Stress and Virulence: West Nile Virus Encephalitis

Ben-Nathan, D.*

Department of Virology, Kimron Veterinary Institute, Beit-Dagan, Israel.

* **Corresponding author:** Dr. David Ben-Natan, Department of Virology, Kimron Veterinary Institute, Beit-Dagan, Israel. E-mail: davidbn@013.net.il

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 efficient 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-encephlitis, 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 neurovirulent 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 neuroviru-

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

	WNV			WN-25		
Treatment Group	Mortality D/T[a]	Log_{10} Brain	PFU/organ[b] Spleen	Mortality D/T	Log_{10} Brain	PFU/organ Spleen
Control	17/36	5.8±0.5	2.2±0.5	0/34	1.5±1	0
Isolation	40/42*	8.6±0.6*	3.8±0.1*	16/28*	7.4±0.7*	3.4±0.4*
Cold	22/24*	8.8±0.7*	3.8±0.2*	19/32*	8.9±0.2*	3.7±0.3*

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).
[a] D/T. dead/total mice.
[b] Six mice were used for virus level.
* P<0.01 compared to control.

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

	MORTALITY			
Treatment	WN-25		SVN	
group	D/T	%	D/T	%
Control	0/18	0	0/12	0
Cold	13/20	65	13/18	72
Isolation	16/20	80	14/18	78
Corticosterone	6/12	50	7/12	58

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.
Modified from Ben-Nathan *et al* 1998 (New Frontiers in stress research) (67).

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

	Brain virus levels (log_{10} PFU)	
Treatment group	WN-25	SVN
Control	<2	<2
Cold	8.5±0.4	7.3±0.2
Isolation	7.8±0.3	7.1±0.2
Corticosterone	7.5±0.4	7.0±0.2

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.
Modified from Ben-Nathan *et al* 1998 (New Frontiers in stress research) (67).

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, faciliting 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.

REFERENCES

1. Selye, H.: The physiology and pathology of exposure to stress. Acta. Montreal. 1950.
2. Selye, H.: Implications of stress concept. N. Y. State J. Med. pp. 2139-2145, 1957.
3. Ader, R. and Cohen, N.: Psychoneuroimmunology. Annu. Rev. Psychol. 44:53-85, 1993.
4. Riley, V.: Psychoneuroendocrine influences on immunocompetence and neoplasia. Science. 212:1100-1109, 1981.
5. Dantzer, R. and Kelley, K.W.: Stress and immunity: An integrated view of the relationships between the brain and the immune system. Life Science. 44:1995-2008, 1989.
6. Sternberg, E.M., Chrousos, G.P., Wilder, R.L. and Gold, P.W.: The stress response and the regulation of inflammatory disease. Ann. Int. Med. 117: 854-866, 1992.
7. Mehta, S.K., Cohrs, R.J., Forghhani, B., Zerbe, G., Gilden, D.H. and Pierson, D.L.: Stress induced subclinical reactivation of varicella zoster virus in astronauts. J. Med. Virol. 72:174-179, 2004.
8. Munhoz, C.D., Grcia-Bueno, B., Madrigal, J.L., Lepsch, L.B., Scavone, C. and Leza, J.C.: Stress-induced neuroinflammation mechanisms and new pharmacological targets. Braz. J. Med. Biol. Res. 41:1037-1046, 2008.
9. Welsh, C.J., Steelman, A.J., Mi, W., Young, C.R., Dean, D.D., Storts, R., Welsh, T.H. jr. and Meagher, W.M.: Effects of stress on immune response to Theiler's virus. Implications for virus-induced autoimmunity. Neuroimmunomodulation. 17:169-172, 2010.
10. Dobbs, C.M., Vasquez, M., Glaser, R. and Sheridan, J.F.: Mechanisms of stress-induced modulation of viral pathogenesis and immunity. J. Neuroimmunol. 48:151-160, 1993.
11. Weigent, D.A. and Blalock, E.: Interactions between the neuroendocrine and the immune systems: common hormones and receptors. Immunol. Rev. 100:79-107, 1987.
12. Roszman, T. and Carlson, S.L.: Neural-immune interactions: circuits and networks. Prog. NeuroEndocrinImmunology. 4:69-78, 1991.
13. Rouabhia, M., Chakir, J. and Deschaux, P.: Interaction between the immune and endocrine systems: Immunomodulatory effects of luteinizing hormones. Prog. NeuroEndocrinImmunology. 4:86-91, 1991.
14. Swilling, B.: Stress affects disease outcomes. Features. 58: 23-25, 1992.
15. Marsland, A.L., Bachen, E.A., Cohen, S., Rabin, B. and Manuck, S.B.: Stress, Immune reactivity and susceptibility to infectious disease. Physiol. Behav. 77:711-7116, 2002.
16. Blalock, J.E.: Virus induced increase in plasma corticosterone. Science. 238:1424-1425, 1987.
17. Smith, E.M., Meyer, W.J. and Blalock, J.E.: Virus induced corticosterone in hypophysectomized mice: A possible lymphoid adrenal axis. Science. 218:1311-1312, 1982.

