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Central IL-1 differentially regulates peripheral IL-6 and TNF synthesis

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Abstract. Centrally given interleukin (IL)-1 is known to induce a rapid rises in blood IL-6. To extend this and to examine the mechanism by which this occurs, the effects of intracerebroventricular (icv) injection of human recombinant IL-1β on mRNA expression of IL-6 and tumour necrosis factor (TNF) in the spleen and liver were examined in rats. Icv injection of IL-1 produced a rapid rise of the tissue mRNA levels of IL-6 and TNF in both organs, prior to and/or in parallel with an increase in their serum levels. Pretreatment with chlorisondamine, a ganglionic blocking agent, inhibited the IL-6 responses, while it had little influence on the TNF responses. The results suggest that brain IL-1 induces peripheral production of IL-6, but not of TNF, through autonomic nervous system activation.

Key words. Interleukin-1 (IL-1); interleukin-6 (IL-6); tumour necrosis factor (TNF); autonomic nervous system; cytokine.

Interleukin (IL)-1 is one of the key mediators of host-defense responses not only in peripheral tissues but also in the central nervous system. For example, intracranial administration of IL-1 produces a broad spectrum of nonimmune and immune responses such as fever, anorexia, leukocytosis, reduction of natural killer cell activity and impaired mitogenic activity of lymphocytes [1]. De Simoni et al. [2] previously reported that the circulating level of IL-6 is increased after intracerebroventricular (icv) injection of IL-1. Since IL-6 acts as a crucial cytokine in various immune responses [3], their reports suggest that brain IL-1 can widely regulate peripheral host-defense responses through IL-6 synthesis in peripheral tissues. In fact, we previously demonstrated a significant increase of blood haptoglobin, a typical hepatic acute phase protein induced by IL-6, following a rapid rise of plasma IL-6 after icv injection of IL-1 [4]. However, little is known about the mechanism of the brain IL-1-mediated regulation of peripheral cytokine production.

Brain IL-1 is known to activate several neuroendocrine systems, including the hypothalamicpituitary-adrenocortical axis (HPA) and the sympathetic-adrenal-medullary axis (SAM) [5], both of which may influence peripheral cytokine production and possibly be involved in the pathway from brain IL-1 to peripheral tissues. Because the serum IL-6 response to IL-1 is not abolished but exaggerated by hypophysectomy [2], hypophysis-related mechanisms, including the HPA, are not prerequisite to the pathway. On the other hand, some researchers have reported that peripheral IL-6 production is modified by catecholamines, suggesting a significant role for the SAM [6–8]. In this study, to obtain more direct evidence for the autonomic mediation of brain IL-1 to peripheral cytokine production, we first examined mRNA expression of IL-6 in the spleen

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and liver as well as their serum levels after icv injection of IL-1, and next the effects of chemical ganglionic blockade on the cytokine responses. In parallel with IL-6 responses, serum and tissue responses of tumour necrosis factor (TNF), another major inflammatory cytokine and an inducer of hepatic acute phase protein synthesis [9], to brain IL-1 were also investigated.

Materials and methods

Experimental animals. Female Wistar rats weighing 180–210 g were housed in plastic cages at 24 ± 1 °C with a 12-h light-dark cycle (lights on 0700–1900 h) and given free access to laboratory chow and water. Rats were implanted with a stainless steel cannula in the right lateral cerebral ventricle under pentobarbital anaesthesia [40 mg/kg, intraperitoneal (ip)], and used for the experiments 2 weeks later. The experimental procedure and care of animals were in accordance with the guidelines of the Animal Care and Use Committee of Hokkaido University.

Cytokines. Human recombinant IL-1β (2×10^7 U/mg protein), IL-6 (5×10^6 U/mg protein) and TNFα (2.55×106 U/mg protein) were kindly provided by Dr. Y. Hirai (Otsuka Pharmaceutical, Tokushima, Japan), Dr. K. Yasukawa (Tosoh, Ayase, Japan) and Dr. M. Kitaura (Dainippon Pharmaceutical, Osaka, Japan), respectively. Endotoxin levels in these cytokine preparations were less than 0.1 ng/mg protein. Inactivated IL-1 was prepared by heating an IL-1β solution at 100 °C for 2 h.

