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VIRUS AND HOST FACTORS THAT MEDIATE THE CLINICAL AND BEHAVIORAL SIGNS OF EXPERIMENTAL HERPETIC ENCEPHALITIS

A SHORT AUTO-REVIEW*

T. BEN-HUR, R. CIALIC and J. WEIDENFELD

Department of Neurology, The Agnes Ginges Center for Human Neurogenetics, Hadassah-Hebrew University Hospital, Jerusalem, Israel

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Experimental models that mimic the clinical syndrome of human viral encephalitis and represent HSV-1 neurotropism were utilized to investigate neuro-pharmacologic changes mediating clinical and behavioral manifestations of encephalitic infection of the central nervous system with HSV-1-induced rapid activation of the hypothalamic – pituitary – adrenocortical (HPA) axis and production of brain derived interleukin-1 (IL-1) and prostaglandin E_2 (PG-E_2), independently of viral replication. HSV-1 infection induced clinical signs of fever, motor hyperactivity and aggressive behavior. These manifestations were dependent on a permissive action of circulating glucocorticoids and not related to the degree of viral replication in the brain. Hyperthermia and HPA axis activation were also specifically dependent on HSV-1-induced brain IL-1 and PG-E_2. The chronic neurological sequel or fatal outcome of HSV-1 encephalitis may be due to viral replication and brain tissue destruction, which are dependent on virus encoded virulence genes. In contrast, the clinical and behavioral signs in the acute phase are a result of activation of neurochemical systems, including cytokines, prostaglandinds and catecholamines. Circulating glucocorticoids play an essential role in mediating the physiologic actions of HSV-1-induced brain products and the clinical syndrome of encephalitis.

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Herpes simplex virus type 1 (HSV-1) is the most common cause of acute, non-epidemic viral encephalitis. The disease presents with fever, behavioral

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changes, such as psychotic state and focal neurological signs and it still carries significant morbidity and mortality despite antiviral therapy. Following inoculation to peripheral sites, such as the cornea, nasal epithelium, and skin, the virus travels by axonal transport to the brain and induces acute encephalitis. The pathogenesis of infection, clinical manifestations and outcome of disease are dependent on multiple factors, including viral genes, host factors and environmental factors. Studies from our laboratory and from others showed that several viral genes determine HSV-1 virulence for experimental animals [1, 2, 9, 13, 19]. These genes enable the virus to overcome host defense systems, to invade the nervous system and to induce lethal encephalitis. For example, we have mapped a DNA region in the viral genome, which enables the virus to replicate and induce a cytopathic effect in cells of the mononuclear-phagocytic system [6]. A virus strain that lacks this region is cleared rapidly by local macrophages after inoculation to the peritoneum. The viral ability for productive infection in the peritoneal macrophages (i.e. expression of viral immediate early genes, DNA replication and cytopathic effect) was rescued, along with partial virulence in an intratypic recombinant virus, in which this region (isolated from a virulent strain) had been inserted back [6]. Other viral genes were associated with neuroinvasiveness and neurovirulence [3, 10, 17]. While these viral genes were associated with their pathogenicity, as determined mainly by the fatal outcome of infection, the mechanisms by which HSV-1 causes the behavioral and clinical signs of encephalitis are not well studied. Specifically, little is known on host brain responses to HSV-1 infection in terms of the neuroanatomic sites and pathways and neurochemical or neuroendocrine systems that are activated during infection. It is not known how HSV-1 induces these host brain responses and in turn, how these responses are related to the production of the symptoms and signs of encephalitis.

It is well established now that the hypothalamic-pituitary-adrenocortical (HPA) axis is highly sensitive to viral and microbial infections. This axis consists of corticotrophin-releasing hormone (CRH) which is released in the median eminence from neurons of which their cell bodies are situated in the hypothalamic paraventricular nucleus (PVN). CRH induces the secretion of ACTH from the anterior pituitary, which in turn stimulates the secretion of glucocorticoids from the adrenal gland. Regulation of this neuroendocrine axis is dependent on several brain structures and pathways. The HPA axis can be activated by systemic stimuli, such as pro-inflammatory cytokines [15, 16], bacterial endotoxin [20], and also by various neurogenic stressful stimuli, such as immobilization, photic and acoustic stimuli. The HPA axis responses to these stimuli are mediated mainly by catecholaminergic and serotonergic pathways that originate in the brain stem [12, 24].