18. Dunn, A.J., Powell, M.L., Meitin, C. and Small, P.A.: Virus infection as a stressor: influenza virus elevates plasma concentration of corticosterone, and brain concentrations of MHPG and tryptophan. Physiol. Behav. 45:591-594, 1989.
19. Escobar, M.R. and Swenson, P.D.: Mechanisms of viral immunopathology. In: The reticuloendothelial system. Vol. 4. Immunopathology. Rose, N.R. and Siegel, B.V. (eds). Plenum Press, New York, pp. 201-253, 1983.
20. Ben-Nathan, D. and Feuerstein, G.: The influence of cold or isolation stress on resistance of mice to West Nile virus. Experientia. 46:285-292, 1990.
21. Lynden, D.L. and Huber, S.A.: Aggravation of coxsackie virus, group B, type 3 induced myocarditis increase in cellular immunity to myocyte antigen in pregnant Balb/c mice and animals treated with progesterone. Cell Immunol. 87:462-472, 1984.
22. Johanson, K.M.: Arenaviruses. In: Virology. Fields, B.N., Knipe, D.M., Chanock, R.M., Melnick, J.L., Roizman, B. and Shope, R.R. (eds). Raven Press, New York. p. 1046, 1985.
23. Clamam, H.M.: Anti-inflammatory effects of corticosteroids. Clin. Immunol. Allergy. 4:317-329, 1984.
24. Parillo, J.E. and Fauci, A.S.: Mechanisms of glucocorticoid action on immune processes. Ann. Rev. Pharmacol. Toxicol. 9:179-201, 1979.
25. Ben-Nathan, D., Lustig, S. and Feuerstein, G.: The influence of cold or isolation stress on neuroinvasiveness and virulence of an attenuated variant of West Nile virus. Arch. Virol. 109: 1-10, 1989.
26. Grossman, C.J.: Interaction between the gonadal steroids and the immune system. Science. 227:257-260, 1985.
27. Ben-Nathan, D., Lustig, S., Kobiler, D., Danenberg, H., Lupu, E. and Feuerstein, G.: Dehydroepiandrosterone protects mice inoculated with West Nile virus and exposed to cold stress. J. Med. Virol. 38:159-166, 1992.
28. Rasmussen, A.F., March, J.T. and Brill, N.Q.: Increased susceptibility to herpes simplex in mice subjected to avoidance-learning stress or restrain. Proc. Soc. Exp. Biol. Med. 96:183-189, 1957.
29. Johnson, T., Lavender, J.F., Multin, E. and Rasmussen, F.: The influence of avoidance-learning stress on resistance to Coxsackie B virus in mice. J. Immunol. 91:569-575, 1963.
30. Jensen, M.M. and Rasmussen, A.F.: Stress and susceptibility to viral infections. II. Sound stress and susceptibility to vesicular stomatitis virus. J. Immunol. 90:21-23, 1963.
31. Ilback, N.G., Friman, G., Beisel, W.R., Johanson, A.J. and Berendt, R.F.: Modifying effects of exercise on clinical course and biochemical response of the myocardium in influenza and tularemia in mice. Infect. Immun. 45:498-504, 1984.
32. Feng, N., Pagniano, R., Tovar, C.A., Bonneau, R.H., Glaser, R. and Sheridan, J.F.: The effect of restraint stress on the kinetics, magnitude, and isotype of the humoral immune response to influenza virus infection. Brain, Behavior and Immunity 5:370-382, 1991.
33. Hermann, G., Tovar, C.A., Beck, F.M. and Sheridan, J.F.: Kinetics of glucocorticoid response to restraint stress and/or experimental influenza viral infection in two inbred strains of mice. J. Neuroimmunol. 49:25-33, 1994.
