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Synergistic Roles of Interleukin-6, Interleukin-1, and Tumor Necrosis Factor in the Adrenocorticotropin Response to Bacterial Lipopolysaccharide *in Vivo**

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ABSTRACT

Administration of lipopolysaccharide (LPS) results in activation of the hypothalamic-pituitary-adrenal axis. LPS induces the release of a number of proinflammatory cytokines, *i.e.* interleukin-1 (IL-1), IL-6, and tumor necrosis factor (TNF), which activate the hypothalamic-pituitary-adrenal axis as well and may mediate the effects of LPS. Variations in the kinetics of appearance of IL-1, TNF, and IL-6 after LPS challenge suggested that these cytokines may play different roles at different times. To elucidate the mutual dependence and contribution of individual cytokines in the course of LPS-induced ACTH release, we used blocking antibodies to IL-6, TNF, and the IL-1 receptor. Our results demonstrate that anti-IL-6 antibody abrogated ACTH induction throughout the course of the response both 2 and 4 h after LPS challenge. In contrast, anti-IL-1 receptor and anti-TNF antibody, given individually, blocked ACTH production at 4 h, but not at 2 h. Only combined administration of these two antibodies diminished, but did not eliminate, ACTH release at 2 h. This is the first demonstration that all three inflammatory cytokines are obligatory for LPS-induced elevation of plasma ACTH. In addition, these results suggest that IL-1, IL-6, and TNF play different roles in LPS-induced ACTH release. (*Endocrinology* **132:** 946-952, 1993)

INFLAMMATION and/or infection lead to activation of the hypothalamic-pituitary-adrenal (H-P-A) axis (1, 2). For many years, this phenomenon was studied in models employing lipopolysaccharide (LPS), a component of bacterial cell walls of gram-negative bacteria (3–6). More recently, a number of proinflammatory cytokines, *i.e.* interleukin-1 (IL-1), IL-6, and tumor necrosis factor (TNF), were shown to similarly activate the H-P-A axis both *in vivo* and *in vitro* (7–9). The finding that these biochemically distinct cytokines had similar effects suggested redundancy. Our previous work, however, indicated that interaction of these cytokines was required for ACTH induction (10, 11).

More specifically, we demonstrated in C3H/HeN mice that within 2 h of ip administration, IL-1 is a potent inducer of ACTH, whereas pharmacological amounts (up to 10 μg) of IL-6 induced only a negligible response (10). However, the combination of IL-1 and IL-6 produced a synergistic response within 30 min of injection (10), and IL-1 induces IL-6 within 2 h of injection (11–13). Together, these results suggested that IL-1 may need to interact with the IL-6 it induces endogenously in stimulating ACTH release. This hypothesis was further supported by our finding that pretreatment with murine monoclonal anti-IL-6 antibody blocked the IL-1-induced ACTH response (11).

LPS induces the release of IL-1, TNF, and IL-6 (14), which may mediate its stimulatory effect on the H-P-A axis. Therefore, the use of cytokine blocking antibodies to modulate the LPS-induced ACTH response should aid in elucidating the mutual dependence and contribution of endogenously produced individual cytokines. Indeed, Rivier *et al.* (5) reported that monoclonal anti-IL-1 receptor antibody partially blocks the H-P-A response to LPS in mice (5). In addition, depletion of cytokines, in particular IL-1, by destruction of macrophages using liposome-encapsulated dichloromethylene diphosphonate blocks the H-P-A response to subpyrogenic amounts of LPS in rats (6).

Moreover, variations in the kinetics of appearance of IL-1, TNF, and IL-6 after LPS challenge have been observed (13, 15–21), suggesting that these cytokines may play different roles at different times. TNF levels were consistently found to peak approximately 1 h after LPS administration and then rapidly declined (13, 15–20), in part probably because TNF release is especially sensitive to negative feedback by the glucocorticoid end product of H-P-A activation (18, 19). In contrast, IL-1 and IL-6 levels were found to peak somewhat later (within 2–4 h) and were sustained longer (15, 17–19, 21). It was, therefore, postulated that TNF initiates, while IL-1 and IL-6 sustain, H-P-A activation after LPS

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exposure (8).

In this report, we present results which indicate that IL-1, IL-6, and TNF are required for LPS-induced ACTH induction, and their relative contributions depend on the time interval after LPS challenge.

Materials and Methods

Experimental animals

Female C3H/HeN mice were purchased from the Animal Genetics and Production Branch, NCI (Frederick, MD). Mice were handled as previously described (10).

In the first set of experiments, groups of four to six mice were injected ip with vehicle (0.5 ml pyrogen-free normal saline), control antibody, or antibodies directed against the IL-1 receptor, IL-6, or TNF at 1630 h on day 1. At 0800 h the next morning (day 2), LPS was administered ip to all of the pretreated groups as well as a group that had not received any pretreatment. Either 2 or 4 h later, unanesthetized mice were decapitated (model 130 Rodent Decapitator, Harvard Apparatus, South Natick, MA) with minimal stress to obtain plasma samples for ACTH.

In the second set of experiments, groups of four to six mice were injected ip with vehicle, recombinant human IL-1α (rhIL-1α), rhIL-6, recombinant human TNFα (rhTNFα), or combinations of these cytokines and decapitated 30–180 min later. In a final set of experiments, groups of four to six mice were pretreated with vehicle and antibodies on day 1, as described in the preceding paragraph, injected with a combination of rhIL-1α and rhTNFα at 0800 h on day 2, and decapitated 120 min later.