Responses of IL-6 and TNF. IL-1 was diluted with sterilized 20 mM phosphate-buffered saline (PBS) and injected intracerebroventricularly (10 µl) at a dose of 200 ng/rat. Chemical ganglionic blockade was performed by intraperitoneal injection (100 µl) of chlorisondamine (3 mg/kg, Ciba-Geigy, Basel, Switzerland) in PBS 1 h before the IL-1 injection. Because chlorisondamine is a noncompetitive and long-acting antagonist against the nicotinic acetylcholine receptors in both ganglions and the adrenal medulla, but does not readily penetrate into the central nervous system [10, 11], this drug has been widely used to deliberately block ganglionic transmission [12, 13]. Before, 1 and 2 h after the IL-1 or PBS injection, rats were killed by decapitation, and the blood, spleen and liver were taken for IL-6 and TNF analysis. In some experiments, rats were given IL-1 (200 ng/100 µl per rat) intraperitoneally, and heat-inactivated IL-1 (200 ng/10 µl per rat) and PBS intracerebroventricularly, and were killed 2 h later.

The concentration of serum IL-6 was measured using the IL-6-dependent cell line MH60.BSF2 (a gift from Dr. T. Matsuda, Osaka University, Suita, Japan) as previously described [14]. The concentration of serum TNF was measured by cytotoxicity assay using cell line WEHI 164 (a gift from Dr. Y. Yokomizo, National Institute of Animal Health, Tsukuba, Japan) as previously described [15]. The minimum concentrations of serum IL-6 and TNF detectable by these methods were 2.0×10^{-2} U/ml (4.0 pg/ml) and 3.0×10^{-2} U/ml (12 pg/ml), respectively.

Northern blot analysis. Total RNA was extracted by the guanidine isothiocyanate method using TRIzol solution (Gibco BRL, Gaithersburg, MD, USA), according to the manufacturer's directions. Poly $(A)^+$ RNA was prepared using oligo-d(T) cellulose columns (Clontech, Palo Alto, CA, USA), denatured at 70 °C, separated on 1% agarose/formaldehyde gel, and transferred to and fixed on a nylon membrane (Amersham, Buckinghamshire, UK). Complementary DNA (cDNA) probes for rat IL-6 and TNFα were prepared by reverse transcription-polymerase chain reaction from total RNA extracted from rat spleen, and were labelled with (α-^{32}P) dCTP using a multiprime DNA labelling kit (Amersham). Nylon membranes were hybridized with the labelled cDNA probes, washed and exposed to X-ray films for 1 or 2 weeks. The radioactivity was quantified using a BAS-1000 bioimage analyser (Fuji Film, Tokyo, Japan). The membranes were also hybridized with a human β-actin cDNA probe (Wako, Osaka, Japan) as a reference.

Statistics. All values were expressed as means $\pm$ SE. Statistical comparison was made by analysis of variance, followed by Scheffe's F test or Fisher's protected least significant difference test.

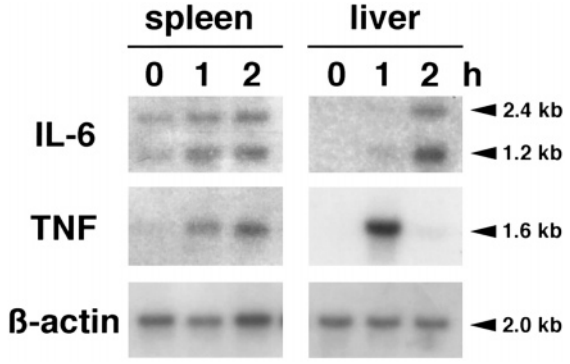

Figure 1. Changes in the peripheral IL-6, TNF and β-actin mRNA levels after icv injection of IL-1. Poly$(A)^+$ RNA (spleen, 3 µg; liver, 12 µg) isolated at 0, 1 and 2 h after icv injection of IL-1 (200 ng/rat) was subjected to Northern blot analysis. Two IL-6 bands of 1.2 kb and 2.4 kb correspond to the IL-6 mRNAs polyadenylated at the different sites [30].

