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Glutamate Augments Retrovirus-Induced Immunodeficiency Through Chronic Stimulation of the Hypothalamic-Pituitary-Adrenal Axis

Michael Graham Espey and Anthony S. Basile

The mechanisms for activating the hypothalamic-pituitary-adrenal (HPA) axis and the roles glucocorticoids play in the pathogenesis of chronic infectious disease are largely undefined. Using the LP-BM5 model of retrovirus-induced immunodeficiency, we found alterations in HPA axis function, manifested as an increase in circulating levels of adrenocorticotropic hormone and corticosterone, beginning after only 3 mo of infection. These changes occurred contemporaneously with a shift in the profile of circulating cytokines from a Th1-dominant (IFN-γ) to Th2-dominant (IL-4, IL-10) phenotype. No significant changes in either circulating IL-1β, IL-6, or TNF-α levels were observed in infected mice. Administering the N-methyl-D-aspartate receptor antagonist MK-801 to infected mice normalized plasma adrenocorticotropic hormone and corticosterone levels, indicating that glutamate was a major activator of the HPA axis. Moreover, MK-801 treatment of late-stage mice also reversed the type 1 to type 2 cytokine shift to a degree comparable or superior to treatment with the glucocorticoid receptor antagonist RU-486. These findings indicate that HPA axis activation during LP-BM5 retrovirus infection is mediated by the chronic hyperactivation of glutamatergic pathways in the hypothalamus. Through this mechanism, the degree of peripheral immunodeficiency observed in the late-stage disease is profoundly augmented. The Journal of Immunology, 1999, 162: 4998–5002.

A bidirectional relationship exists between the immune and neuroendocrine systems that governs both the character and extent of the cellular and humoral responses mounted against pathogens. A major route of communication between these two systems is the hypothalamic-pituitary-adrenal (HPA) axis. Activation of glutamatergic neurons in the paraventricular nuclei (PVN) of the hypothalamus (1–4) results in the release of corticotropin-releasing factor from terminals in the median eminence. Corticotropin-releasing factor reaches the anterior pituitary via the hypophysial portal circulation, stimulating corticotrophs to release adrenocorticotropic hormone (ACTH), which induces the secretion of glucocorticoids from the adrenal cortex. In addition to their effects on general metabolism, glucocorticoids act in feedback loops to modulate cytokine networks (5–9) and the proliferation, development, and trafficking of leukocytes (10–12).

Defining how specific mediators activate and are regulated by the HPA axis has been paramount in conceptualizing the neuroimmunologic mechanisms involved in infectious and autoimmune diseases. During the acute inflammatory response to infection, peripheral leukocytes produce IL-1β, IL-6, and TNF-α. These cytokines can activate the HPA axis either directly by stimulating ACTH release from the anterior pituitary (13–15) or indirectly by signal transduction cascades mediated through the cerebral vasculature (16–18) or those brain regions projecting to the PVN, including the ventrolateral medial hypothalamus, the area postrema, and the vagus/nucleus tractus solitarius (19, 20). Synthesis of acute inflammatory cytokines is subsequently down-regulated by the actions of glucocorticoid-coupled pathways (21), suppressing this phase of the immune response (7, 22).

Whether such a reciprocal relationship between cytokines and glucocorticoids continues to function in chronic infectious diseases has not been determined. Moreover, little information exists on the role of intrinsic mediators within the brain in activating the HPA axis under chronic pathologic conditions (23). Thus, we examined the status of cytokine networks and the HPA axis in mice infected with the LP-BM5 retrovirus mixture. LP-BM5-infected mice develop a progressive immunodeficiency syndrome that impacts both the immune and central nervous systems (CNS) over a course of 16 wk (24–34). During this time, mice infected with LP-BM5 develop many of the pathological features observed in humans with AIDS, including: impaired T and B lymphocyte responses to antigenic stimuli; enhanced susceptibility to infection; development of lymphoma and paraneoplasia; polyclonal B lymphocyte activation and expansion; and hypergammaglobulinemia. The effect of in vivo treatment with either the N-methyl-D-aspartate (NMDA) receptor antagonist MK-801 or the glucocorticoid type II receptor antagonist RU-486 on blood cytokine levels were compared to determine whether retrovirus-induced neurodegeneration mediated by glutamate indirectly influences the degree of immunodeficiency in the periphery by an HPA axis mechanism.

Materials and Methods

Animals

Male C57BL/6 mice (National Cancer Institute, Frederick, MD) were housed and fed according to National Institute of Health-American Association for the Accreditation of Laboratory Animal Care guidelines, which were also followed when the mice were sacrificed. These mice were inoculated i.p. at 4–6 wk of age with 0.1–0.2 ml of LP-BM5 murine leukemia virus stocks prepared from infected SC-1 embryonic fibroblasts (21, 29). Uninfected littermates of mice infected for 4–16 wk with LP-BM5 were
used in this investigation. Because the results obtained from these age-matched animals did not vary with time, the data from control animals was pooled for statistical purposes.

