IFNγ) (1×10^7 I.U./mg protein) was a gift from Professor K. Kumagai of this school. Recombinant mouse granulocyte-macrophage-colony-stimulating factor (GM-CSF) (5×10^7 units/mg protein) was

Table 1. Induction of HDC and ODC activities by cytokines in the spleen of mice*

Stimulants	Dose (μg/kg)	HDC activity (pmol/hr)	ODC activity (nmol/hr)
Saline	—	4 ± 1	<0.1
IL-2	1500†	7 ± 2	<0.1
IFNγ	1000†	11 ± 1‡	0.21 ± 0.06
GM-CSF	1†	13 ± 2‡	<0.1
TNFα	100	18 ± 3‡	0.40 ± 0.10‡
IL-1α	100	32 ± 2‡	0.75 ± 0.05‡
IL-1β	100	34 ± 5‡	1.0 ± 0.25‡
LPS	100	73 ± 9‡	1.2 ± 0.25‡

* Mice were killed at 4 hr after the injection of each stimulant. Each value is the mean ± SD of three mice.

† Injected intravenously, because intraperitoneal injections were less effective.

‡ $P < 0.05$ vs control mice (saline injected).

purchased from the Genzyme Corp. (Boston, MA, U.S.A.). One unit of GM-CSF equals the amount that produces a single colony from 7.5×10^4 murine bone marrow cells in soft agar in 7 days. A lipopolysaccharide (LPS) derived from *Escherichia coli* 055:B5, prepared by the Boivin method, was obtained from Difco Laboratories (Detroit, MI, U.S.A.). Test samples were diluted in sterilized saline. Male ddY mice were purchased from the Shizuoka Agricultural Association for Laboratory Animals (Shizuoka, Japan).

Assay of HDC and ODC activities. Test samples were injected intraperitoneally, unless stated otherwise, into male ddY mice (6- to 7-weeks-old, 24–27 g, 0.2 to 0.3 ml/mouse). The mice were killed by decapitation at indicated time intervals, and tissues were removed rapidly and kept in a dry-ice box until assayed. Bone marrow cells were obtained from both femur and tibia by pushing saline into the bones and collecting the cells by centrifugation (1500 *g*, 5 min). HDC and ODC activities were assayed simultaneously by fluorometrical determinations of histamine and putrescine formed in the reaction mixture as described previously [10, 11] and expressed as pmol or nmol per hr per mg protein. Protein concentration was measured by the method of Lowry *et al.* [12]. Statistical analysis was made using Student's *t*-test.

RESULTS

Abilities of various cytokines to induce HDC and ODC activities. In addition to macrophages, lymphocytes produce various cytokines. Preliminary experiments showed that a lymphocyte cell line also produces a factor(s) capable of inducing HDC and ODC activities when it was injected into mice [13]. Therefore, the abilities of some lymphokines (IL-2, IFNγ and GM-CSF) to induce HDC and ODC activities were also tested and compared with those of IL-1α, IL-1β and TNFα. Since inductions of HDC and ODC activities were highly sensitive in spleen, the effects of these cytokines in the spleen are shown in Table 1. IL-2 was not effective even at a high dose. IFNγ induced significant HDC activity only at a high dose, while GM-CSF induced significant HDC activity at a low dose (1 μg/kg, intravenous injection). GM-CSF also induced HDC activity in the bone marrow, liver and lung at this dose (2 to 3 times higher activities than those in control mice). The experiment using higher doses of GM-CSF is too expensive to carry out extensively at present. On the one hand, the abilities of IL-1α, IL-1β and TNFα were potent in the induction of both HDC and ODC activities. Although LPS was the most potent in ability, the injection of 1 ng/kg of LPS (more than a 10 times higher amount contained in 1 mg of the preparations of these cytokines; see *Materials*) did not induce HDC or ODC activity in various tissues tested (bone marrow, spleen, lung and liver), indicating that the effects of these cytokines are not due to the endotoxin or LPS that is contained in the preparations.