Table 1. Serum IL-6 and TNF levels after IL-1 injection.

	Serum level (U/ml)		
	time (h)	IL-6	TNF
Control		0.18 ± 0.01	0.14 ± 0.03
IL-1 icv	1	0.50 ± 0.13	0.16 ± 0.03
IL-1 icv	2	5.80 ± 1.70*	0.47 ± 0.08*
IL-1 icv	4	1.49 ± 0.21*	0.31 ± 0.04
PBS icv	2	0.08 ± 0.01	0.11 ± 0.04
Heat-inactivated IL-1 icv	2	0.16 ± 0.03	0.15 ± 0.05
IL-1 ip	2	0.28 ± 0.04	0.07 ± 0.02

IL-1 (200 ng/rat), heat-inactivated IL-1 (200 ng/rat) or PBS was given intracerebroventricularly (icv) or intraperitoneally (ip). Values are means $\pm$ SE for 5–7 rats. Control rats were also implanted with a cannula in the lateral cerebral ventricle but were uninjected. *$P < 0.05$ compared with the control.

Results

Response of serum IL-6 and TNF to icv injection of IL-1. Intracranial injection of IL-1 has been reported to produce a rapid rise in serum IL-6 levels [2, 16]. To confirm the stimulatory effect of brain IL-1 on peripheral cytokines [2], we injected 200 ng of recombinant human IL-1β intracerebroventricularly, and measured the serum levels of IL-6 and TNF. As shown in table 1, after icv injection of IL-1, the serum IL-6 level did not change in 1 h, but increased substantially in 2 h, and returned to a lower level in 4 h. A smaller but significant increase was also seen in the serum TNF level. Neither intraperitoneal injection of IL-1 at the same dose, icv injection of vehicle (PBS) alone nor icv injection of heat-inactivated IL-1 significantly influenced serum IL-6 and TNF levels.

Response of IL-6 and TNF mRNA to icv injection of IL-1. The observed increase in serum cytokines may be a result of increased synthesis in some tissues. Significant contributions of the spleen and liver have been proposed in the production of some cytokines in response to inflammatory stimuli and immobilization stress [17–19]. To examine the effect of brain IL-1 on peripheral cytokine expression, the mRNA levels of IL-6 and TNF were measured in the spleen and liver by Northern blot analysis (fig. 1). Before IL-1 injection, the tissue levels of IL-6 mRNA were very low in both the spleen and liver. Icv injection of IL-1 increased the IL-6 mRNA levels in 1 to 2 h in both tissues. The TNF mRNA levels in the spleen and liver were also very low before the IL-1 injection, but increased in both tissues after the IL-1 injection. The time course of the response seemed different between the spleen and liver; that is, in the liver the mRNA level increased transiently in 1 h, whereas in the spleen it increased gradually in 2 h. There was no noticeable and consistent change in the β-actin mRNA level after the IL-1 injection.

Effects of ganglionic blockade on IL-6 and TNF response. To explore the possible role of the autonomic nervous system in the induction of IL-6 and TNF by icv IL-1, the effect of chlorisondamine, a long-lasting ganglionic blocker, was examined. When chlorisondamine was given intraperitoneally, the IL-6 mRNA response in both the spleen and liver were significantly attenuated (fig. 2). With chlorisondamine treatment, the serum IL-6 level was still increased after the IL-1 injection, but the response was greatly depressed, being only one-seventh of that without the treatment. In contrast to the IL-6 response, the tissue and serum TNF response to IL-1 was influenced little by pretreatment