**Treatments**

The noncompetitive NMDA receptor antagonist (+)-MK-801 maleate (dizocilpine; Research Biochemicals, Natick, MA) was continuously administered via s.c. implanted osmotic minipumps (Alza, Palo Alto, CA) at a dosage of 1 mg/kg/day for 14 days. The glucocorticoid type II receptor antagonist RU-486 (mifepristone; Sigma-Aldrich, St. Louis, MO) was administered at a dose of 25 mg/kg/day for 7 days by osmotic minipumps (Alza). Control animals were implanted with minipumps containing vehicle (ethanol:Alkamuls EL-620 [Rhone-Poulenc, Cranbury, NJ]:water, 7:2:1). No significant differences were observed in ACTH and corticosterone levels in uninfected mice either with or without vehicle containing pump implants (data not shown). Cytokine-treated (uninfected) mice were sacrificed 2 h after receiving either 100 or 400 ng of IL-4 or IL-10 (R&D Systems, Minneapolis, MN) administered by i.p. injection.

**Blood collection**

Blood samples were taken between 9:00 and 10:30 a.m. Blood (250 μl) was collected from the right atrium of mice within 3–4 min of an i.p. injection of 1 mg sodium pentobarbitol and dispensed into a tube either with (plasma) or without (serum) heparin (1 USP Unit, Monoject Scientific, St. Louis, MO). Samples were placed on wet ice, then centrifuged at 750 × g for 4 min. The supernatant was retained and stored at −70°C until analyzed.

**Cytokine and hormone analysis**

Concentrations of ACTH and corticosterone in plasma were determined by ELISA (Peninsula Laboratories, Belmont, CA) and RIA (Amersham, Arlington Heights, IL), respectively. Plasma samples were heated to 60°C for 30 min before analysis of corticosterone. Levels of IL-1β, IL-4, IL-6, IL-10, IFN-γ, and TNF-α in serum were determined by ELISA (Endogen, Woburn, MA; IL-1β, CYT Immune, Gaithersburg, MD).

**Results**

**HPA axis activation coincides with cytokine shifts**

The time-course of changes in cytokine levels in the peripheral blood of mice infected with LP-BM5 are shown in Fig. 1. Serum IFN-γ concentrations were elevated early in the disease course and persisted at a level of 600% above control values until 12 wk postinoculation (PI) (Fig. 1A). Following this period, a downward trend in IFN-γ levels was observed, and these values were no longer significantly different from control. In contrast with the end-stage levels of IFN-γ, a reciprocal pattern was observed in the concentrations of IL-4 and IL-10 (Fig. 1, B and C). Significant increases in the serum concentrations of these cytokines were evident beginning at 12 wk PI, rising to 3500% and 3600%, respectively, above control levels in end-stage mice. Surprisingly, HPA axis hormones were not elevated until the last 2 wk of the infection course (Fig. 2), when plasma levels of ACTH were increased 160% and 200% above control at 14 and 16 wk PI and corticosterone levels were raised 90% and 130% above control at these time points.

**HPA axis activation is not cytokine mediated**

Because the rise in IL-4 and IL-10 titers was accompanied by a significant increase in the circulating levels of both ACTH and corticosterone, the capacity of these cytokines to activate the HPA axis was tested. Peripheral administration of either IL-1β or TNF-α stimulates the HPA axis in a linear, dose-dependent manner (20). A bolus injection of either IL-4 or IL-10 at concentrations 100-fold higher than those observed in the peripheral blood of mice with end-stage LP-BM5 infection had no significant effect (p < 0.05; one-way ANOVA, Dunnett’s multiple comparison test; n = 6) on plasma concentrations of either ACTH (saline, 2.7 ± 0.2; IL-4, 1.7 ± 0.2; IL-10, 2.2 ± 0.2) or corticosterone (saline, 50.5 ± 9.4; IL-4, 64.8 ± 20.0; IL-10, 49.5 ± 7.5) in normal mice.

**FIGURE 1.** Time course of changes in cytokine levels following infection with LP-BM5. The concentration (pg/ml ± SEM) of IFN-γ (A), IL-4 (B), and IL-10 (C) were determined in serum samples collected from C57BL/6 mice infected from 0 to 16 wk with LP-BM5 by ELISA as described in Materials and Methods. *, p < 0.05; **, p < 0.01, significantly different from control (t = 0) values; one-way ANOVA followed by Tukey’s multiple comparison test (n = 6–32/time point).