A central regulatory mechanism in this system is negative feedback exerted by serum glucocorticoids on CRH and ACTH secretion [12]. This circuit is tightly regulated by extra-hypothalamic structures, such as the hippocampus. The feedback inhibition can be demonstrated by the dexamethasone suppression test, where injection of this glucocorticoid causes a significant decrease in basal and stress-induced serum ACTH and corticosterone levels.

To elucidate some of the neuroendocrine processes that occur in the brain during encephalitis, we have studied the HPA axis responses to HSV-1. Following corneal inoculation with sublethal virus doses, HSV-1 activated the HPA axis (as determined by increased serum levels of ACTH and corticosterone) when the virus was transported from the cornea to the brain stem, by trigeminal routes [4]. We found that following HSV-1 inoculation to the cornea, the HPA axis became resistant to the negative feedback, as dexamethasone failed to decrease the elevated ACTH and corticosterone levels in the serum. Also, the HSV-1 infected animals became non-responsive to neural stressful stimuli, such as photic and acoustic stresses [4]. Preliminary data suggest that the glucocorticoid resistance is associated with down regulation of glucocorticoid receptors in the hippocampus, which develops within 3 days after inoculation. The neuroendocrine changes were induced only by a virulent HSV-1 strain, and not by a non-virulent strain. The HPA axis activation occurred in the infected animals before development of fever or other clinical signs of disease and in the absence of any inflammatory infiltrates in histopathological sections of the brain (and specifically in the PVN). In addition, systemic inoculation of similar amounts of virus by the intraperitoneal route did not induce any HPA axis changes. The HSV-1-induced HPA axis changes were associated with induction of interleukin-1 (IL-1) gene expression and of prostaglandin E_2 (PG-E_2) production in the brain. Also, HPA axis activation by corneal inoculation with HSV-1 could be blocked by ablation of noradrenergic innervation of the hypothalamus by stereotaxic injection of the neurotoxin 6-hydroxy-dopamine into the ventral noradrenergic bundle [5]. Ablation of the serotonergic innervation of the hypothalamus by injection of the serotonergic neurotoxin 5, 7-dihydroxytryptamine into the raphe nucleus did not block HPA axis activation by HSV-1. In sum, it can be concluded that HSV-1-induced HPA axis activation is not mediated by a systemic inflammatory process but rather by central mechanisms, which include brain IL-1 and prostaglandin production and intact noradrenergic input to the hypothalamus.

We further characterized the roles of HSV-1 and brain IL-1β in mediating the adrenocortical axis response to viral infection. For this purpose we examined the acute HPA axis responses following intracerebroventricular (ICV) HSV-1 in-

fection [7]. We found that the HPA axis was already maximally activated within 3 hours post-infection in a virus-dose dependent manner. Sucrose-gradient purified HSV-1 virions induced the same neuroendocrine responses, indicating that this effect was not due to products of infected cells, that are present in crude virus extracts. UV-irradiated purified virions induced a similar but transient HPA-axis response, although the virus was completely inactivated. The HPA axis activation by the purified virions was completely blocked by ICV pre-treatment with the IL-1β receptor antagonist (IL-1ra).

Based on the prominent negative feedback effect of glucocorticoids it has been predicted that adrenalectomized animals will exhibit an exaggerated ACTH response to stressors. However, removal of endogenous glucocorticoids by adrenalectomy resulted in lack of ACTH response to stimuli such as ICV injection of IL-1 or administration of indomethacin, although these rats continued to respond to other stressful stimuli, such as ether [22, 23]. Also, HPA axis responses to hemodynamic stress [18] and certain neural stimuli, but not to metabolic stimuli required the presence of circulating glucocorticoids [22]. To evaluate the role of glucocorticoids in HSV-1-induced HPA axis activation, we inoculated purified virions into adrenalectomized rats that are deficient in circulating glucocorticoids. In these rats, the purified virions did not induce an increase in ACTH level above that is observed after adrenalectomy [7]. The lack of hypothalamic-pituitary response to HSV-1 in these rats was not due to the already high ACTH levels following adrenalectomy, since administration of the bacterial endotoxin LPS to adrenalectomized animals induced a further increase in ACTH levels.