In addition, 5–10 noninjected control mice were killed on the day of each experiment.

Cytokines and LPS

rhIL-1α (117-271 Ro 24-5008, lot IL-1 2/88; SA, 3×10^8 U/mg) was generously provided by Dr. Peter Lomedico, Hoffman LaRoche, Inc. (Nutley, NJ). rhIL-6 (SDZ 280-969, batch PPG 9001; SA, 5.2×10^7 U/mg) was a gift from Dr. E. Liehl, Sandoz, Vienna, Austria). rhTNFα (lot CP4026P08; SA, 9.6×10^6 U/mg) was provided by Biogen (Cambridge, MA). LPS (protein free; prepared from *Escherichia coli* K235 by the phenol-water extraction method) was kindly provided by Dr. Stefanie Vogel, Uniformed Services University of the Health Sciences (Bethesda, MD). The recombinant cytokines were diluted in 0.5 ml pyrogen-free saline on the day of injection.

Antibodies

Rat monoclonal antibody to mouse rIL-6 (MP5 20F3) was prepared using semipurified Cos-7 mouse IL-6 as an immunogen, as previously described (22). Rat monoclonal antibody to β-galactosidase (GL 113) was used as an isotype control. Rat monoclonal immunoglobulin G1, antimurine IL-1 receptor (anti-IL-1R) antibody (35F5) (23) was generously provided by Dr. R. Chizzonite, Hoffman LaRoche. Hamster monoclonal antibody to murine TNFα (TN3.19.12) (24) was a kind gift from Dr. R. Schreiber, Washington University (St. Louis, MO). The antibodies were diluted in 0.5 ml pyrogen-free saline on the day of injection. The amount of antibody injected (anti-IL-6, anti-IL-1R, and anti-TNF) was approximately the same as the quantity we used in earlier work to block LPS-, IL-1-, and TNF-induced radioprotection (11, 25). Moreover, the amount of anti-IL-1R antibody used (250 μg) was similar to the quantity of the same antibody (200 μg) found to be effective by Rivier *et al.* (5) in partially blocking LPS-induced ACTH release and reducing by 90% IL-1-induced leucocytosis. None of the antibodies injected by themselves had an effect on ACTH release.

Measurement of ACTH in plasma

ACTH was assayed in plasma from decapitated mice using an ^{125}I RIA kit (INCSTAR Corp., Stillwater, MN), as previously described (10). The ACTH antibody used in this assay is derived from rabbits immunized against ACTH-(1–24), a region that is identical in human and murine ACTHs. The threshold sensitivity of this assay was 8 pg/ml.

Statistical analysis

In Figs. 1 and 3, evaluation of the results was carried out using analysis of variance, followed by the Scheffe F test. In Figs. 2 and 4, comparison of the response to each cytokine treatment at each time point with the response to simultaneously injected vehicle was made using Student's *t* test. Comparison of the response to combined cytokine treatment with the sum of the responses to each cytokine treatment given separately at each time point was made as a 1 degree of freedom contrast. For each time point, each *P* value stated reflects a Bonferroni correction for the number of tests run.

Results

LPS-induced ACTH release

The ACTH levels in the plasma of mice receiving various amounts of LPS at 2, 4, and 6 h are presented in Table 1. The administration of all doses of LPS resulted in a maximal ACTH response at 2 h, which progressively diminished at 4 and 6 h. All maximal ACTH responses at 2 h were similar. Therefore, we chose 1 μg LPS to study the modulation of the 2 h ACTH response to LPS. The 4 h ACTH response was similar after 5–50 μg LPS. Therefore, we chose 5 μg LPS to study the modulation of the 4 h ACTH response to LPS. The 6 h ACTH response after all doses of LPS injected was not substantial enough to allow further study. Thus, the magnitude of the ACTH response to LPS in C3H/HeN mice is less than that observed in BALB/c mice by Rivier *et al.* (5). This probably is related to genetic differences between these two strains.

Effect of antibody pretreatment on the plasma level of ACTH 2 h after challenge with LPS

Figure 1A demonstrates the effect of pretreatment with anti-IL-6 antibody, anti-IL1R antibody, anti-TNF antibody, the combination of anti-IL-1R antibody and anti-TNF antibody, or antigalactosidase antibody on the 2 h ACTH response to 1 μg LPS. Pretreatment with anti-IL-6 antibody completely blocked the response to LPS, while the combination of anti-TNF antibody and anti-IL-1R antibody only partially blocked the response. In contrast, pretreatment with

TABLE 1. Plasma ACTH levels after ip injection of LPS

LPS dose (μg)	2 h	4 h	6 h
Vehicle	69.2 ± 8.8	66.3 ± 8.51	68.6 ± 3.86
1	185.7 ± 6.0	109.8 ± 8.4	65.6 ± 6.3
2	211.0 ± 18.7	102.0 ± 5.8	65.0 ± 3.0
5	166.8 ± 15.8	142.9 ± 7.6	69.4 ± 5.3
10	170.8 ± 7.3	130.4 ± 4.3	61.0 ± 2.9
25	178.2 ± 12.2	135.6 ± 8.7	102.0 ± 17.9
50	170.0 ± 15.0	145.0 ± 11.1	105.0 ± 9.4

Values are expressed as picograms per ml. Female C3H/HeN mice received various amounts of LPS ip and then were decapitated to obtain plasma for ACTH measurements 2, 4, or 6 h later. Each value shown is the mean ± SEM for 5 animals, except for the vehicle values, which represent 10 animals each.