The ability of IL-1β, IL-6, and TNF-α to stimulate the HPA axis has been previously reported (7). Nonetheless, high serum levels of either IL-1β, IL-6, or TNF-α were only detected in an insignificant percentage (5–15%; p > 0.05; one-way ANOVA, Dunnett’s multiple comparison test; n = 30/cytokine) of mice infected with LP-BM5 (data not shown), and may reflect paracrine or transient expression of these cytokines peripherally. Although cytokines synthesized within the brain in response to systemic inflammation can modulate HPA axis function (e.g., IL-1β; Ref. 23), in situ hybridization and PCR studies indicate that de novo synthesis of cytokines does not occur in the CNS during LP-BM5 infection (IL-1β, TNF-α, IFN-γ; M. Herkenham and Y. Sei, unpublished observations). Thus, cytokines, whether of central or peripheral origin, do not appear to be involved in the dysregulation of the HPA axis observed in LP-BM5-infected mice.

**Glutamate receptor antagonist treatment blocks HPA axis activation**

Infection with LP-BM5 leads to gliosis and neurodegenerative changes consistent with hyperactivation of glutamatergic pathways...
(29–33) and coincide with increased levels of extracellular glutamate in the brain parenchyma and cerebrospinal fluid (34). The involvement of CNS glutamate in HPA axis activation was investigated by administering the NMDA receptor antagonist MK-801 to mice infected with LP-BM5 for 12 wk. Plasma ACTH levels were reduced 40% and corticosterone levels were decreased 75% following MK-801 administration for 2 wk (Fig. 3). The effect of MK-801 on the basal concentrations of ACTH and corticosterone in uninfected mice was less pronounced, reducing these levels 7% and 40%, respectively.

CNS glutamate indirectly augments immunodeficiency through the HPA axis

The effects of treatment with either MK-801 or the glucocorticoid type II receptor antagonist RU-486 on the aberrant serum cytokine profile in mice with late-stage disease (14 wk PI) are compared in Fig. 4. MK-801 administration increased serum IFN-γ levels 215% above the titer observed in untreated mice infected with LP-BM5. While IFN-γ levels also increased following RU-486 treatment (81% above values in untreated infected mice), this change was not significant. In contrast to the augmentation of IFN-γ levels by MK-801 in late-stage mice, serum IL-4 and IL-10 levels were reduced ~60%. RU-486 was slightly more effective than MK-801 in reducing IL-4 concentration (~78%) and had a comparable effect on IL-10 levels (~62%). Neither MK-801 nor RU-486 significantly altered basal peripheral blood cytokine concentrations in uninfected mice. The parallel results with either MK-801 or RU-486 treatment support the hypothesis that the effects of MK-801 are mediated through its inhibition of tonic HPA axis stimulation by glutamate, subsequently decreasing both ACTH and glucocorticoid production.

Discussion

The present studies demonstrate that chronic stimulation of NMDA receptors in the CNS during retrovirus infection hyperactivates the HPA axis. Glutamate is an intrinsic activator of neurosecretory cells in the hypothalamus (1–4). Met-enkephalin and substance P levels are significantly depleted and the expression of constitutive nitric oxide synthase is decreased in the hypothalamus after 8 wk of infection with LP-BM5, a decline that continues as the disease progresses (32, 35). Concurrent with these changes in neurotransmitter and second messenger systems are increases in glutamate concentrations in the cerebrospinal fluid and extracellular spaces of the brain (34), which may lead to the chronic hyperactivation of the PVN, increasing blood levels of ACTH, and corticosterone. Peripheral manifestations of disease, such as lymphadenopathy and splenomegaly (24, 28), may also contribute to the activation of the HPA axis by tonically exciting vagal afferent fibers, which would chronically stimulate the PVN via the nucleus tractus solitarius.

The nature and duration of pathogenic stimulation greatly influence the role the HPA axis may play in disease outcome. In resolving bacterial and viral infections, glucocorticoid-coupled diminution of type 1 cytokine synthesis (7, 22, 36) and leukocyte
Directly suppress IFN-γ lymphocyte growth and clonal expansion (40–42), ACTH can.

Steroids are a major factor contributing to the type 2 cytokine patterns. The dramatic reduction in IL-4 and IL-10 levels following RU-486 treatment suggests that glucocorticoids caused by virus infection. The dramatic reduction in IL-4 and IL-10 levels following RU-486 treatment suggests that glucocorticoids are essential for the recovery or expansion of leukocyte populations into type 2-biased phenotypes through the actions of both ACTH and glucocorticoids (6, 22, 29, 43). These findings have important therapeutic implications, particularly in dealing with syndromes such as AIDS dementia complex, where chronic glucocorticoid hyperactivity in the CNS is superimposed upon a peripheral immunodeficiency.

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**References**


GLUTAMATERGIC ACTIVATION OF THE HPA AXIS


