Stress and Virulence: West Nile Virus Encephalitis

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ABSTRACT
Stress has been found to exacerbate the outcome of bacterial and viral infectious diseases in humans and animal models. The effect of stress on the immune system in human and animal studies is suppressive with changes in the activity of a wide range of immune system components. The data suggests that immune changes induced by stress appear to be great enough to be a health risk. The interaction of the immune system with the brain plays a significant role in the outcome of infectious diseases. The results reported demonstrate that stress paradigms are proposed to increase the fluidity of cell membranes in the central nervous system (CNS), and consequently disrupt the blood-brain-barrier, promoting viral neuroinvasion causing encephalitis and death in mice infected with attenuated West Nile Virus (WNV). Exposure of infected mice to various stress paradigms have been found to induce immunosuppression, increased replication of the virus and caused penetration of non-invasive viruses into the brain.

Keywords: Animal models, stress, immunosuppression, viral diseases, west nile virus.

INTRODUCTION
Stress has been defined as any external or internal stimulus that disrupts the internal environment and leads to suppression of the immune system. Stress is a term defining the reaction of the body to a variety of emotional and physical stimuli which threaten homeostasis (1-2). The effect of stress on the humoral immune response has been reported in numerous studies: the direction of the effect varying greatly and depending upon the stress patterns (type and duration). The outcome of stress on the immune system in human and animal studies is most commonly suppressive, with changing production and activity of a wide range of immune system components (3-8). Physiologic responses to stressors are mediated by the sympathetic nervous system and the hypothalamic-pituitary-adrenal axis. Glucocorticoids are major mediators in the reaction to stress and in stress-induced immunosuppression (9). It has been shown that stress-induced elevation of serum corticosterone during influenza infection suppressed IFNγ production and increased mortality (10).

Stress stimuli affect the immune system and cause immunosuppression through involution of lymphoid organs possibly mediated by the increased activity of the pituitary adrenocortical axis (11). Evidence has been provided demonstrating connections between stress and the immune system (3-5) and between the immune system and the central nervous system (CNS) (11-13). The interaction of the immune system with the brain plays a significant role in the outcome of infectious diseases (14-15).

Glucocorticoids that are released in response to stress are the major mediators on the effect on viral replication, changes in virulence and viral penetration into the CNS. It has been demonstrated that viral infections cause a stress reaction with resultant elevation of endogenous glucocorticoid levels (16-18), involution of lymphoid organs and generalized immunosuppression (4, 19-20).

The administration of glucocorticoids during viral infection leads to higher viral titers and more severe symptomatology (21-24), morbidity and mortality (4, 25-26). The effect is similar to that of stress on viral infections (4, 20, 27-28). It appears that stress acts by enhancing virus replication in
lymphocytes as shown in the spleen of stressed mice (20, 25). Since activated lymphocytes can pass through the blood brain barrier, the larger fraction of infected lymphocytes in stressed mice could also contribute to higher virus titers in the brain.

A variety of stress paradigms have been shown to exacerbate the effects of several infectious agents, including herpes simplex virus (28), coxsackie B1 virus (29), vesicular stomatitis virus (30), cytomegalovirus (CMV), influenza virus (31-35), mengovirus (36), human immunodeficiency virus (HIV) (37) and encephalitic viruses (20, 25, 27, 38). Viruses are known to flourish in the stressed host, with increased morbidity and mortality (20, 38-41). The effect is attributed to elevated corticosterone levels which is known to be augmented in viral infection. It is suggested that stress induced blood-brain-barrier breakdown is due to the release of agents that can alter the blood-brain-barrier permeability (42). Furthermore, stress paradigms are proposed to increase fluidity of cell membrane in the CNS, and consequently disrupt the blood-brain-barrier, promoting viral neuroinvasion causing encephalitis and death (42).

Numerous studies have investigated the effects of environmental and psychological stress on the immune system and on susceptibility to disease (15, 41, 43-47). Since stress alters the chemical balance of the body, it probably influences the development of diseases through its effect on the immune system (14, 15, 43, 48-49).

In animals, acute exposure to cold stress, electric shock, isolation, handling and loud noise have all been shown to suppress some aspects of immunity. Social stress in chickens induces Newcastle Disease Virus (NDV) and E. coli infection (50). In addition, chickens, turkeys, and other poultry exposed to stressors and infectious diseases cause impairment of innate and acquired immunity potentially affecting efficiency production (50). The effect of stress on susceptibility of animal to bacterial and viral infections has been reviewed by Dantzer and Kelley (5), Sheridan et al. (39), Cohen and Williamson (46) and Dhabhar et al. (43).