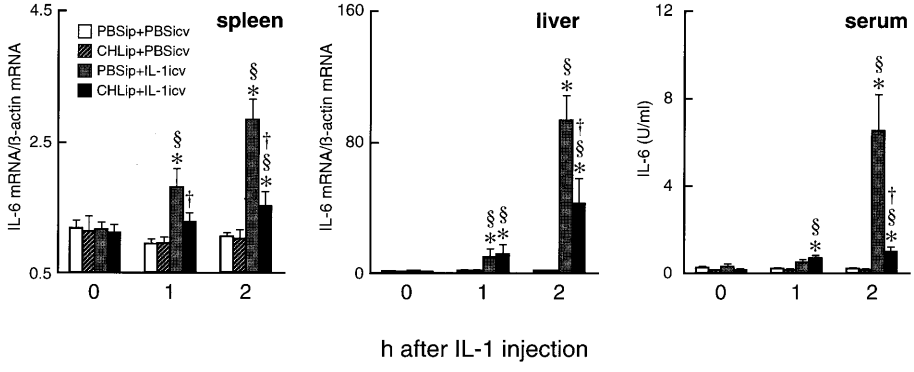

Figure 2. Effect of chlorisondamine (CHL) on the tissue and serum IL-6 responses to icv IL-1 injection. Rats were injected with CHL (3.0 mg/kg) or PBS intraperitoneally 1 h before icv injection of IL-1 (200 ng/rat) or PBS. IL-6 mRNA was analysed using poly(A)$^+$ RNA (spleen, 10 μg; liver, 15 μg) as in figure 1. For quantitative analysis, the IL-6 mRNA level was normalized by β-actin mRNA and expressed as a value relative to the average of the untreated control rats (see table 1). There was no significant alteration in the β-actin mRNA level induced by CHL injection itself. Values are means $\pm$ SE for four (spleen), five (liver), and five to seven (serum) rats. *$P < 0.05$, §$P < 0.05$, †$P < 0.05$ compared with the 0 h, PBS icv and PBS ip values, respectively.

with chlorisondamine (fig. 3). The effect of chlorisondamine on the basal levels of IL-6 and TNF was also examined without IL-1 injection. From 0 to 3 h after chlorisondamine injection, the tissue mRNA and serum levels of IL-6 and TNF showed no noticeable change, remaining at the low basal levels (Figs. 2 and 3). Thus, the IL-6 responses to icv IL-1 were depressed by ganglionic blockade, but the TNF responses were not.

Discussion

Previous reports have indicated that intracranial administration of IL-1 or bacterial lipopolysaccharide (LPS), a stimulant of inflammatory cytokine synthesis in glial cells, induces high circulating levels of IL-6 and TNF [2, 4, 20, 21]. This was confirmed in the present study. Since the identical dose of IL-1 given intraperitoneally did not elevate the serum IL-6 and TNF levels, it is unlikely that the serum cytokine response induced by icv injection of IL-1 is due to the passage of IL-1 into the circulation through the blood-brain barrier. Our results indicate that IL-1 injected into the brain is able to regulate various circulating cytokines, and thereby to modulate peripheral immune functions. For example, hepatic synthesis and secretion of acute phase proteins are stimulated by IL-6 and TNF in vitro [9] and, in fact, are increased by icv administration of IL-1 [4].

The present results demonstrate that icv injection of IL-1 produces a rapid increase in the mRNA levels of IL-6 and TNF in the spleen and liver, which are known to be the major sources of circulating cytokines in inflammation and physiological stress situations [17–19]. Similar responses of IL-6 mRNA to intracranial administration of LPS have also been reported in the adrenal gland and lymph nodes [20]. Since tissue mRNA responses were prior to and/or in parallel with serum responses, it seems likely that rise of serum IL-6 and TNF levels a consequence of increased synthesis in these peripheral tissues.

In an attempt to clarify the pathway from brain IL-1 to peripheral tissues, De Simoni et al. [2] demonstrated that the serum IL-6 response to IL-1 is not abolished, but exaggerated, by hypophysectomy and/or adrenalectomy. Thus, hypophysis-related mechanisms involving the HPA are not essential to the pathway. On the other hand, growing evidence has accumulated that various cellular immune functions are directly influenced by the SAM. For example, Sundar et al. [13] reported that central IL-1 produced a marked reduction of peripheral lymphocyte activities, and that the suppressive effect was attenuated after ganglionic blockade. Similarly, the suppressive effects of foot-shock stress [22], intracranial administration of interferon-α [23], and hypothalamic stimulation [24] on splenic lymphocytes and natural killer cells are also known to be mediated through sympathetic activation. Our results are similar to these observations in that the peripheral IL-6 response induced by brain IL-1 was much depressed by ganglionic blockade, indicating a significant role for the autonomic nervous system in the response. In addition, when comparable amounts of IL-1 were given intracerebroventricularly, the sympathetic nerve activity assessed by norepinephrine turnover was increased in both the spleen and liver [5, and unpublished observations]. All these results collectively suggest that brain IL-1 induces the expression of IL-6 in some

