INTRACEREBROVENTRICULAR INJECTION OF INTERLEUKIN 1 INDUCES HIGH CIRCULATING LEVELS OF INTERLEUKIN 6

BY MARIA GRAZIA DE SIMONI,* MARINA SIRONI,† ADA DE LUIGI,* ALFREDO MANFRIDI,* ALBERTO MANTOVANI,† AND PIETRO GHEZZI‡

From the Unit of Neurotransmitter Metabolism and the Laboratory of Immunology, Istituto di Ricerche Farmacologiche "Mario Negri," 20157 Milan, Italy

There is a growing interest for connections between the central nervous system (CNS) and systemic immune and/or inflammatory responses. The inflammatory cytokine IL-1 has a wide spectrum of targets, which include the CNS. Intracerebroventricular as well as systemic administration of IL-1 were reported to induce central effects including fever (1), slow-wave sleep (2), anorexia (3), and activation of the hypothalamus-pituitary axis leading to release of adrenal corticosteroids (4, 5). Moreover, different brain cells such as astrocytes, microglia, and neurons can respond and/or have been found to contain various cytokines like IL-1, IL-2, IL-6, and TNF, and there is evidence that these factors act on neuronal survival, growth, and differentiation (1, 3, 6–10).

It is also possible that some of the systemic activities of IL-1 are, at least in part, centrally mediated. In fact, early reports indicated that central administration of crude leukocytic endogenous mediators (presumably containing, among other cytokines, IL-1) induced an increase of acute-phase proteins (11). The mechanism by which centrally administered IL-1 can activate the synthesis of hepatic acute-phase proteins is still unknown, and the present study was aimed at revisiting this early observation by investigating how intracerebroventricularly administered rIL-1 induced a systemic response. Our attention was focused on IL-6, since this cytokine, induced by IL-1, plays a crucial role in the acute-phase response as a hepatocyte-stimulating factor, and the levels of circulating IL-6 were reported to correlate with the levels of acute-phase proteins in some infective and inflammatory diseases (12–14).

Materials and Methods

Male rats (250–300 g) (CD-COBS, Charles River Breeding Laboratories, Inc., Calco, Como, Italy) were used. They were housed with free access to food and water, under a 12-h light/dark cycle with constant temperature (21–23°C) and humidity (20–25%).

IL-1 (human rIL-1-β; a kind gift of Sclavo, Siena, Italy) was injected either intraperitoneally, intravenously, or intracerebroventricularly through one polyethylene cannula permanently implanted in the lateral ventricle 3 d before the experiment (15). IL-1 was dissolved in sterile,
pyrogen-free saline containing 0.1% BSA and administered at the dose of 200 ng/5 µl for each rat. Control rats were given the same volume of vehicle.

Indomethacin (Chiesi Farmaceutici, Parma, Italy) was administered at the dose of 20 mg/kg, i.p., 1 h before IL-1.

10 d before the experiment, one group of rats was hypophysectomized according to the procedure described by Falconi and Rossi (16); another group was adrenalectomized and given 1% NaCl dissolved in drinking water. For each experiment, appropriate sham-operated rats were used as controls.

Rats were killed by decapitation, blood was collected, and serum was prepared. IL-6 in serum samples was measured as hybridoma growth factor using the 7TD1 cell line obtained through the courtesy of Dr. J. Van Snick, Bruxelles, Belgium, as previously described (17). 1 U in the 7TD1 assay corresponded to 1 pg human rIL-6. The sensitivity of the assay with rat serum was 50 U/ml.

Results and Discussion

Fig. 1 shows the effects of intracerebroventricular administration of IL-1 (200 ng/rat) on serum IL-6 levels at 2 h after IL-1. The results of two different experiments are reported, where the effect of intracerebroventricular administration was compared with that of systemic administration (intraperitoneal or intravenous) of the same amount of IL-1. It is clear that centrally administered IL-1 induced markedly higher levels of IL-6 than systemically given IL-1. The time course of IL-6 induction by IL-1 given intravenously or intracerebroventricularly was comparable, as shown in Fig. 2, with a peak at 2 h. When heat-inactivated (90°C, 20 min) IL-1 was injected intracerebroventricularly, no IL-6 induction was observed (IL-6 levels were <50 U/ml), thus ruling out the possibility that endotoxin contamination of the rIL-1 preparation could be responsible for the observed effect.