In humans, acute stressors such as examinations, social isolation, sleep deprivation and trauma have been shown to impact on immune parameters (7, 15, 37, 51, 52). In addition, it was reported that stressed individuals proved more likely to develop clinical illness, and the rate of both respiratory infection and clinical cold symptoms increased in a dose-dependent manner with increases in the degree of psychological stress (14).

**Stress and virulence of attenuated WNV**

West Nile virus (WNV) is one of the most widespread of the Japanese encephalitis serogroup of the genus flavivirus. The virus is maintained in nature in a mosquito-bird-mosquito cycle (primarily Culex), with humans, horses and other mammals serving as incidental hosts (53). The virus infects wild birds and domestic birds (geese, ducks, turkey and chicken). WNV is a mosquito-borne disease found most commonly in Africa, West Asia, and the Middle East, where up to 40% of the human population possesses antibodies (54). It is an emerging disease in the United States, since 1999 and has spread throughout the US and Canada (55-61).

Symptoms of WNV infection in humans range from asymptomatic seroconversion to fatal meningo-encephalitis, with symptoms including cognitive dysfunctions, muscle weakness and paralysis (56, 57). Elderly individuals and those with depressed immunity are at greatest risk of developing severe neurological disease (56, 57, 62).

Neuro-invasiveness and neurovirulence are the crucial properties that determine the capacity of a virus to cause encephalitis (60). The major routes for virus spread are through nerves or via the bloodstream. In immunodeficient mice of the µMT strain which are genetically deficient in B cells and antibody have been found to be highly susceptible to infection and developed high virus titers in the blood and the CNS after infection with low dose of WNV (60). In addition it has been shown that mice lacking both B and T cells (SCID (63) and RAG1 mice (64, 65)), are highly susceptible to infection and that T and B lymphocytes both protect against WNV infection. In both reports it was shown that SCID mice infected with WNV developed a high and prolonged viremia together with a CNS infection. It was suggested that early antibody response limits disseminated infection of WNV in the CNS (38). Similarly as reported earlier, the absence of both B and T cells as in SCID mice but not that of T cells alone (nude mice) increased mortality associated with WNV infection (60, 64).

In a mouse model using the wild type strain of WNV it was shown that exposure of mice to cold water (1±0.5°C) for 8-10 days increased the mortality from 47% in control to 92%-100% in stressed groups (38). Cold or isolation stress increased blood, spleen and brain virus levels by 2-3 logs as compared to control non-stressed mice. WNV which was isolated from the brain of moribund stressed mice was found...
to be more virulent when inoculated intraperitoneally into normal non-stressed mice. Moreover, lymphoid organs such as spleen and thymus showed a severe loss of weight (Table 1).

In another study the experimental stress model used comprised two arboviruses that would not usually cause fatal infections to normal non-stressed mice when administered peripherally: WN-25, a noninvasive variant of the West Nile virus and SVN, a neuroviral non-invasive Sindbis virus (66, 67). These two viruses will not cause fatal infections when administered peripherally. Since glucocorticoids levels are elevated during stress, the effects of stress or cortisone administration were investigated in relation to neurovirulence and encephalitis by the attenuated arboviruses.

As shown in Table 2, stress paradigms induced mortality of 65%, 80% and 50% (WN-25) and 75%, 78% and 58% (SVN) in cold, isolation and cortisone administration, respectively. No mortality was seen in non-stressed intraperitoneally inoculated mice. The virus level in the brain of stressed mice was 8.5, 7.8 and 7.5 (WN-25) 7.3, 7.1, and 7.0 (SVN) logs 10 PFU (Plaque Forming Units) in cold, isolation and for cortisone administration respectively. No virus was detected in non-stressed inoculated mice (Table 3).

Isolation of a neuroinvasive strain from the brains of stress-exposed mice suggested that the induction of infection is attributed to immunesuppression with subsequent increased viral replication and selection of neuroinvasive strains. The WN-25 virus extracted from the brain of the moribund mice showed a change in its neuroinvasive properties, raising the possibility of stress-enhanced proliferation and selective processes leading to reversion to neurovirulence.

