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REPLICATION OF LANGAT VIRUS IN IMMUNOCOMPETENT CELLS OF MICE SUBJECTED TO IMMOBILIZATION STRESS

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Summary. — Immobilization stress (hypokinesis) in Balb/c mice may aggravate asymptomatic infection with Langat virus (strain TP-21) as evidenced by 4-fold increased lethality in comparison with control animals. The virus levels in the spleen and brain of stressed and infected mice and the *in vitro* yield of the virus in immunocompetent cells derived from stressed mice were significantly higher than in controls. Enhanced virus replication in latter cells may contribute to increased accumulation of the infectious agent in lymphatic tissues, which would facilitate virus invasion into CNS followed with acute disease and death of animals.

Key words: *Langat virus*; *emotional stress*; *asymptomatic infection*; *immunocompetent cells*

Introduction

Stress may aggravate the course of viral and bacterial infections and enhance the risk of tumour development (Selye, 1976; Ader, 1981; Frolov *et al.*, 1986).

It has been reported that stress alters the activity of immunocompetent effector cells suppressing the functioning of natural killer cells, of antibody producing B cells, of T helper cells and interferon synthesis (Frolov *et al.*, 1985; Okimura, 1986). The mechanisms of this phenomenon are not known. According to several authors, one of the reasons for development of stress-induced alterations is the activation of T suppressors (Regine *et al.*, 1984; Okimura, 1986). However, several data indicate a decreased T suppressor activity during stress (Frolov *et al.*, 1985). We have shown previously that activation of asymptomatic experimental Langat virus infection by means of stress is associated with the occurrence in the body of nonspecific to the antigen T suppressors, with depression T lymphocyte functions and mature antibody producers (Ozherelkov *et al.*, 1987). In addition, in the recent paper we show that enhanced virus replication in immunocompetent cells of stressed mice may participate in the aggravation of the course of infection.

Table 1. Dynamics of Langat virus replication in the brain, in spleen, and in the splenocytes of stressed and control mice

Experimental conditions	Lethality (per cent)	Virus titre (LD_{50}/0.03 ml)[1]		Replication in splenocytes[2] days p.i.	
		Brain	Spleen	0	3
Stress	80 ± 10.42	6.78 ± 0.42*	5.00 ± 0.31*	1.43 ± 0.09	5.74 ± 0.19*
No stress	20 ± 3.8	2.89 ± 0.18*	2.83 ± 0.12*	1.25 ± 0.07	3.15 ± 0.19*

[1] day 6 p.i.; [2] derived from stressed or control mice, respectively.
Note: differences between values in stressed and control animals significant at $p \leq 0.05$ — indicated by asterisks.

Materials and Methods

Viruses. Langat virus (strain TP-21) was prepared from brain suspension of suckling mice after intracerebral inoculation. The virus was titrated by serial dilutions in 2—3-day-old SPF suckling mice and the titre was calculated according to Reed and Münch.

Animals. Balb/c males weighing 18—20g were obtained from breeding farm Stolbovaya (Acad. Med. Sci. U.S.S.R.).

Emotional stress was induced by immobilization (hypokinesis) as described (Ozherelkov *et al.*, 1986). During 10 days for 12 hr the mice were kept in plastic chambers (8.5×4×2 cm), while control mice were kept in standard cages (42×14.5×11.5 cm), ten in each. In all cases the stress was determined according to development of the increased weight of adrenals. This was estimated by the index

$$\frac{\text{body weight}}{\text{adrenal weight}} \times 100$$

which considerably increased in stressed mice as compared to controls. The condition of stress has been fixed also according to decreased weights of spleen and thymus in immobilized mice as compared with controls. On day 10 the stressed and control mice were infected and then all animals were kept under standard conditions. Splenocytes for *in vitro* experiments were removed after 10 days stressor application.

Control mice were kept under standard conditions.

Virus infection. Stressed and control mice were inoculated intravenously with 10^3LD_{50}/0.3 ml of Langat virus. Splenocytes from stressed and control mice were partially purified by centrifugation, washed with medium No. 199 in Hank's solution and then they were inoculated with 100 LD_{50} of Langat virus per cell. After 1 hr adsorption, the cells were resuspended in RPMI-1640 and incubated at 37 °C. The viability of cells was checked by trypan blue; no difference was found between the stressed and control group. Virus titres in the brain and spleen were determined on day 6 post-infection (p.i.), in the splenocytes on days 0 and 3 post-inoculation. Statistical evaluations were made by Student's test.