The higher serum IL-6 levels observed when IL-1 was administered intracerebroventricularly rather than systemically clearly rule out the possibility that induction of
circulating IL-6 by intracerebroventricularly administered IL-1 might be due to a passage of IL-1 into the circulation through the blood brain barrier.

The effect of IL-1 on hypothalamic thermoregulatory centers is known to be mediated by prostaglandins (1, 18). It was therefore important to evaluate whether products of arachidonate metabolism were involved in induction of systemic IL-6 by intracerebroventricular IL-1. We have studied the effect of pretreatment with an inhibitor of prostaglandin synthesis, indomethacin (20 mg/kg, i.p., 1 h before IL-1), which was previously shown to inhibit the pyrogenic action of IL-1 administered intracerebroventricularly (19, 20). The results reported in Fig. 3 show that indomethacin did not abolish the effect of centrally administered IL-1 on serum IL-6 levels. It should be noted that indomethacin alone increased serum IL-6 levels, although to a lesser extent than IL-1. Maximal induction of IL-6 by indomethacin alone was at 2 h, and was also observed with 30 mg/kg of another cyclooxygenase inhibitor, ibuprofen (data not shown). One possibility for the inducing effect of cyclooxygenase inhibitors on IL-6 levels is that prostaglandins provide an inhibitory signal for IL-6 synthesis, as it was reported for IL-1 (21).

IL-1 activates the hypothalamus-pituitary axis, causing release of pituitary hormones and, ultimately, glucocorticoids (4, 5). It was therefore important to ascertain whether the activation of the hypothalamus-pituitary axis is responsible for the induction of serum IL-6 by centrally administered IL-1. For this purpose, we have studied the induction of IL-6 in hypophysectomized rats. As shown in Fig. 4 A, hypophysectomy did not block the induction of IL-6 by centrally administered IL-1. Indeed, in all the experiments, hypophysectomy increased the IL-6 response. We have also considered the possibility that IL-6 could be released by the adrenals, which were reported to contain high levels of IL-1 that could be released by degranulation of catecholaminergic terminals (22). Results obtained using adrenalectomized rats are shown in Fig. 4 B. Adrenalectomy also increased the induction of IL-6 by centrally administered IL-1. It should be pointed out that adrenalectomy, like hypophysectomy, increased IL-6 levels even after intraperitoneally administered IL-1 (data not shown), confirming our previous reports of a higher sensitivity of adrenalectomized animals to IL-1, probably due to the absence of feedback mechanisms mediated by corticosteroids (23).

Taken together, these data rule out the possibility that induction of serum IL-6
by centrally administered IL-1 is secondary to its pyrogenic action mediated by prostaglandins or to the stimulation of the hypothalamus-pituitary axis.

The finding that intracerebroventricular administration of IL-1 induces circulating IL-6 levels extends the list of central activities of IL-1 and indicates the existence of a novel pathway that could explain how infections or lesions confined to the CNS result in systemic alterations of acute-phase response parameters. The role played by IL-1 under conditions involving disturbances in the neurotransmission has been up to now poorly investigated. Glia cells are known to synthesize and store IL-1, as well as other cytokines (6-10), and reactive gliosis with elevated IL-1 activity has been recently reported after brain injury and in neuropathological diseases like Down's syndrome and Alzheimer's disease (24, 25). It was also shown that elevated levels of IL-6 are present in the cerebrospinal fluid of patients with meningitis (26), and both IL-1 and IL-6 were found in cerebrospinal fluid of patients with HIV-1 infection of the CNS (27).

The origin of the high blood levels of IL-6 induced by intracerebroventricular IL-1 remains to be established. IL-6 could be produced in the brain, for instance, by microglial cells (8, 10) or endothelial cells in the plexus chorioideus; alternatively, via an yet undefined pathway, production could be induced at peripheral sites.