Table 1: Compression between virulent and attenuated strain of West Nile Virus in mice exposure to cold or isolation stress

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>WNV</th>
<th>WN-25</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>17/36</td>
<td>5.8±0.5</td>
</tr>
<tr>
<td>Isolation</td>
<td>40/42*</td>
<td>8.6±0.6*</td>
</tr>
<tr>
<td>Cold</td>
<td>22/24*</td>
<td>8.8±0.7*</td>
</tr>
</tbody>
</table>

Isolation – one mouse per cage.
Cold stress treatment (5 min. a day at 1±0.5°C) was administered on the day of virus inoculation and afterwards (Modified from Ben-Nathan et al 1996).

Table 2: Effect of stress treatments on mortality of mice inoculated with attenuated arboviruses.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>MORTALITY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WN-25</td>
</tr>
<tr>
<td></td>
<td>D/T</td>
</tr>
<tr>
<td>Control</td>
<td>0/18</td>
</tr>
<tr>
<td>Cold</td>
<td>13/20</td>
</tr>
<tr>
<td>Isolation</td>
<td>16/20</td>
</tr>
<tr>
<td>Corticosterone</td>
<td>6/12</td>
</tr>
</tbody>
</table>

D/T - Dead from total inoculated mice.
Cold stress treatment (5 min. a day at 1±0.5°C) was administered on the day of virus inoculation and afterwards.
Isolation - one mouse per cage.
Corticosterone, 10 mg/kg injected i.v. one day after virus inoculation.

Table 3: Virus levels in brains of stressed mice inoculated with attenuated viruses

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Brain virus levels (log_{10} PFU)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WN-25</td>
</tr>
<tr>
<td>Control</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Cold</td>
<td>8.5±0.4</td>
</tr>
<tr>
<td>Isolation</td>
<td>7.8±0.3</td>
</tr>
<tr>
<td>Corticosterone</td>
<td>7.5±0.4</td>
</tr>
</tbody>
</table>

Cold stress treatment (5 min. a day at 1±0.5°C) was administered on the day of virus inoculation and afterwards.
Isolation - one mouse per cage.
Corticosterone, 10 mg/kg injected i.v. one day after virus inoculation.
Six mice were tested in each group - all mice were sacrificed on day 7.
lence (42, 67). The WN-25 virus which was extracted from the brain of moribund stressed mice was extremely virulent. Intraperitoneal inoculation of as little as 10 PFU to normal non-stressed mice caused encephalitis and death. In the case of SVN, no changes were detected in the neuroinvasive properties of recovered progeny virus extracted from the moribund mice (42, 67).

In stressed mice, these viruses (both lacking neuroinvasive properties) invade the brain and the virus levels were found to be greater than 10^6 PFU/brain in all moribund mice inoculated with WN-25 or with SVN, whereas no virus was detected in the brain of control inoculated mice (67). These titers were determined by PFU in tissue cultures and by in vivo assay in which mice were inoculated intracerebrally or intraperitoneally. In the in vivo assay of the virus isolated from a stressed mouse, the intraperitoneal LD50 increased by 10^6 indicating an extreme increment in virulence (67). The data clearly showed that in stressed mice the avirulent WN-25 strain become virulent and reached a titer similar to the original WNV in control mice (data not shown). We suggest that cold stress conditions in mice induce a selection process in which the attenuated strain replicates and kills mice similar to the wild virulent WNV strain (67).

It was suggested that the neurovirulent non-neuroinvasive viruses can serve as markers for blood-brain-barrier permeability, and aid in studying pathological processes of the brain (42). Moreover, we suggest that the use of these viruses as reliable pathogenic indicators for stress in animals. This susceptibility of stressed mice to viral infection could be explained by the finding that stress induced suppression of macrophage and T-lymphocyte activities and decreased natural killer cell cytotoxicity (48). We suggest that stress effects can result in an elevated viremia for longer periods, facilitating the penetration of viruses into the CNS.

In summary, there is strong evidence for an association between stress and the immune system and between the immune system and the central nervous system.

Susceptibility to infections is presumed to be primarily mediated by immune function. Since WNV is widely distributed and is capable of endemic spread the effects of stress on WNV infection were studied. It appears that suppressed immune response to stress with defective function of macrophages and lymphocytes, leads to enhancement of viral replication in the blood and peripheral organs, induction of a selective process with the selection of a neuroinvasive strain. The results reported demonstrate that stress paradigms are proposed to increase fluidity of cell membrane in the CNS, and consequently disrupt blood-brain-barrier, promoting viral neuroinvasion causing encephalitis and death.

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