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INDUCTION OF HISTIDINE DECARBOXYLASE IN MOUSE TISSUES BY MITOGENS *IN VIVO*

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Abstract—Various types of mitogenic substances, such as a *Escherichia coli* lipopolysaccharide (LPS), concanavalin A (Con A), pokeweed mitogen, polyI:polyC (a synthetic double-stranded RNA) and 12-*O*-tetradecanoylphorbol-13-acetate (a component of croton oil), induced histidine decarboxylase (HDC) in the liver, spleen and lung of mice at 4.5 hr after injection. Other inflammatory agents without mitogenic activity, such as zymosan, carrageenan, glycogen, D-galactosamine and *N*-acetyl-muramyl-L-alanyl-D-isoglutamine, did not induce the enzyme. Both LPS (a B-cell mitogen) and Con A (a T-cell mitogen) induced HDC also in nude mice that lack T-cells, indicating that T-cells are not required for HDC induction by mitogens. C3H/HeJ mice, which are LPS-low responder mice in various immunological tests, were quite a bit less responsive to LPS also in the HDC induction. These results show that mitogens with different properties can induce HDC as a common characteristic. On the basis of these results, the possible participation of macrophages in the process of HDC induction by mitogens was discussed.

Histamine has been associated generally with immediate hypersensitivity reactions, and its release from mast cells and basophils has been studied for many years. Recent studies suggest that histamine also plays a role in the regulation of cell-mediated immunity or delayed-type hypersensitivity responses [1, 2]. Histamine suppresses lymphocyte responses to mitogens [3–7]. However, these results are based on the effects of histamine injected into animals or in experiments *in vitro*, and the source of endogenous histamine that may participate in such a regulation is not clear.

Injection of *Escherichia coli* lipopolysaccharide (LPS)* into mice produces induction of histidine decarboxylase (HDC), with a resulting elevation of histamine levels in various tissues and blood [8]. LPS is an inflammatory agent and also a potent mitogen, stimulating non-specific immune responses. The present study examined (a) the ability of various inflammatory agents with or without mitogenic activity to induce HDC and (b) HDC inductions in nude mice that lack T-cells and in C3H/HeJ mice whose response to LPS is very poor in many immunological tests.

MATERIALS AND METHODS

Materials. LPS derived from *E. coli* 055:B5, prepared by the Boibin method, was obtained from Difco Laboratories, Detroit, MI, U.S.A. PWM was from the E-Y Laboratories, San Mateo, CA, U.S.A. Double-stranded pIpC was from P-L Biochemicals Inc., Milwaukee, WI, U.S.A. Carrageenan (Seakem No 202) was supplied by Marine Colloid Inc., Springfield, NJ, U.S.A. Zymosan (from *Saccharomyces cerevisiae*), Con A, TPA and MDP were purchased from the Sigma Chemical Co., St. Louis, MO, U.S.A. Other reagents were purchased from Wako Pure Chemical Ind., Osaka, Japan.

Agents, except for TPA, were dissolved or suspended in saline and injected into mice intravenously or intraperitoneally (0.1 ml/10 g body weight). TPA was dissolved in ethanol, mixed with 5 vol. of saline, and injected intraperitoneally (0.1 ml/mouse).

The properties of the inflammatory agents examined for HDC induction in this study are shown in Table 1.

Animals. C3H/HeJ mice were donated by Dr. R. Yoshida (Department of Medical Chemistry, Kyoto University, Kyoto, Japan) and propagated in our laboratory under conventional conditions. Male ddI mice were obtained from the Mouse Center of our University. C3H/He, BALB/c and BALB/c-nu/nu mice were purchased from the Shizuoka Agricultural Cooperative Association for Laboratory Animals, Shizuoka, Japan. The latter two strains were raised under specific pathogen-free conditions. Male mice, 5- to 7-weeks-old, of all these strains were used for experiments.

Methods. Mice were decapitated and a single tissue of each mouse was assayed for HDC activity as described in Ref. 8. The activity was expressed as $nmoles \cdot hr^{-1} \cdot (g\ tissue)^{-1}$ for the liver or $nmoles \cdot hr^{-1} \cdot organ^{-1}$ for the spleen and lung, because the weight of the spleen, increased by the treatment with mitogens, and the weight of lung varied by the infusion of blood at decapitation.

RESULTS

HDC inductions following the injection of various inflammatory agents into mice. HDC activities in the

* Abbreviations: LPS, lipopolysaccharide; HDC, histidine decarboxylase; Con A, concanavalin A; PWM, pokeweed mitogen; pIpC, polyI:polyC; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; and MDP, *N*-acetyl-muramyl-L-alanyl-D-isoglutamine.