The mechanism by which IL-1 can stimulate IL-6 production by a CNS-mediated pathway is still unclear. Studies are in progress to investigate the brain areas and the neurotransmitters implicated in this effect of IL-1 on the CNS and the source...
of the IL-6 released. Whatever the cellular origin and pathways involved, the observations reported herein provide a link whereby IL-1 produced intracerebrally or reaching the CNS can elicit a systemic acute-phase response.

Summary

IL-1 is known to have a central role in the induction of acute-phase response, and some of its activities (including induction of some acute-phase proteins) were reported to be mediated by an induction of IL-6. Administration to rats of 200 ng of human rIL-1 by intracerebroventricular injection resulted in a more marked induction of circulating IL-6 than the same dose of IL-1 administered systemically (intravenously or intraperitoneally). Induction of serum IL-6 by centrally administered IL-1 was also observed in hypophysectomized or adrenalectomized rats, suggesting that activation of the hypothalamus-pituitary-adrenal axis is not essential for this effect of IL-1. IL-6 induction was also observed after pretreatment with indomethacin, indicating that the effect was dissociated from the pyrogenic activity of IL-1. Induction of IL-6 by a central action could represent a novel pathway in IL-1-induced acute-phase response.

Received for publication 23 January 1990.

References

1. Dinarello, C. A., J. G. Cannon, and S. M. Wolff. 1988. New concepts on the pathogenesis of fever. Rev. Infect. Dis. 10:168.
2. Krueger, J. M., J. Walter, C. A. Dinarello, S. M. Wolff, and L. Chedid. 1984. Sleep-promoting effects of endogenous pyrogen (interleukin-1). Am. J. Physiol. 246:R994.
3. Plata-Salaman, C. R., Y. Oomura, and Y. Kai. 1988. Tumor necrosis factor and interleukin-1: Suppression of food intake by direct action in the central nervous system. Brain Res. 448:106.
4. Besedovsky, H., A. Del Rey, E. Sorkin, and C. A. Dinarello. 1986. Immuno-regulatory feedback between interleukin-1 and glucocorticoid hormones. Science (Wash. DC) 233:652.
5. Sapolsky, R., C. Rivier, G. Yamamoto, P. Plotsky, and W. Vale. 1987. Interleukin-1 stimulates the secretion of hypothalamic corticotropin-releasing factor. Science (Wash. DC). 238:522.
6. Breder, C. D., C. A. Dinarello, and C. B. Saper. 1988. Interleukin-1 immunoreactive innervation of the human hypothalamus. Science (Wash. DC). 240:321.
7. Araujo, D. M., P. A. Lapchak, B. Collier, and R. Quirion. 1989. Localization of interleukin-2 immunoreactivity and interleukin-2 receptors in the rat brain: interaction with the cholinergic system. Brain Res. 498:257.
8. Frei, K., U. V. Malipiero, T. P. Leist, R. M. Zinkernagel, M. E. Schwab, and A. Fontana. 1989. On the cellular source and function of interleukin 6 produced in the central nervous system in viral diseases. Eur. J. Immunol. 19:689.
9. Hama, T., M. Miyamoto, H. Tsukui, C. Nishio, and H. Hatanaka. 1989. Interleukin-6 as a neurotrophic factor for promoting the survival of cultured basal forebrain cholinergic neurons from postnatal rats. Neurosci. Lett. 104:340.
10. Righi, M., L. Mori, G. De Libero, M. Sironi, A. Biondi, A. Mantovani, S. D. Donini, and P. Ricciardi-Castagnoli. 1989. Monokine production by microglial cell clones. Eur. J. Immunol. 19:1443.
11. Bailey, P. T., F. B. Abeles, E. C. Hauer, and C. A. Mapes. 1976. Intracerebroventricular
administration of leukocytic endogenous mediators (LEM) in the rat. Proc. Soc. Exp. Biol. Med. 153:419.