Induction of HDC and ODC activity by IL-1 and TNFα. Time courses of the HDC induction by IL-1β, TNFα and LPS were very similar to each other (Fig. 1). However, in spite of lower maximal HDC inductions by IL-1β, significant HDC inductions ($P < 0.05$) occurred at 1 hr in the liver, spleen and lung by IL-1β but not by LPS, suggesting that the HDC induction by IL-1β in these tissues is more rapid than that by LPS.

HDC activities induced at 4 hr after the injection of various doses of IL-1β and TNFα are shown in Fig. 2. In the spleen and bone marrow, as little as 0.1 μg/kg of IL-1β produced a significant ($P < 0.05$) increase in HDC activity. On the other hand, inductions of HDC activity were far less sensitive to TNFα than to IL-1β in all tissues tested.

Since it is known that LPS is a potent stimulator of macrophages to produce IL-1 and TNFα, the effect of the combination of IL-1α and IL-1β or the combination of IL-1β and TNFα was examined (Fig. 3). The potency of IL-1α (molecular weight: 17,100) to induce HDC activity was similar to that of IL-1β (molecular weight: 18,100), and their combination with the same amount produced no potentiation of HDC induction. In contrast, although the ability of TNFα (molecular weight: 17,400) was less potent than IL-1α and IL-1β, the combination of TNFα with IL-1β produced higher HDC activities than those induced by the combination of IL-1α and IL-1β.

It should be noted that the HDC activity in the bone marrow was very high even in control mice.

Induction of ODC activity by IL-1 and TNFα. In addition to HDC activity, macrophage products (IL-1α, IL-1β and TNFα) induced ODC activity in the liver and spleen (Table 1, Fig. 4). Time courses of the ODC induction by IL-1β, TNFα and LPS are shown in Fig. 4, top panels. In this experiment, the rise of ODC activity induced by IL-1β in the liver was more rapid than that induced by LPS. The ODC inductions in the liver and spleen by IL-1β were more transient than those by LPS.

ODC activities were induced by IL-1β and TNFα in a dose-dependent manner (Fig. 4, bottom panels). The ability of TNFα to induce ODC activity was less potent than that of IL-1β at lower doses (1–100 μg/kg), but a higher dose of TNFα (1 mg/kg) produced a marked induction of ODC activity. There was no potentiation of ODC induction in the liver by the

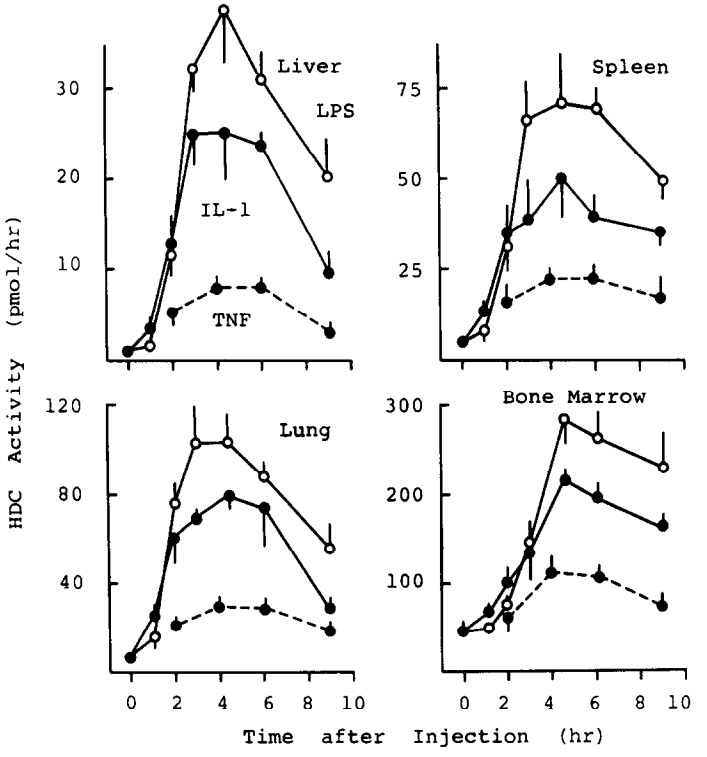

Fig. 1. Time course of the induction of HDC activity by IL-1β, TNFα and LPS. Mice were killed at indicated time intervals after the injection of IL-1β (50 μg/kg), TNFα (100 μg/kg) or LPS (100 μg/kg). Each value is the mean ± SD of three mice.