Table 1. Inflammatory agents examined for HDC induction

Agents	Properties
LPS	Endotoxin, B-cell mitogen [9, 10]
Con A	Protein, lectin, T-cell mitogen [10]
PWM	Protein, lectin, T-cell-dependent B-cell mitogen [10, 11]
pIpC	Synthetic double-stranded RNA, B-cell mitogen [12]
TPA	A component of croton oil, T- and/or B- cell mitogens [13]
MDP	A minimum unit with adjuvant activity of BCG [14]
Zymosan	Polysaccharide [15]
Carrageenan	Galactose polymer [16]
Glycogen	Glucose polymer
D-Galactosamine	An inducer of experimental hepatitis [17]

Table 2. HDC inductions by various inflammatory agents in mouse tissues*

Agents	Doses (mg/kg)	HDC activities Liver	Spleen	Lung
Saline		<0.2	<0.1	<0.05
LPS	0.5	6.8 ± 0.4	0.91 ± 0.08	1.67 ± 0.32
Con A	5	1.2 ± 0.2	1.09 ± 0.15	0.22 ± 0.03
PMW	5	2.9 ± 0.4	1.14 ± 0.13	0.78 ± 0.09
pIpC	5	2.9 ± 0.3	0.29 ± 0.04	0.42 ± 0.06
TPA†	0.4	3.0 ± 1.1	0.54 ± 0.12	1.10 ± 0.40
MDP	5	<0.2	<0.1	<0.05
Zymosan	5	<0.2	<0.1	<0.05
Carrageenan	5	<0.2	<0.1	<0.05
Glycogen	100	<0.2	<0.1	<0.05
D-Galactosamine	400	<0.2	<0.1	<0.05

* Mice (ddI) were killed at 4.5 hr after intravenous injections of the agents. Each value is the mean ± S.D. of five mice. HDC activities are expressed as nmoles per hr per g tissue for the liver and nmoles per hr per organ for the spleen and lung.

† Injected intraperitoneally.

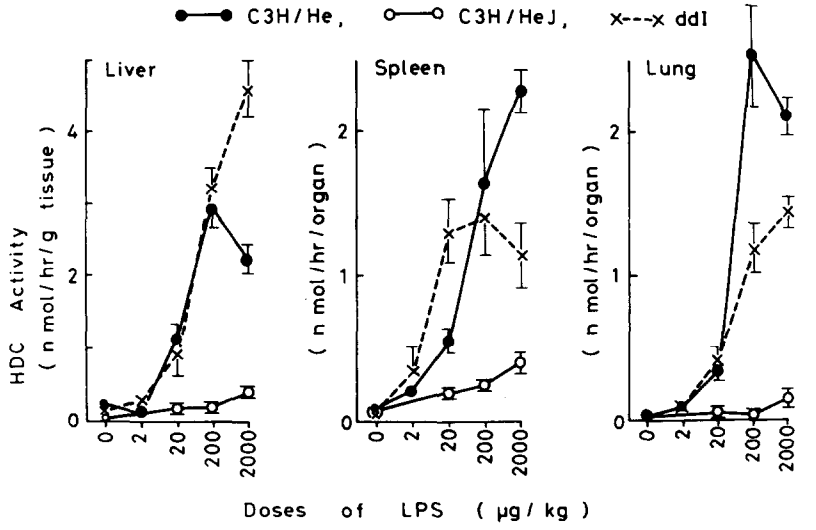

Fig. 1. HDC induction in C3H/HeJ, C3H/He and ddI mice by LPS. Mice were killed at 4.5 hr after the intraperitoneal injection of LPS. Each value is the mean ± S.D. (N = 3 in C3H/HeJ and C3H/He, N = 5 in ddI).

Table 3. HDC induction in nude mice by Con A and LPS*

	HDC activities		
	Liver	Spleen	Lung
BALB/c-nu-nu			
Saline	0.41 ± 0.04	0.05 ± 0.01	0.06 ± 0.00
Con A	5.9 ± 2.3	1.0 ± 0.4	2.6 ± 0.4
LPS	8.0 ± 1.2	1.8 ± 0.2	1.4 ± 0.0
BALB/c			
Saline	0.32 ± 0.09	0.05 ± 0.01	0.05 ± 0.01
Con A	1.2 ± 0.1	1.1 ± 0.1	2.9 ± 0.4
LPS	6.2 ± 1.1	0.9 ± 0.1	0.7 ± 0.1

* Mice were killed at 4.5 hr after the intravenous injection of Con A (20 mg/kg) or LPS (0.5 mg/kg). Each value is the mean ± S.D. of three mice. HDC activities are expressed as nmoles per hr per g tissue for the liver and nmoles per hr per organ for the spleen and lung. All levels of HDC induced by Con A and LPS are significnatly greater than those of control mice (saline injected) ($P < 0.001$, Student's t-test).

liver, spleen and lung of normal mice are very low. However, after the intravenous injection of LPS into mice, HDC activities in these tissues increase within 1.5 hr, peak at 3–6 hr and return to the basal levels within 15 hr [8]. In the present study, therefore, the mice were killed at 4.5 hr after the injection of each inflammatory agent, and HDC activities in these tissues were assayed (Table 2).