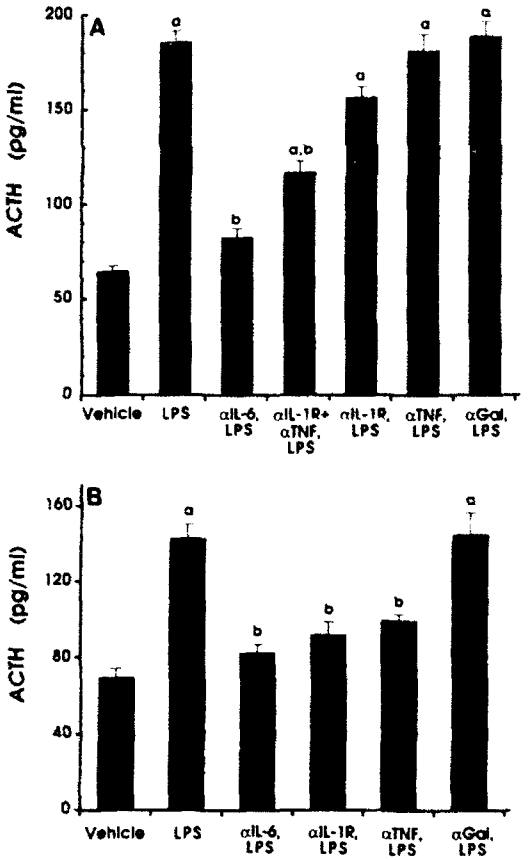

FIG. 1. C3H/HeN mice received ip injections of antibody [600 μg anti-IL-6 (αIL-6), 600 μg α-galactosidase (αGal), 250 μg αIL-1R, 100 μg αTNF, or 250 μg αIL-1R and 100 μg αTNF combined] 16 h before ip challenge with 1 μg LPS (A) or 5 μg LPS (B). Other mice were administered vehicle, 1 μg LPS (A), or 5 μg LPS (B) without antibody pretreatment. Blood samples were obtained 2 h (A) or 4 h (B) after LPS or vehicle alone. Each *bar* represents the mean ± SEM for 8–34 animals. a, $P < 0.05$ *vs.* vehicle alone; b, $P < 0.05$ *vs.* αGal plus LPS.

anti-TNF, anti-IL-1R, or control antibody did not attenuate the ACTH response.

Effect of antibody pretreatment on the plasma level of ACTH 4 h after challenge with LPS

Figure 1B demonstrates the effect of pretreatment with anti-IL-6 antibody, anti-IL-1R antibody, anti-TNF antibody, or antigalactosidase antibody on the 4 h ACTH response to 5 μg LPS. Pretreatment with any of the three anticytokine antibodies alone substantially blocked the ACTH response to LPS, while pretreatment with control antibody had no effect. All of the anticytokine antibodies were equally effective.

Release of ACTH after the injection of a combination of rhIL-1α and rhTNFα

Preliminary experiments indicated that rhTNFα administered ip to mice by itself induced a minimal ACTH response. Therefore, we examined the effect of the combined injection of suboptimal amounts of rhIL-1α and rhTNFα. Combined administration of 10 ng rhIL-1α and 1 μg rhTNFα resulted in a significant increase in circulating ACTH at 30, 60, 120, and 180 min compared with the response to simultaneously injected vehicle (Fig. 2). The responses to simultaneously injected vehicle were inconsequential (Fig. 2). When the responses to the rhIL-1/rhTNF combination were compared with those achieved with 10 ng rhIL-1α or 1 μg rhTNFα given separately, the responses to the combined injection were significantly greater than the sum of the responses to each cytokine injected alone at 120 and 180 min (Fig. 2).

Effect of antibody pretreatment on plasma ACTH 2 h after the combined injection of rhIL-1α and rhTNFα

Figure 3 demonstrates the effect of pretreatment with anti-IL-6 antibody, anti-IL-1R antibody, or antigalactosidase antibody on the 2 h ACTH response to the combined injection of 10 ng rhIL-1α and 1 μg rhTNFα. Pretreatment with anti-IL-6 antibody was as effective as anti-IL-1R antibody in blocking the ACTH response to the combined rhIL-1/rhTNF injection. Pretreatment with either of these antibodies produced a significant decline compared to pretreatment with control antibody.

Release of ACTH after the injection of a combination of rhTNFα and rhIL-6

We previously observed that suboptimal amounts of rhIL-1α and rhIL-6 synergistically stimulate the release of ACTH (12). To determine whether a similar interaction occurs between rhTNFα and rhIL-6, we evaluated the effect of the combined injection of rhTNFα and rhIL-6. After the combined administration of 1 μg rhTNFα and 1.25 μg rhIL-6, a significant increase in circulating ACTH was observed at 30, 60, 120, and 180 min compared with the response to simultaneously injected vehicle (Fig. 4). The responses to simultaneously injected vehicle were inconsequential (Fig. 4).