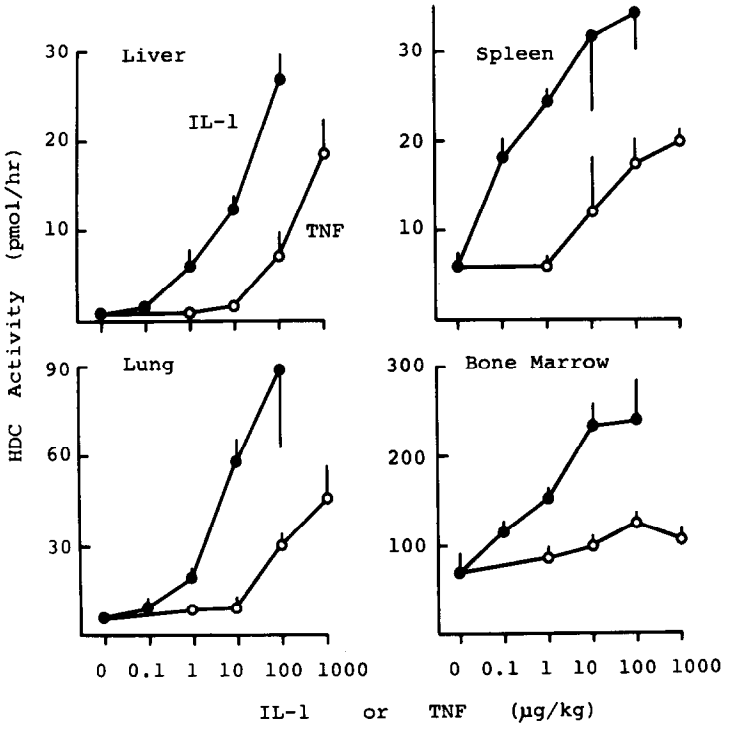

Fig. 2. Dose-dependent induction of HDC activity by IL-1β and TNFα. Mice were killed at 4 hr after the injection of various doses of IL-1β or TNFα. Each value is the mean ± SD of three mice.

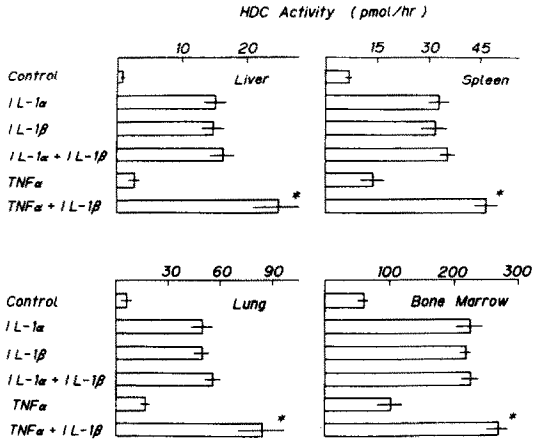

Fig. 3. Effects of combination of cytokines on the induction of HDC activity. Mice were killed at 4 hr after the injection of IL-1α (10 μg/kg), IL-1β (10 μg/kg), TNFα (20 μg/kg), IL-1α plus IL-1β (each 10 μg/kg) or TNFα (20 μg/kg) plus IL-1β (10 μg/kg). Each value is the mean ± SD of four mice. Key: * $P < 0.05$ vs IL-1α + IL-1β.