In summary, both viable and UV-inactivated HSV-1 can acutely activate the HPA axis before and independently of any viral replication. HSV-1-induced HPA axis activation depends on a permissive action of circulating glucocorticoids and on host derived brain interleukin-1. These findings are consistent with the notion that physiological levels of glucocorticoids are required to permissively enhance some immune and stress related brain functions [14].

We further investigated host brain responses associated with the clinical signs of experimental HSV-1 encephalitis and the role of glucocorticoids on the pathogenesis of infection. To measure motor activity and body temperature, battery operated biotelemetric transmitters were implanted in the peritoneal cavity of rats. The output was monitored by a receiver board placed under each animal's cage and fed into a peripheral processor connected to a computer. Intracerebroventricular inoculation of HSV-1 in rats induced fever and behavioral changes, including motor hyperactivity and aggressive behavior, that were reminiscent of the clinical picture observed in human patients during the course of viral encephalitis.

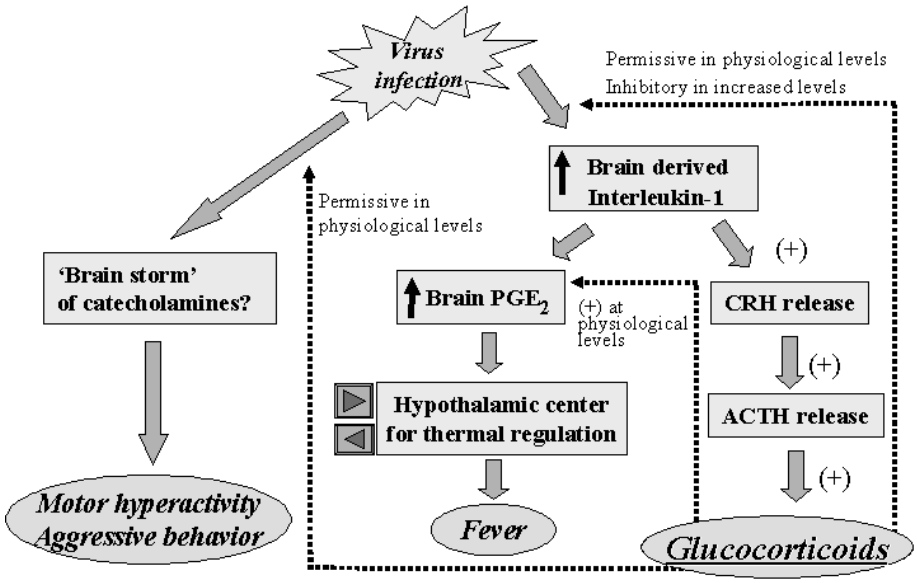

Figure 1. Summary of neurochemical changes involved in the pathogenesis of the clinical manifestations of HSV-1 encephalitis

This experimental system may therefore be useful to study neurochemical alterations and anatomic structures and pathways that are involved in the pathogenesis of the clinical syndrome of encephalitis. We found that in adrenalectomized rats HSV-1 failed to induce clinical signs of encephalitis, and in particular the animals did not develop fever, motor hyperactivity or aggressive behavior [8]. However, mortality rate and virus titers in the brain of adrenalectomized rats were identical to those of sham-operated rats. Surgical hypophysectomy (after which the animals lack any circulating ACTH or glucocorticoids) or blockade of glucocorticoid receptors (by the type II glucocorticoid receptor antagonist RU38486) also prevented the HSV-1-induced febrile response. We next determined whether the inhibitory effect of adrenalectomy on the clinical signs of HSV-1 encephalitis is due to the absence of circulating glucocorticoids per se, or to other factors associated with adrenalectomy, such as depletion of adrenal medulla catecholamines or hypersecretion of ACTH. To this aim we examined whether glucocorticoid replacement therapy in adrenalectomized rats can restore the HSV-1-induced fever and behavioral abnormalities. In adrenalectomized rats, which received replacement therapy by daily injections of the synthetic glucocorticoid dexamethasone, HSV-1-induced hyperthermia proved to be similar as in intact rats [8]. Similarly,

dexamethasone replacement therapy in adrenalectomized rats restored the aggressive behavior and the increased motor activity in response to HSV-1 infection, similar to intact, HSV-1-infected rats. Thus, these results indicated that development of fever and behavioral abnormalities during HSV-1 encephalitis depend on the presence of systemic glucocorticoids.