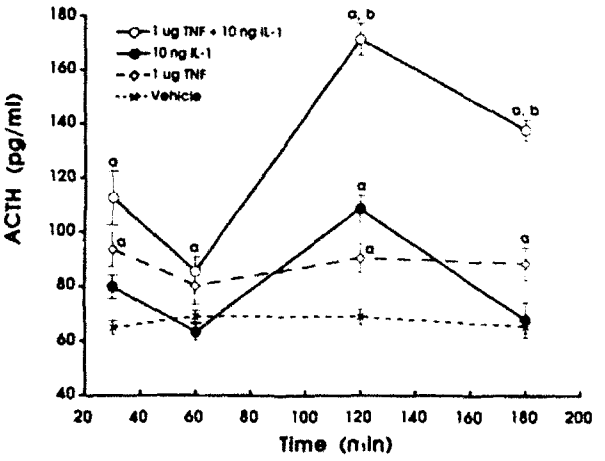

FIG. 2. Comparison of the time course of increase in plasma ACTH in C3H/HeN mice after ip injection of 10 ng rhIL-1α combined with 1 μg rhTNFα, 10 ng rhIL-1α, or 1 μg rhTNFα. The mean vehicle responses at each time point are also shown. Each time point represents the mean ± SEM of hormone determinations for 6–28 animals. a, $P < 0.05$ *vs.* the response to simultaneously injected vehicle; b, $P < 0.05$ *vs.* the sum of the responses to rhIL-1α and rhTNFα injected separately.

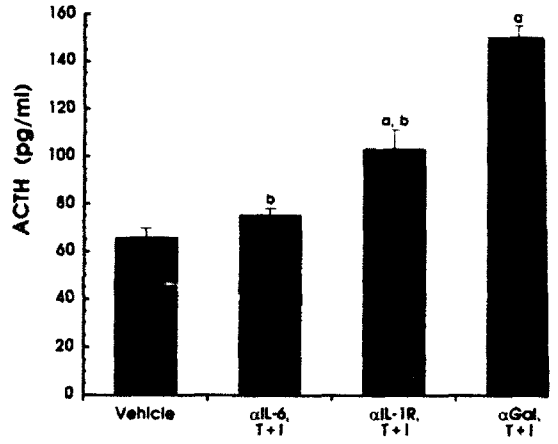

FIG. 3. C3H/HeN mice received ip injection of antibody [600 μg anti-IL-6 (αIL-6), 600 μg α-galactosidase (αGal), or 250 μg αIL-1R] 16 h before ip injection of 10 ng rhIL-1α combined with 1 μg rhTNFα (T+I). Other mice were administered vehicle without antibody pretreatment. Blood samples were obtained 2 h after T+I or vehicle alone. Each *bar* represents the mean ± SEM for 13–15 animals. a, $P < 0.05$ *vs.* vehicle alone; b, $P < 0.05$ *vs.* αGal plus T+I.

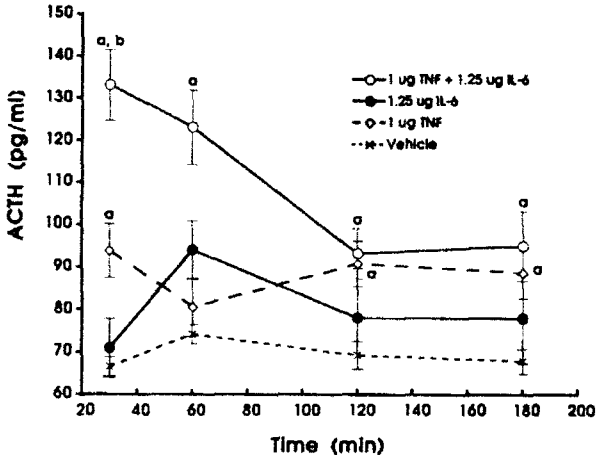

FIG. 4. Comparison of the time course of increase in plasma ACTH in C3H/HeN mice after ip injection of 1 μg rhTNFα combined with 1.25 μg rhIL-6, 1 μg rhTNFα, or 1.25 μg rhIL-6. The mean vehicle responses at each time point are also shown. Each time point represents the mean ± SEM of hormone determinations for 7–28 animals. a, $P < 0.05$ *vs.* the response to simultaneously injected vehicle; b, $P < 0.05$ *vs.* the responses to rhTNFα or rhIL-6 injected separately.

When the early responses to the rhTNF/rhIL-6 combination were compared with those to 1 μg rhTNFα or 1.25 μg rhIL-6 given separately, the responses to the combined injection were significantly greater than the responses to each cytokine injected alone (but not significantly greater than the sum of the responses to each cytokine injected alone; Fig. 4).

Discussion

Previous studies demonstrate that IL-1, IL-6, and TNF each stimulate the H-P-A axis *in vivo* via a CRH-dependent mechanism (7–9, 26–32) and *in vitro* at the level of the hypothalamus and pituitary (7–9, 27, 33, 34). On a molar basis, IL-1 is a more potent stimulator than TNF or IL-6 (31, 35). Our results demonstrate that IL-6 plays a fundamental role in LPS-induced ACTH release, but the participation and interaction of IL-1 and TNF are also required. In addition, the relative importance of these three cytokines varies at different times after LPS challenge.