Table 2. Effects of combination of cytokines on the induction of ODC activity*

Treatments	ODC activity (nmol/hr) Spleen	Liver
Saline	<0.1	<0.01
IL-1α	0.75 ± 0.15	0.19 ± 0.04
IL-1β	0.78 ± 0.08	0.18 ± 0.03
IL-1α + IL-1β	0.85 ± 0.10	0.20 ± 0.04
TNFα	0.22 ± 0.05	0.02 ± 0.01
TNFα + IL-1β	1.20 ± 0.10†	0.21 ± 0.03

* ODC activities were determined simultaneously with the HDC activities in the experiment shown in Fig. 3.
† $P < 0.05$ vs IL-1α + IL-1β.

combination of IL-1α and IL-1β nor by the combination of TNFα and IL-1β (Table 2). In the spleen, however, the combination of TNFα with IL-1β produced a higher ODC activity than that produced by the combination of IL-1α and IL-1β.

Through these experiments, it was noticed that, in spite of a high HDC activity in the bone marrow,

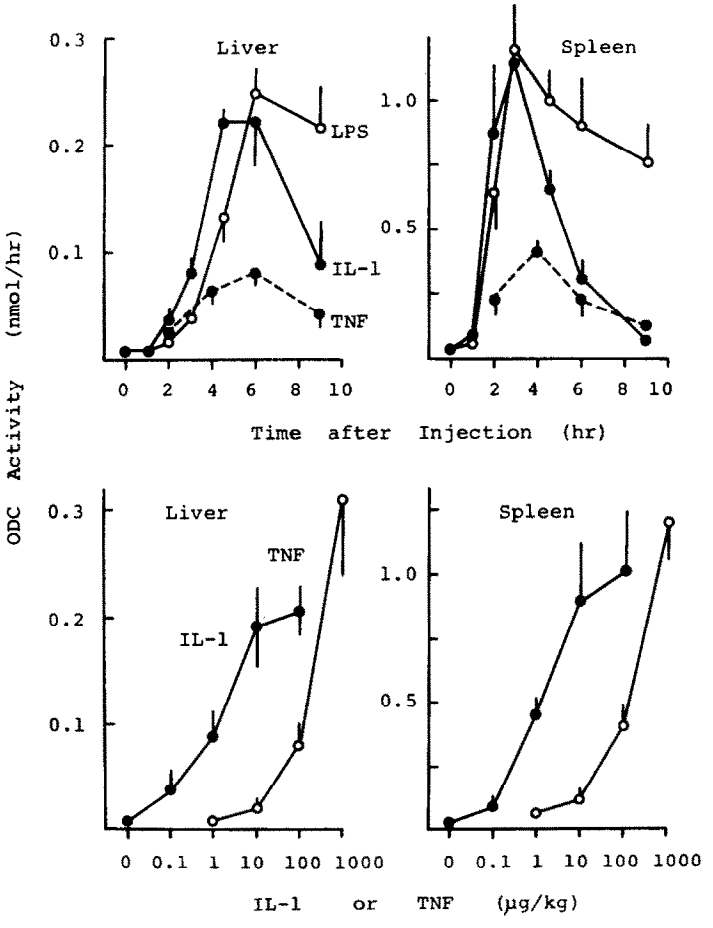

Fig. 4. (Top panels) Time course of the induction of ODC activity by IL-1β, TNFα and LPS. Mice were killed at indicated time intervals after the injection of IL-1β (50 μg/kg), TNFα (100 μg/kg) or LPS (100 μg/kg). Each value is the mean ± SD of three mice. (Bottom panels) Dose-dependent induction of ODC activity by IL-1β and TNFα. Mice were killed at 4 hr after the injection of various doses of IL-1β or TNFα. Each value is the mean ± SD of three mice.

ODC activity in this tissue was below a detectable level (less than 0.1 nmol/hr/mg protein), and there was no detectable induction of ODC activity after the injection of IL-1α, IL-1β, TNFα or even a high dose of LPS (1 mg/kg).