34. Tseng, R.J., Padgett, DA., Dhabhar, F.S., Engle, H. and Sheridan, J.F.: Stress-induced modulation of NK activity during influenza viral infection: role of glucocorticoids and opioids. Brain Behav. Immun. 19:153-164, 2005.
35. Murphy, E.A, Davis, J.M., Carmichael, M.D., Gangemi, J.D., Ghaffar, A. and Mayer, E.P.: Exercise stress increases susceptibility to influenza infection. Brain Behav. Immun. 22:1152-1155, 2008.
36. Clausing, P., Bocker, T., Diekgerdes, J., Gartner, K., Guttner, J., Haemisch, A., Veckenstedt, A. and Weimer, A.: Social isolation modifies the response of mice to experimental mengo virus infection. J. Exper. Animal Sci. 36:37-54, 1994.
37. Leserman, J.: Role of depression, stress and trauma in HIV disease progression. Psychosom. Med. 70:539-545, 2008.
38. Ben-Nathan, D., I. Huitinga, I., Lustig, S., Van Rooijen, N. and Kobiler, D.:. West Nile virus neuroinvasion and encephalitis induced by macrophage depletion in mice. Arch. Virol. 141:459–69, 1996.
39. Sheridan, J.F., Dobbs, C., Brown, D. and Swilling, B.: Psychoneuroimmunology: Stress effects on pathogenesis and immunity during infection. Clin. Microbiol. Rev. 7:202-212, 1994.
40. Friedman, S.B., Glasgow, L.A. and Ader, R.: Differential susceptibility to viral agent in mice housed alone or in group. Psychosom. Med. 32: 285-299, 1970.
41. Rogers, M.P. Dubey, D. and Reich, P.: The influence of the psyche and the brain on immunity and susceptibility: A critical review. Psychosom. Med. 41:147-164, 1979.
42. Ben-Nathan, D., Lustig, S. and Danenberg, H.: Stress-induced neuroinvasiveness of a neurovirulent noninvasive Sindbis virus in cold or isolation subjected mice. Life Sci. 48: 1493-1500, 1991.
43. Dhabhar, F.S., Miller, A.H. MacEwen, B.S. and Spencer, R.L.: Effects of stress on immune cell distribution dynamics and hormonal mechanisms. J. Immunol. 154:5511-5527, 1995.
44. Khanshari, D.N., Murgo, A.J. and Faith, R.E.: Effect of stress on the immune system. Immunol.Today. 11:170-175, 1990.
45. Peterson, P.K., Chun, C.C., Molitor, T., Murtaugh, M., Strgar, F. and Sharp, M.: Stress and pathogenesis of infectious disease. Rev. Infect. Dis. 13:710-720. 1991.
46. Cohen, S. and Williamson, G.M.: Stress and infectious disease in human. Psychol. Bull. 109:5-24, 1991.
47. Koolhaas, J.M.: Coping style and immunity in animals: making sense individual variation. Brain Behav. Immun. 22:662-667, 2008.
48. Shavit, Y., Lewis, J.W. and Terman, G.W.: Opioid peptides mediate the suppressive effect of stress on natural killer cell cytotoxicity. Science. 223:188-190, 1984.
49. Gonzales, XF. Deshmukh, A., Pulse, M., Johnson, K. and Jones, HP.: Stress-induced differences in primary and secondary resistance against bacterial sepsis corresponds with diverse corticotrophin releasing hormone receptor expression by pulmonary CD11c+ MHC ll+ and CD11c- MHC ll+ APCs. Brain Behav. Immun. 22:552-564, 2008.
50. Hoerr, FJ.: Clinical aspects of immunosuppression in poultry. Avian Dis. 54:2-15, 2010.
51. Frey, D.J., Fleshner, M. and Wright, K.P. Jr.: The effects of 40 hours of total sleep deprivation on inflammatory markers in healthy young adults. Brain Behav. Immun. 21:1050-1057, 2007.
52. Marin, M.F., Lord, C., Andrews, J., Juster, R.P., Sindi, S., Arsenault-Lapierre, G., Fiocco, A.L. and Lupien, S.J.: Chronic stress