Pretreatment with anti-IL-6 antibody completely abrogated the ACTH response to LPS 2 and 4 h after injection. Furthermore, the synergistic induction of ACTH after the combined injection of rhTNFα and rhIL-1α was blocked by anti-IL-6 antibody.

Inexplicably, although we were able to eliminate LPS-induced ACTH responses by pretreatment with anti-IL-6 antibody, ip administration of large doses (10 μg) of IL-6 to mice elicited only a minimal response (10). This suggests that IL-6 in the circulation may require an additional factor(s) to induce ACTH release. Alternatively, it is possible that systemic IL-6 does not reach the necessary local site(s) in the brain, whereas the anti-IL-6 antibody neutralizes LPS- or IL-1-stimulated IL-6 produced in the hypothalamus and/or pituitary gland (36–39). If a cofactor(s) is required for IL-6 to stimulate ACTH release, it is not clear at what level the interaction takes place, *e.g.* at the target cell level or due to facilitated transport across the blood-brain barrier.

Our results suggest that both IL-1 and TNF play important roles as IL-6 cofactors. The ACTH response 2 h after LPS challenge was not blocked by pretreatment with anti-IL-1R or anti-TNF antibody given separately, but was diminished by the combination of these antibodies; moreover, pretreatment with either anti-IL-1R or anti-TNF antibody alone totally blocked the 4 h ACTH response. Our previous observations that IL-1 and IL-6 synergize in inducing ACTH release (10) and that the 2 h ACTH response to IL-1 may be dependent upon an obligatory interaction between IL-1 and the IL-6 it induces endogenously (11) further suggest that IL-1 is an important cosecretagogue for IL-6 in stimulating the H-P-A axis. The coinjection of rhTNFα and rhIL-6 resulted in a greatly augmented (but not synergistic) ACTH response, suggesting a lesser role for TNF-IL-6 interaction in ACTH induction.

Since anti-IL-6 antibody totally abrogated ACTH release, while the combination of anti-IL-1R and anti-TNF antibodies only partially blocked the ACTH response 2 h after LPS administration, it is possible that in addition to IL-1 and TNF, other factors cooperate with IL-6. Among these, the arachidonic acid cascade metabolites, *i.e.* prostaglandins, leukotrienes, and epoxygenase products, which have been shown to modulate CRH release from the hypothalamus (40) and ACTH release from the pituitary (41) *in vitro*, seem likely candidates. Other possible factors are histamine (3) and IL-2 (8, 9, 42).

In addition to directly stimulating the hypothalamus and pituitary in conjunction with IL-6, LPS-induced IL-1 and TNF also contribute to stimulation of the H-P-A axis by inducing IL-6 production. In contrast to observations with anti-IL-6 antibody, pretreatment with either anti-IL-1R or anti-TNF antibody blocked the 4 h, but not the 2 h, ACTH response to LPS. The greater efficacy of these antibodies at 4 h may be due in part to their ability to interfere with TNF/

IL-1 induction of IL-6. There is ample evidence that LPS-induced elevation of IL-6 depends upon IL-1 and TNF induced by LPS. LPS stimulates the release of IL-1 and TNF *in vitro* (43, 44), including the production of IL-1 in the hypothalamus and pituitary (45, 46) and TNF in central nervous system microglial cells (47). *In vivo*, serum levels of TNF peak before IL-1 and IL-6 after LPS administration (13, 15–21). TNF and IL-1, in turn, both stimulate the release of IL-6 (11–13, 43, 44, 48–50). TNF is a much less potent inducer of IL-6 than IL-1 in mice (11). This may help to explain why ip injection of TNF stimulated only a minimal ACTH response in mice, in contrast to reports of more substantial ACTH responses after iv TNF administration to rats (32, 51). At the local level, subpopulations of nonneuronal cells in both the hypothalamus (36) and pituitary (37, 38) of rats spontaneously produce IL-6, and IL-1 enhances the release of IL-6 from cultures of rat anterior pituitary cells (39).

The synergistic induction of ACTH after coinjection of rhIL-1α and rhTNFα was completely blocked by pretreatment with anti-IL-6 antibody, suggesting that these cytokines synergistically induced IL-6 to produce the ACTH response. Indeed, recent *in vivo* (13) and *in vitro* (52) studies have demonstrated that IL-1 and TNF can synergistically stimulate IL-6 production.

Previous work employing blocking antibodies to TNF or IL-1 provides further support for the hypothesis that increased IL-6 levels during inflammation are dependent on TNF and IL-1. Results from our own laboratory (unpublished), as well as reports by a number of other investigators (13, 15, 17, 22) show that pretreatment with anti-TNF antibody substantially diminished IL-6 2–4 h after administration of LPS [as well as LPS-induced increases in IL-1 (15, 17)]. In addition, we observed that pretreatment with anti-IL-1R antibody markedly diminished the IL-6 response 4 h after LPS administration (unpublished). Similarly, pretreatment with anti-IL-1R antibody significantly attenuated the plasma IL-6 response to a turpentine-induced sterile abscess in mice (53).