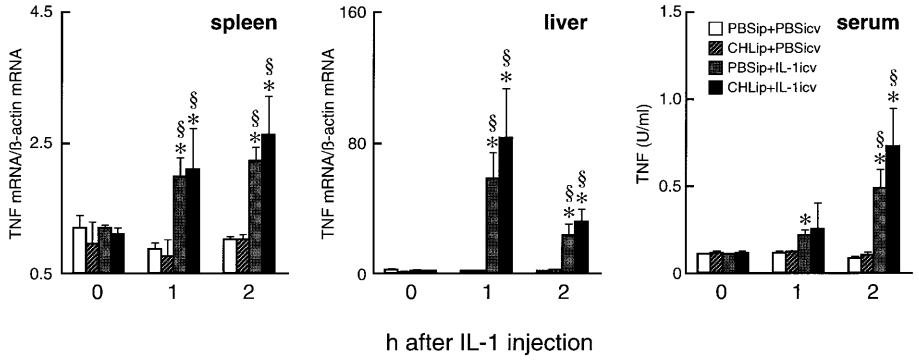

Figure 3. Effect of CHL on the tissue and serum TNF responses to icv IL-1 injection. The TNF mRNA level was analysed as in figures 1 and 2. Values are means ± SE for four (spleen), five (liver) and five to seven (serum) rats. *$P < 0.05$ and §$P < 0.05$ compared with the 0 h and PBS icv values, respectively.

peripheral organs and the resulting rise in its blood level through sympathetic activation. This is also consistent with the recent report of Finck et al. [8] that the plasma IL-6 response to icv LPS injection is attenuated by pretreatment with an α-adrenoreceptor antagonist. Liao et al. [25] showed that epinephrine is able to potentiate LPS-induced IL-6 production in isolated perfused rat liver. Thus, it seems quite likely that the IL-1-induced IL-6 production is attributable to the direct action of catecholamines released from the sympathetic nerve endings and/or the adrenal medulla. A possible role for the SAM is also proposed in the plasma IL-6 response to some stressors [6, 7].

It has been reported that lymphocytes have nicotinic acetylcholine receptors [26], and that nicotine induces IL-6 production in peripheral blood mononuclear cells in vitro [27]. It thus seems possible that the nicotinic mechanism in immune cells, which might also be blocked by chlorisondamine, is involved in the IL-6 response. However, splenic innervation consists mostly of noradrenergic fibres and has few cholinergic components [28, 29]. Moreover, it is well known that circulating acetylcholine is negligible in physiological responses because of its very short half-life. Hence, it is unlikely that the IL-6 response is mediated through nicotinic receptors on the cytokine-producing cells, at least in the spleen.

In contrast to IL-6, the TNF responses to brain IL-1 were not significantly influenced by ganglionic blockade, suggesting a minor role for the autonomic nervous system in the modulation of TNF. This is consistent with a report that catecholamines are not involved in TNF induction in isolated perfused rat liver [25]. Moreover, in our preliminary experiments, the serum TNF response after icv IL-1 injection was not attenuated by adrenalectomy. Thus, the HPA might not be involved in the IL-1-induced increase in TNF production. Since brain IL-1 is known to influence secretion of various hypothalamic pituitary hormones such as corticotropin-releasing hormone, growth hormone-releasing hormone thyroid-stimulating hormone and adrenocorticotropic hormone [1], the possible involvement of these hormones in peripheral TNF production remains to be investigated.

In conclusion, icv injection of IL-1 into rats increases the expression of IL-6 and TNF in the spleen and liver, followed by a rise in their serum levels. The peripheral responses of IL-6, but not of TNF, may be mediated through autonomic nervous system activation.

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