12. J. Gauldie, C. Richards, D. Harnish, P. Lansdorp, and H. Baumann. 1987. Interferon-β/B-cell stimulatory factor type 2 shares identity with monocyte-derived hepatocyte-stimulating factor and regulates the major acute phase protein response in liver cells. Proc. Natl. Acad. Sci. USA. 84:7251.

13. Nijsten, M. W. N., E. R. de Groot, H. J. ten Duis, H. J. Klasen, C. E. Hack, and L. A. Aarden. 1987. Serum levels of interleukin-6 and acute phase responses. Lancet. 17:921.

14. Ueno, Y., N. Takano, H. Kanegane, T. Yokoi, A. Yachie, T. Miyawaki, and N. Taniguchi. 1989. The acute phase nature of interleukin 6: Studies in Kawasaki disease and other febrile illnesses. Clin. Exp. Immunol. 76:337.

15. De Simoni, M. G., V. Guardabasso, K. Misterek, and S. Algeri. 1982. Similarities and differences between d-ALA2 MET S enkephalin amide and morphine in the induction of tolerance to their effects on catalepsy and on dopamine metabolism in the rat brain. Naunyn Schmiedebergs Arch. Pharm. (Berlin). 321:105.

16. Falconi, G., and G. L. Rossi. 1964. Transauricular hypophysectomy in rats and mice. Endocrinology. 74:301.

17. Sirioni, M., F. Brevisario, P. Proserpio, A. Biondi, A. Vecchi, J. Van Damme, E. Dejana, and A. Mantovani. 1989. IL-1 stimulates IL-6 production in endothelial cells. J Immunol. 142:549.

18. Morimoto, A., T. Nakamori, T. Watanabe, T. Ono, and N. Murakami. 1988. Pattern differences in experimental fevers induced by endotoxin, endogenous pyrogen, and prostaglandins. Am. J. Physiol. 254:R633.

19. Stitt, J. T., and H. A. Bernheim. 1985. Differences in endogenous pyrogen fevers induced by iv and icv routes in rabbits. J. Appl. Physiol. 59:342.

20. Morimoto, A., N. Murakami, T. Nakamori, Y. Sakata, and T. Watanabe. 1989. Possible involvement of prostaglandin E in development of ACTH response in rats induced by human recombinant interleukin-1. J. Physiol. 411:245.

21. Knudsen, P. J., C. A. Dinarello, and T. B. Strom. 1986. Prostaglandins posttranscriptionally inhibit monocyte expression of interleukin 1 activity by increasing intracellular cyclic adenosine monophosphate. J. Immunol. 137:3189.

22. Schultzberg, M., C. Andersson, A. Unden, M. Troye-Blomberg, S. B. Svenson, and T. Bartfai. 1989. Interleukin-1 in adrenal chromaffin cells. Neuroscience. 30:805.

23. Bertini, R., M. Bianchi, and P. Ghezzi. 1988. Adrenalectomy sensitizes mice to the lethal effects of interleukin 1 and tumor necrosis factor. J. Exp. Med. 167:1708.

24. Giulian, D., and L. B. Lachman. 1985. Interleukin-1 stimulation of astroglial proliferation after brain injury. Science (Wash. DC). 228:497.

25. Griffin, W. S. T., L. C. Stanley, C. Ling, L. White, V. MacLeod, L. J. Perrot, C. L. White III, and C. Araoz. 1989. Brain interleukin 1 and S-100 immunoreactivity are elevated in Down syndrome and Alzheimer disease. Neurobiology (Oxford). 86:7611.

26. Houssiau, F. A., K. Bukasa, C. J. M. Sindic, J. Van Damme, and J. Van Snick. 1988. Elevated levels of the 26K human hybridoma growth factor (interleukin 6) in cerebrospinal fluid of patients with acute infection of the central nervous system. Clin. Exp. Immunol. 71:320.

27. Gallo, P., K. Frei, C. Rordorf, J. Lazdins, B. Tavolato, and A. Fontana. 1989. Human immunodeficiency virus type 1 (HIV-1) infection of the central nervous system: an evaluation of cytokines in cerebrospinal fluid. J. Neuroimmunol. 23:109.