It is also likely that LPS directly stimulates the release of IL-6, especially 2 h after injection. The ability of LPS to induce IL-6 in various cell cultures has been observed by a number of investigators (54, 55), including nonneuronal cells in the hypothalamus (36) and pituitary (37, 39), and Romero *et al.* (56) reported that IL-1 receptor antagonist blocks IL-1β-induced, but not LPS-induced, IL-6 release from cultures of rat anterior pituitary cells. In addition, in a recent *in vivo* study, IL-1 receptor antagonist did not block IL-6 induction after the administration of sublethal amounts of LPS (57).

In summary, in mice injected with sublethal amounts of LPS, IL-6, IL-1, and TNF play different roles in initiating and sustaining an ACTH response. The presence of IL-6, derived from the direct effects of LPS and/or its induction by TNF/IL-1, is obligatory at both time points studied. However, to elicit an ACTH response, an interaction with another factor may be required. IL-1 and TNF appear to be essential in sustaining the IL-6 levels required to maintain an ACTH response, especially 4 h after LPS administration and, in addition, may play an interactive role with IL-6 at both time points. The definitive explanation of how these cytokines mediate the activation of the H-P-A axis by LPS will have to take into account the contribution of cytokines produced in the hypothalamus and pituitary gland, and how and if they are induced by circulating cytokines originating in the periphery.

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References

1. **Dinarello CA** 1984 Interleukin-1 and the pathogenesis of the acute-phase response. N Engl J Med 311:1413–1418
2. **Sibbald WJ, Short A, Cohen MP, Wilson RF** 1977 Variations in adrenocortical responsiveness during severe bacterial infections. Unrecognized adrenocortical insufficiency in severe bacterial infections. Ann Surg 186:29–33
3. **Nakano K, Suzuki S, Oh C** 1987 Significance of increased secretion of glucocorticoids in mice and rats injected with bacterial endotoxin. Brain Behav Immunol 1:159–172
4. **Yasuda N, Greer MA** 1978 Evidence that the hypothalamus mediates endotoxin stimulation of adrenocorticotropic hormone secretion. Endocrinology 102:947–953
5. **Rivier C, Chizzonite R, Vale W** 1989 In the mouse, the activation of the hypothalamic-pituitary-adrenal axis by a lipopolysaccharide (endotoxin) is mediated through interleukin-1. Endocrinology 125:2800–2805
6. **Derijk R, Van Rooijen N, Tilders FJH, Besedovsky HO, Del Rey A, Berkenbosch F** 1991 Selective depletion of macrophages prevents pituitary-adrenal activation in response to subpyrogenic, but not to pyrogenic, doses of bacterial endotoxin in rats. Endocrinology 129:330–338
7. **Whitnall MH**, Regulation of the hypothalamic corticotropin-releasing hormone neurosecretory system. Prog Neurobiol, in press
8. **Eskay RL, Grino M, Chen HT** 1990 Interleukins, signal transduction, and the immune system-mediated stress response. Adv Exp Med Biol 274:331–343
9. **Imura H, Fukata J, Mori T** 1991 Cytokines and endocrine function—an interaction between the immune and neuroendocrine systems—review. Clin Endocrinol (Oxf) 35:107–115
10. **Perlstein RS, Mougey EH, Jackson WE, Neta R** 1991 Interleukin-1 and interleukin-6 act synergistically to stimulate the release of adrenocorticotropic hormone *in vivo*. Lymphokine Cytokine Res 10:141–146
11. **Neta R, Perlstein R, Vogel SN, Ledney GD, Abrams J** 1992 Role of interleukin 6 (IL-6) in protection from lethal irradiation and in endocrine responses to IL-1 and tumor necrosis factor. J Exp Med 175:689–694
12. **Neta R, Vogel SN, Sipe JD, Wong GG, Nordan RP** 1988 Comparison of *in vivo* effects of human recombinant IL 1 and human recombinant IL 6 in mice. Lymphokine Res 7:403–412
13. **Shalaby MR, Waage A, Aarden L, Espevik T** 1989 Endotoxin, tumor necrosis factor-α and interleukin 1 induce interleukin 6 production *in vivo*. Clin Immunol Immunopathol 53:488–498
14. **Vogel SN, Hogan MM** 1990 Role of cytokines in endotoxin-mediated host responses. In: Oppenheim JJ, Shevach EN (eds) Immunophysiology, Role of Cells and Cytokines in Immunity and Inflammation. Oxford University Press, Oxford, pp 238–258
15. **Fong Y, Tracey KJ, Moldawer LL, Hesse DG, Manogue KB, Kenney JS, Lee AT, Kuo GC, Allison AC, Lowry SF, Cerami A**