cognitive functioning and mental health. Neurobiol Learn. Mem, 96:583-595, 2011.
53. Hayes, E.B., Komar, N., Montgomery, S.P., OLeary, D.R. and Campbell, G.L.: Epidemiology and transmission dynamics of West Nile virus disease. Emerg. Infect. Dis. 11:1167-1173, 2005.
54. Cohen, D., Zaide, Y., Karasenty, E., Schwarz, M., LeDuc, J.W., Slepon, R., Ksiazek, T.G., Shemer, J. and Green, M.S.: Prevalence of antibodies to West Nile fever, sandfly fever Sicilian, and sandfly fever Naples viruses in healthy adults in Israel. Public Health Rev. 27:217-230, 1999.
55. Smithburn, K.C., Hughes, T.P., Burke, A.V.V. and Paul, G.H.: A neutrotropic virus isolated from the blood of a native of Uganda. Am. J. Trop. Med. 11:471-472, 1940.
56. Hayes, E.B. and Gubler, D.J.: West Nile virus: epidemiology and clinical features of an emerging epidemic in the United States. Annu. Rev. Med. 57:181-194. 2006.
57. Sejvar, J.J., Haddad, M.B., Tierney, B.C., Campbell, G.L., Marfin, A.A., Van Gerpen, J.A., Fleischauer, A., Leis, A.A., Stokic, D.S. and Petersen, L.R.: Neurologic manifestations and outcome of West Nile virus infection. JAMA. 290:511-515, 2003.
58. Ben-Nathan, D., Lustig, S., Tam, G., Robinzon, S., Segal, S. and Rager-Zisman, B.: Prophylactic and therapeutic efficacy of human intravenous immunoglobulin in treating West Nile virus infection in mice. J. Infect. Dis. 188:5-12, 2003,
59. Diamond, M.S., Mehlhop, E., Oliphant, T. and Samuel M.A.: The host immunologic response to West Nile encephalitis virus. Front. Biosci. 14:3024-3034, 2009.
60. Diamond, M. S., Shrestha, B., Marri, A., Mahan, D. and Engle, M.: B cells and antibody play critical roles in the immediate defense of disseminated infection by West Nile encephalitis virus. J. Virol. 77, 2578-2586, 2003.
61. Goldblum, N., Sterk, V.V. and Paderski, B.: West Nile fever; the clinical features of the disease and the isolation of West Nile virus from the blood of nine human cases. Am. J. Hyg. 59:89-103, 1954.
62. Carson, P.J., Konewko, P., Wold, K.S., Mariani, P., Goli, S., Bergloff, P. and Crosby, R.D.: Long-term clinical and neuropsychological outcomes of West Nile virus infection. Clin Infect. Dis. 43:723-730. 2006.
63. Halevy, M., Akov, Y., Ben-Nathan, D., Kobiler, D., Lachmi, B. and Lustig, S.: Loss of active neuroinvasiveness in attenuated strains of West Nile virus: pathogenicity in immunocompetent and SCID mice. Arch. Virol, 137:355-370, 1994.
64. Diamond, M.S. and Klein, R.S.: West Nile virus: crossing the blood–brain barrier. Nature Med. 10, 1294-1295, 2004.
65. Shrestha, B. and Diamond, M.S.: The role of CD8+ T cells in the control of West Nile virus infection. J. Virol. 78, 8312-8321, 2004
66. Lustig, S., Halevy, M., Ben-Nathan, D. and Akov, Y.: A novel variant of Sinbis virus (SV) is both neurovirulent and neuroinvasive in adult mice. Arch. Virol. 122: 237-248, 1992.
67. Ben-Nathan, D., Kobiler, D., Loria, R.M. and Lustig, S.: Stress-induced central nervous system penetration by non-invasive attenuated encephalitis viruses. In New Frontiers in stress research, Modulation of brain function. Levy, A., Grauer, E., Ben-Nathan, D. and Ronald de Kloet, E. (eds). Harwood Academic Publishers, Amsterdam, pp. 277-283, 1988.