Results

As shown in Table 1, immobilization stress in mice converted the symptomless infection with Langat virus to a clinically overt disease. In stressed animals the lethality increased 4-fold. The clinical recrudescence of asymptomatic infection had been preceeded by accumulation of virus in the spleen of stressed mice. On day 6 p.i. the Langat virus titre in the spleen of stressed mice was 1000 times higher than in controls. Under stress conditions the conversion of symptomless virus infection to overt disease is accompanied with intensive virus replication in the CNS of previously immobilized mice. In such animals the virus titre in brain on day 6 p.i. was 1000 times higher than in mice kept under control conditions throughout.

The *in vitro* replication of Langat virus in splenocytes derived from stressed and control mice showed the virus reaching considerably higher titres in immunocompetent cells of former mice. On day 3 p.i. the supernatant of infected splenocytes prepared from immobilized mice contained 160 times more virus as compared to controls (Table 1).

Discussion

During stress of man and animals transient defects occur in the T and B cell responses. The most studied mechanism of stress-induced modulation of the immune response are the cytotoxic effects of stress hormones and the

suppression of interferon synthesis; both cause a more severe course of i
fection (Selye, 1976; Frolov, 1986). In our former work we have shown th
nonspecific T suppressors appearing in immobilized mice may contribute
stress-induced activation of symptomless infection with Langat virus
mice. These suppressors depress the function of effector T lymphocytes a
mature antibody producers (Ozherelkov *et al.*, 1987). Recent data using t
same model allowed to conclude that enhanced virus replication in immu
competent cells of stressed animals represented an important link in t
mechanism of stress-induced aggravation of virus infection. Enhanced vi
replication in the spleen may contribute to the invasion of virus into C
facilitating the development of acute encephalitis and death. The increas
virus replication in immunocompetent cells of the stressed host may oc
due to infection of higher number of cells or due to more intensive vi
growth in individual infected cells or by combination of both. It may
assumed that more intensive virus replication in individual immunoco
petent cells and/or involvement of further subpopulations of previously n
permissive cells would affect their functions. These alterations along w
the activation of stress-induced suppressors may worsen the severity of i
munodeficiency developing during stress and may allow the transiti
to virus-induced secondary immunodeficiency. Considering the ubiquit
stressors in human life one could assume that these mechanisms would c
tribute to the pathogenesis of human virus infections. It should be und
lined that stress-induced activation of virus infection may be specific
relation to the infectious agent. This question is currently under study.

References

Ader, R. (1981): *Psychoneuroimmunology*, pp. 185—287, Academic Pres New York.

Frolov, B. A., Filipov, V. K., and Meerson, F. Z. (1985): Activating effect of stress on
mediated hyposensitivity in mice. *Immunologiya* **1985** (**2**), 39—41 (in Russian).

Frolov, B. A., Chetverikova, L. K., Kramskaya, T. A., and Polyak, P. Ya. (1986): Effec
immobilization stress on the course of experimental influenza and interferon formation.
Mikrobiol. **1986** (**3**), 53—56 (in Russian).

Okimura, T. (1986): Stress and Immune Response. *Jap. J. Pharmacol.* **41**, 229—247.

Ozherelkov, S. V., Khozinsky, V. V., and Semenov, B. F. (1986): The course of experime
infection by Langat virus in mice due to immobilization stress. *Vopr. Virusol.* **31** (**5**), 632—
(in Russian).

Ozherelkov, S. V., Khozinsky, V. V., and Semenov, B. F. (1987): Antigen nonspecific
pressors elicited during stress in the pathogenesis of infection of mice inoculated with La
virus. *Zh. Mikrobiol.* **1987** (**11**), 80—83 (in Russian).

Regine, M. A., Landmann, A., Muller, F. B., Peritini, Ch., Wesp, M., Erne, P., and Bu
F. R. (1984): Changes of immunoregulatory cells induced by psychological and phys
stress. *Clin. exp. Immunol.* **58**, 1227—1235.

Selye, H. (1976): *Stress in Health and Disease*, pp. 712—720. Butterworth, Boston, Lon