1989 Antibodies to cachectin/tumor necrosis factor reduce interleukin 1β and interleukin 6 appearance during lethal bacteremia. J Exp Med 170:1627–1633
16. **Waage A, Halstensen A, Shalaby MR, Brandtzaeg P, Kierulf P, Espevik T** 1989 Local production of tumor necrosis factor alpha, interleukin 1, and interleukin 6 in meningococcal meningitis. Relation to the inflammatory response. J Exp Med 170:1859–1867
17. **Zanetti G, Heumann D, Gerain J, Kohler J, Abbet P, Barras C, Lucas R, Glauser M-P, Baumgartner J-D** 1992 Cytokine production after intravenous or peritoneal gram negative bacterial challenge in mice. J Immunol 148:1890–1897
18. **Chensue SW, Terebuh PD, Remick DG, Scales WE, Kunkel SL** 1991 *In vivo* biologic and immunohistochemical analysis of interleukin-1 alpha, beta and tumor necrosis factor during experimental endotoxemia. Am J Pathol 138:395–402
19. **Zuckerman SH, Shellhaas J, Butler LD** 1989 Differential regulation of lipopolysaccharide-induced interleukin 1 and tumor necrosis factor synthesis: effects of endogenous and exogenous glucocorticoids and the role of the pituitary-adrenal axis. Eur J Immunol 19:301–305
20. **Michie HR, Manogue KR, Spriggs DR, Revhaug A, O'Dwyer S, Dinarello CA, Cerami A, Wolff SM, Wilmore DW** 1988 Detection of circulating tumor necrosis factor after endotoxin administration. N Engl J Med 318:1481–1486
21. **Fong Y, Moldawer LL, Marano M, Wei H, Tatter SB, Clarick RH, Santhanam U, Sherris D, May LT, Sehgal PB, Lowry SF** 1989 Endotoxemia elicits increased circulating β2-IFN/IL-6 in man. J Immunol 142:2321–2324
22. **Starnes HF, Pearce MK, Tewari A, Yim JH, Zou J-C, Abrams JS** 1990 Anti-IL-6 monoclonal antibodies protect against lethal *Escherichia coli* infection and lethal tumor necrosis factor-α challenge in mice. J Immunol 145:4185–4191
23. **Chizzonite R, Truitt T, Kiliar. PL, Stern AS, Nunes P, Parker KP, Kaffka KL, Chua AO, Lugg DK, Gubler U** 1989 Two high-affinity interleukin-1 receptors represent separate gene products. Proc Natl Acad Sci USA 86:8029–8033
24. **Sheehan KCF, Ruddle NH, Schreiber RD** 1989 Generation of hamster monoclonal antibodies that neutralize tumor necrosis factors. J Immunol 142:3884–3893
25. **Neta R, Oppenheim JJ, Schreiber RD, Chizzonite R, Ledney GD, MacVittie TJ** 1991 Role of cytokines (interleukin 1, tumor necrosis factor, and transforming growth factor β) in natural and lipopolysaccharide-enhanced radioresistance. J Exp Med 173:1177–1182
26. **Spangelo BL, MacLeod RM** 1990 Regulation of the acute phase response and neuroendocrine function by interleukin 6. Progr Neuroendocrin Immunol 3:167–175
27. **Lyson K, McCann SM** 1991 The effect of interleukin-6 on pituitary hormone release *in vivo* and *in vitro*. Neuroendocrinology 54:262–266
28. **Naitoh Y, Fukata J, Tominaga T, Nakai Y, Tamai S, Mori K, Imura H** 1988 Interleukin-6 stimulates the secretion of adrenocorticotropic hormone in conscious, freely-moving rats. Biochem Biophys Res Commun 155:1459–1463
29. **Whitnall MH, Perlstein RS, Mougey EH, Neta R** 1992 Effects of interleukin-1 on the stress-responsive and -nonresponsive subtypes of corticotropin-releasing hormone neurosecretory axons. Endocrinology 131:37–44
30. **Dunn AJ** 1990 Interleukin-1 as a stimulator of hormone secretion. Prog Neuroendocrin Immunol 3:26–34
31. **Besedovsky HO, Del Rey A, Sorkin E, Dinarello CA** 1986 Immunoregulatory feedback between interleukin-1 and glucocorticoid hormones. Science 233:652–654
32. **Bernardini R, Kamilaris TC, Calogero AE, Johnson EO, Gomez ET, Gold PW, Chrousos GP** 1990 Interactions between tumor necrosis factor-alpha, hypothalamic corticotropin-releasing hormone, and adrenocorticotropin secretion in the rat. Endocrinology 126:2876–2881
33. **Lyson K, McCann SM** 1992 Involvement of arachidonic acid cascade pathways in interleukin-6-stimulated corticotropin-releasing factor release *in vitro*. Neuroendocrinology 55:708–713
34. **Navarra P, Pozzoli G, Brunetti L, Ragazzoni E, Besser M, Grossman A** 1992 Interleukin-1β and interleukin-6 specifically increase the release of prostaglandin E_2 from rat hypothalamic explants *in vitro*. Neuroendocrinology 56:61–68
35. **Warren RS, Fletcher H, Starnes J, Alcock N, Calvano S, Brennan MF** 1988 Humoral and metabolic response to recombinant human tumor necrosis factor in rat: *in vitro* and *in vivo*. Am J Physiol 255:E206–E212
36. **Spangelo BL, Judd AM, MacLeod RM, Goodman DW, Isakson PC** 1990 Endotoxin-induced release of interleukin-6 from rat medial basal hypothalami. Endocrinology 127:1779–1785