In addition to LPS, every mitogenic substance, i.e. Con A, PWM, pIpC and TPA, induced HDC in all tissues tested. The HDC inductions in the spleen of ddI mice by LPS, Con A and PWM appeared to reach maximum at the doses tested (see the results in Fig. 1 for LPS). LPS seems to be the most potent HDC inducer of these mitogens. Since a lower dose of TPA injected intraperitoneally produced higher HDC activities, this agent also appears to be a potent HDC inducer. TPA was reported to induce HDC in the skin when it was painted on the skin [18]. Both intravenous and intraperitoneal injections of LPS (0.5 mg/kg) produced maximum HDC activities in these tissues at similar periods, i.e. 3–6 hr after the injections (data not shown). Intraperitoneal injections of the same doses of these agents that are shown in Table 2 also produced HDC inductions, although the activities were lower than those by intravenous injections (examples for LPS are shown in Table 2 and Fig. 1).

On the other hand, none of the substances without mitogenic activity induced HDC in these tissues. MDP has an adjuvant activity like LPS, but its mitogenic activity appears to be very low, if present [14]. Zymosan and carrageenan have been used as experimental inducers of inflammatory reactions [15, 16]. These agents, however, were inactive in inducing HDC. Higher doses of glycogen and D-galactosamine also did not induce HDC under the experimental conditions. Although it is possible that higher doses of MDP, zymosan and carrageenan may induce HDC at this period or at a delayed period, it is clear that these agents have very little ability to induce HDC, in comparison with mitogenic substances.

HDC inductions in nude mice and C3H/HeJ mice. Nude mice are known to lack T-cells. Therefore, if T-cells were critically required for HDC induction by mitogens, one would expect the HDC induction by mitogens not to occur in nude mice. However, both Con A (a T-cell mitogen) and LPS induced HDC in nude mice (BALB/c-nu/nu) as well as in normal control mice (BALB/c) (Table 3).

C3H/HeJ mice are known to be low responder mice to LPS in various immunological tests [19, 20]. The mice were also much less responsive to LPS in HDC induction (Fig. 1). The response to LPS was 1/100 to 1/1000 compared with C3H/He or ddI mice, i.e. in C3H/HeJ mice the HDC activities induced by 2 mg/kg of LPS corresponded to those in C3H/He and ddI mice induced by 20 μg/kg or less of LPS. There was no such difference in HDC induction by Con A in mice of these strains.

DISCUSSION

The findings that various mitogens with different properties, as shown in Table 1, could induce HDC at 4.5 hr after injection as a common property and that the agents without mitogenic activity did not induce the enzyme suggest that the induction of HDC is intimately related to immune responses or cell-mediated inflammation.

The experiments on nude mice suggest that T-cells are not necessary for the HDC induction by mitogens. Macrophages are known to be important or required absolutely at an early stage of many immune responses or mitogen reactions [21, 22]. In addition, the macrophages in C3H/HeJ mice have been reported to be poorly responsive to LPS [20]. Therefore, the results in the present study suggest that macrophages may be as important in HDC induction by mitogens as they are in various immune responses.

On the basis of the observations that both basophils and mast cells degranulate in delayed-type hypersensitivity reactions, it has been proposed that the histamine released from these cells may play a regulatory role in the reactions [1]. In a previous study, HDC induction by LPS resulted in a marked elevation of histamine levels in tissues of mice and its diffusion into blood [8]. Although the kind of cells in which HDC induction occurs is not clear at present, it is possible that newly formed histamine participates in the proposed immune regulation.

In the delayed-type hypersensitivity responses caused by antigen challenge to immunized mice, Askenase *et al.* [1] have emphasized that the timing of the histamine injection is critical to obtain its effective suppression, i.e. 3–6 hr after the antigen challenge [1]. This period appears to be the time when the induction of HDC occurs. (Preliminary results of this study, including the effects of some drugs on the HDC induction, are described in Refs 23–25.)

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