Since we have previously shown that HSV-1 infection caused increased production of PG-E_2 and IL-1 gene expression in various brain regions [5], we further examined whether these responses depend on the presence of circulating glucocorticoids. The *ex vivo* production of PG-E_2 was significantly increased (by approximately 2-fold) in tissue slices from the frontal cortex of HSV-1-infected rats as compared to tissues from uninfected control rats. Adrenalectomy had no effect on basal production of PG-E_2 but it completely prevented the increase in brain PG-E_2 synthesis following HSV-1 inoculation. Dexamethasone replacement therapy in adrenalectomized rats fully restored the HSV-1-induced hyperproduction of PG-E_2 synthesis in the brain [8]. The lack of a febrile response to HSV-1 in adrenalectomized rats, in which PG-E_2 production is impaired, may also be due to inability of the rats to respond to PG-E_2. However, ICV injection of PG-E_2 induced a comparable increase in rectal temperature in adrenalectomized and sham operated rats. Thus, these results suggest that HSV-1-induced fever depends on increased brain production of PG-E_2, which requires the presence of circulating glucocorticoids. Furthermore, the failure of adrenalectomized rats to develop fever is not due to an impairement in the response to exogenous PG-E_2.

Since IL-1 is also known to have an important role in mediating the febrile responses to viral infection, we examined whether endogenous glucocorticoids are important also for the induction of IL-1 mRNA in HSV-1 infected brains. HSV-1 induced IL-1β gene expression in the pons of intact rats, as expected. Adrenalectomy alone caused pronounced expression of IL-1β mRNA. This adrenalectomy-induced activation of the IL-1β gene was not associated with febrile or behavioral changes. Importantly, HSV-1 infection did not produce a further increase in IL-1β expression in adrenalectomized rats, as determined by semiquantitative reverse transcriptase (RT)-PCR [8]. To determine whether IL-1β expression in adrenalectomized rats was maximal or could be further stimulated by a different immune challenge, LPS was injected ICV into adrenalectomized and intact rats. LPS induced IL-1β expression in intact rats' brain and further enhanced its expression in adrenalectomized rats by 2–3 fold, as determined by semiquantitative RT-PCR. When recombinant human IL-1β was injected ICV a similar rise in body temperature was observed in intact and adrenalectomized rats, indicating that the febrile response to exogenous IL-1 was not affected by adrenalectomy. In

sum, these studies suggested that adrenalectomy has a suppressive effect on IL-1 production during HSV-1 encephalitis and that this effect might underly the inhibition of the febrile response.

To further study virus-host relations, we examined the effects of purified HSV-1 virions infection on primary cultures of astrocytes *in vitro.* The purified virions induced IL-1β gene expression in the astrocytes within 3 hours after infection. This was associated with translocation of the transcription factor NF-*k*B from the cytoplasm to the nucleus of the infected astrocytes, as determined by immunocytochemistry and western blots. Removal of cortisol from the culture medium caused the translocation of NF-*k*B to the nucleus in uninfected astrocytes and basal expression of IL-1β RNA. However, in the absence of cortisol in the culture medium, HSV-1 infection did not further increase IL-1β expression [8]. Thus, our *in vitro* system's data are similar to the *in vivo* data, where HSV-1 infection of astrocytes induces IL-1 expression. This effect is dependent on a permissive action of glucocorticoids and on the NF-*k*B signal transduction pathway.

In conclusion, we developed an experimental model of HSV-1 encephalitis manifested by fever, motor hyperactivity and aggressive behavior, which are similar to the clinical presentation of human patients. Our studies shed some light on host brain responses to infection with HSV-1. The chronic neurologic sequela or lethal outcome of herpetic encephalitis seem to be associated with viral replication and brain tissue destruction. However, the clinical and neuroendocrine phenomena at the early stages of the disease are not a result of brain damage but rather due to activation of neural systems. The activation of the HPA axis by HSV-1 and development of clinical signs of encephalitis are dependent on infected brain products, such as cytokines, prostaglandins and catecholamines. Circulating glucocorticoids play an essential role in mediating the clinical, behavioral and physiologic actions of these products.

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