37. **Spangelo BL, MacLeod RM, Isakson PC** 1990 Production of interleukin-6 by anterior pituitary cells *in vitro*. Endocrinology 127:582–586
38. **Vankelecom H, Carmeliet P, Van Damme J, Billiau A, Denef C** 1989 Production of interleukin-6 by folliculo-stellate cells of the anterior pituitary gland in a histiotypic cell aggregate culture system. Neuroendocrinology 49:102–106
39. **Spangelo BL, Judd AM, Isakson PC, MacLeod RM** 1991 Interleukin-1 stimulates interleukin-6 release from rat anterior pituitary cells *in vitro*. Endocrinology 128:2685–2692
40. **Bernardini R, Chiarenza A, Calogero AE, Gold PW, Chrousos GP** 1989 Arachidonic acid metabolites modulate rat hypothalamic corticotropin-releasing hormone secretion *in vitro*. Neuroendocrinology 50:708–715
41. **Cowell AM, Flower RJ, Buckingham JC** 1991 Studies on the roles of phospholipase A2 and eicosanoids in the regulation of corticotrophin secretion by rat pituitary cells *in vitro*. J Endocrinol 130:21–32
42. **Cambronero JC, Rivas FJ, Borrell J, Guaza C** 1992 Interleukin-2 induces corticotropin-releasing hormone release from superfused rat hypothalami: influence of glucocorticoids. Endocrinology 131:677–683
43. **Dinarello CA** 1991 Interleukin-1 and interleukin-1 antagonism. Blood 77:1627–1652
44. **Neta R, Sayers TJ, Oppenheim JJ** 1992 Relationship of TNF to interleukins. In: Aggarwal BB, Vilcek J (eds) Tumor Necrosis Factors: Structure, Function, and Mechanism of Action. Marcel Dekker, New York, pp 499–566
45. **Rettori V, Dees WL, Hiney JK, Milenkovic L, McCann SM.** Interleukin-1 alpha (IL-1α)-immunoreactive neurons in the hypothalamus of the rat are increased after lipopolysaccharide (LPS) injection. 74th Annual Meeting of The Endocrine Society, San Antonio TX, 1992, p 185 (Abstract 534)
46. **Koenig JI, Snow K, Clark BD, Toni R, Cannon JG, Shaw AR, Dinarello CA, Reichlin S, Lee SL, Lechan RM** 1990 Intrinsic pituitary interleukin-1 beta is induced by bacterial lipopolysaccharide. Endocrinology 126:3053–3058
47. **Ricciardi-Castagnoli P, Pirami L, Righi M, Sacerdote P, Locatelli V, Bianchi M, Sassano M, Valsasnini P, Shammah S, Panerai AE** 1990 Cellular sources and effects of tumor necrosis factor-α on pituitary cells and in the central nervous system. Ann NY Acad Sci 594:156–168
48. **Libert C, Brouckaert P, Shaw A, Fiers W** 1990 Induction of interleukin 6 by human and murine recombinant interleukin 1 in mice. Eur J Immunol 20:691–694
49. **McIntyre KW, Stepan GJ, Kolinsky KD, Benjamin WR, Plocinski JM, Kaffka KL, Campen CA, Chizzonite RA, Kilian PL** 1991 Inhibition of interleukin 1 (IL-1) binding and bioactivity *in vitro* and modulation of acute inflammation *in vivo* by IL-1 receptor antagonist and anti-IL-1 receptor monoclonal antibody. J Exp Med 173:931–939
50. **Mengozzi M, Bertini R, Sironi M, Ghezzi P** 1991 Inhibition by interleukin 1 receptor antagonist of *in vivo* activities of interleukin 1 in mice. Lymphokine Cytokine Res 10:405–407
51. **Sharp BM, Matta SG, Peterson PK, Newton R, Chao C, McAllen K** 1989 Tumor necrosis factor-alpha is a potent ACTH secretagogue. comparison to interleukin-1 beta. Endocrinology 124:3131–3133
52. **Benveniste EN, Sparacio SM, Norris JG, Grenett HE, Fuller GM** 1990 Induction and regulation of interleukin-6 gene expression in rat astrocytes. J Neuroimmunol 30:201–212
53. **Gershenwald JE, Fong YM, Fahey TJ, Calvano SE, Chizzonite R, Kilian PL, Lowry SF, Moldawer LL** 1990 Interleukin 1 receptor blockade attenuates the host inflammatory response. Proc Natl Acad Sci USA 87:4966–4970

54. **Kotloff RM, Little J, Elias JA** 1990 Human alveolar macrophage and blood monocyte interleukin-6 production. Am J Respir Cell Mol Biol 3:497–505
55. **Jirik FR, Podor TJ, Hirano T, Kishimoto T, Loskutoff DJ, Carson DA, Lotz M** 1989 Bacterial lipopolysaccharide and inflammatory mediators augment IL-6 secretion by human endothelial cells. J Immunol 142:144–147
56. **Romero LI, Lechan RM, Clark BD, Dinarello CA, Reichlin S**, IL-1 receptor antagonist inhibits hIL-1 beta but not bacterial lipopolysaccharide (LPS) stimulated IL-6 secretion by rat anterior pituitary cells. 73rd Annual Meeting of The Endocrine Society, Washington DC, 1991, p 150 (Abstract 479)
57. **Fischer E, Marano MA, Van Zee KJ, Rock CS, Hawes AS, Thompson WA, DeForge L, Kenney JS, Remick DG, Bloedow DC, Thompson RC, Lowry SF, Moldawer LL** 1992 Interleukin-1 receptor blockade improves survival and hemodynamic performance in *Escherichia coli* septic shock, but fails to alter host responses to sublethal endotoxemia. J Clin Invest 89:1551–1557