DISCUSSION

In addition to the role of histamine as a mediator in immediate hypersensitivity reactions, histamine has been shown to act as an immunomodulator in various immune systems [6]. Various inflammatory or mitogenic substances can induce HDC activity and produce histamine in mouse tissues rich in macrophages, such as spleen, lung and liver [1, 2]. On the basis of the observations made using nude mice and C3H/HeJ mice, it was suggested that macrophages may have an important role in HDC induction [2]. LPS is a potent inducer of HDC activity in mouse tissues [2] as well as in rat tissues (unpublished data), and LPS is known to stimulate macrophages to produce IL-1 and TNFα [4, 5]. Recently we found that a murine macrophage cell line produces factors capable of inducing HDC activity and that the factors are very similar to IL-1 [6]. In the present study, it was confirmed that both IL-1α and IL-1β have a potent ability to induce HDC activity, whereas IL-2 and IFNγ, products of lymphocytes, were not effective in inducing HDC activity.

In spite of low homology (28%) between amino acid sequences of IL-1α and IL-1β, it is well known that these factors have similar or identical biological activities [14, 15], and the receptors for IL-1α and IL-1β have been shown to be identical [16]. In the present study, IL-1α and IL-1β exhibited similar potencies when inducing HDC activity in various tissues, and the combination of equal amounts of IL-1α and IL-1β did not exhibit any potentiating effect, suggesting that these molecules induced HDC activity by identical or similar mechanisms. In contrast, although the ability of TNFα to induce HDC activity was far less potent than that of IL-1, its combination with IL-1β produced higher HDC activities than those induced by the combination of IL-1α and IL-1β, suggesting that TNFα and IL-1β either act on different cells or act in a synergistic manner. In any case, these results suggest that, in addition to IL-1, TNFα may be involved in the HDC induction in mice treated with LPS: LPS produces a rapid increase in TNFα activity *in vivo* within 20 min after its injection into rabbits [17].

Macrophage products (IL-1α, IL-1β and TNFα) also induced ODC activity, in addition to HDC activity, in the liver and spleen. It is well known that ODC is induced in response to various stimuli for cell growth or cell functions [7]. IL-1 stimulates proliferation of various cell types such as endothelial cells, epithelial cells, fibroblasts, B-cells and astroglial cells [15, 18] and stimulates hepatocytes to produce various proteins [15]. Although it remains to be clarified in what kind of cells ODC is induced by IL-1, the ODC induction shown in this study may relate to stimulation of cell growth or cell functions. On the other hand, TNFα is known to suppress growth of various tumor cells. Therefore, it was expected that TNFα might suppress the ODC activity induced by IL-1. However, TNFα itself induced ODC activity and produced a higher ODC activity in the spleen by the combination with IL-1β. Recently it was shown that TNFα has proliferative actions on various cells [19] and it was suggested that the stimulation of cell growth may be a physiological function of TNFα [20]. The present results obtained from *in vivo* experiments may support this idea.

In normal mice, HDC activity in the bone marrow was the highest of various tissues tested, and the activity was further enhanced by IL-1 and LPS. In contrast, although bone marrow is a rapidly proliferating tissue, its ODC activity was below a detectable level and neither IL-1 nor LPS induced any detectable ODC activity. Such a low ODC activity, at a time when there is a high rate of cell proliferation, was also pointed out by Rath and Reddi [21, 22], although the reason is not clear. On the one hand, IL-3, a hematopoietic factor produced by T-cells, has been shown to stimulate histamine formation in bone marrow cells *in vitro* [23]. Recently it was reported that stromal cells obtained from bone marrow produce colony-stimulating factors (GM-CSF and G-CSF) in response to LPS and IL-1 [24]. In the present study it was shown that a low dose of GM-CSF could induce HDC activity in the bone marrow. Since it has been suggested that newly formed histamine could be involved in the proliferation of various cells as reviewed in Ref. 25, these observations, taken together, suggest that induction of HDC activity, but not ODC activity, is an early and important biochemical event involved in hematopoiesis in bone marrow.

In conclusion, the present results indicate that inductions of HDC and ODC activities or syntheses of histamine and putrescine are regulated by IL-1 and/or TNFα in inflammatory or immune responses.

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