PHYSIOLOGY

Comparative Analysis of the Effect of Cytokines on the Thymus, Adrenal Glands, and Spleen in Rats with Various Behavioral Characteristics

S. S. Pertsov, E. V. Koplik, and L. S. Kalinichenko

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 150, No. 9, pp. 244-247, September, 2010
Original article submitted August 23, 2009

We compared the effect of a pro-inflammatory cytokine IL-1β and anti-inflammatory cytokine IL-4 on the state of stress-marker organs in rats with various behavioral characteristics in the open-field test. Intraperitoneal injection of the cytokines was followed by a slight decrease in the relative weight of the thymus, adrenal glands, and spleen in behaviorally active and, especially, in passive animals. These changes are probably associated with the effect of the test immunomodulators on apoptosis and migration of cells of the immunocompetent organs. Pretreatment with IL-1β and IL-4 was shown to prevent involution of the thymus and spleen in rats during acute stress on the model of immobilization with simultaneous electrocutaneous stimulation. Hence, these cytokines have the same effects on functional state of stress-marker organs in animals. IL-1β and IL-4 have a greater effect in passive rats than in active specimens, which reflects specific features of immune mechanisms in animals with different emotional reactivity.

Key Words: *IL-1β; IL-4; emotional stress; stress-marker organs; active and passive rats*

Emotional stress is an urgent problem of modern medicine. Stress develops in acute or chronic conflict situations [14], which is followed by cardiovascular disorders, neuroses, endocrine and immune disturbances, and other pathological changes [7].

Various specimens have different resistance to the development of negative consequences of emotional stress [7]. Our studies showed that behavioral activity of rats in the open-field test serves as a reliable prognostic criterion for their resistance to stress exposure. Behaviorally active animals are more resistant to stress than passive rats [5].

P. K. Anokhin Institute of Normal Physiology, Russian Academy of Medical Sciences, Moscow, Russia. ***Address for correspondence:*** s.pertsov@mail.ru. S. S. Pertsov

Psychoemotional stress in mammals is accompanied by immune disorders, including changes in the cytokine profile of the body [3,12]. The state of immune functions is mainly determined by the proportion between pro-inflammatory (IL-1β, IL-2, IL-6, IFN-γ, TNF-α, *etc*.) and anti-inflammatory cytokines (IL-4, IL-10, IL-13, and transforming growth factor-β) [3]. Increasing interest to IL-1β [11] and IL-4 [3] is due to their biological functions in mammals.

Our previous studies revealed specific features of IL-1β involvement in the stress response of animals with different prognostic resistance to stress [2,6]. Much attention was paid to evaluation of the role of IL-4 in immune reactions of the body. However, little is known about its role in the stress response.

0007-4888/11/1503-0277

Here we compared the effects of IL-1β and IL-4 on stress-marker organs (thymus, adrenal glands and spleen) in rats with various behavioral characteristics during acute stress.

MATERIALS AND METHODS

Experiments were performed on 80 male Wistar rats weighing 253.8±3.1 g. The experiment was conducted in accordance with the "Rules of Studies on Experimental Animals" (approved by the Ethics Committee of the P. K. Anokhin Institute of Normal Physiology; protocol No. 1, 3.09.2005), requirements of the World Society for the Protection of Animals (WSPA), and European Convention for the Protection of Experimental Animals.

The animals were housed in cages (6-7 specimens per cage) at 20-22°C and normal light/dark cycle (8.00-20.00, light; 20.00-8.00, darkness). They had free access to water and food. The animals were adapted to laboratory conditions for 5 days after delivery to the laboratory.

Individual and typological characteristics of rats were evaluated in the open-field test for 3 min [5]. The open field was a round area (90 cm in diameter) surrounded by walls (40 cm in height). There were 8 vertical bars (13 cm in height) on the floor. The area was divided into 19 central squares and 18 peripheral squares. It was illuminated with a 100-W lamp. To calculate the index of activity, the sum of crossed peripheral and central squares, peripheral and central rearing postures, and explored objects was divided by the sum of the latency of the first movement and entry into the center of the open field.

Depending on the initial behavior in the open-field test, the animals were divided into active (n=40) and passive (n=40) specimens. These animals differed in the average index of activity (passive rats, 0.52±0.03; active rats, 3.68±0.48). In the follow-up period, behaviorally active and passive rats were divided into 12 groups of 6-7 animals each.

Rat IL-4 (I3650-5UG, Sigma) and recombinant IL-1β (activity 10^8 U/mg; State Research Institute of Highly Pure Biopreparations, Federal Medical and Biological Agency of Russia, St. Petersburg) in doses of 5 μg/kg were dissolved in 1 ml sterile physiological saline. These cytokines or physiological saline (1 ml) were injected intraperitoneally 1 h before stress exposure. Control (nonstressed) rats received these injections 2 h before decapitation.

Immobilization of rats in individual plastic cages and simultaneous delivery of stochastic electrocutaneous stimulation (1 h) served as the model of acute emotional stress. This standard method of stress exposure was described previously [6]. Control (nonstressed) rats were maintained in home cages during this period.

Stressed and control animals were decapitated immediately after the experiment. Stress-marker organs (thymus, adrenal glands and spleen) were sampled after decapitation, isolated from the surrounding tissue, and weighed on an Adventurer™ electronic balance (OHAUS Corp.). The relative weight of these organs was calculated per 100 g body weight.

The significance of between-group differences was evaluated by nonparametric Mann–Whitney test. The data are presented as means and standard errors.

RESULTS

The relative weight of the thymus, adrenal glands, and spleen did not differ in nonstressed active and passive rats receiving physiological saline (Table 1).

Immobilization with simultaneous electrocutaneous stimulation was accompanied by a significant decrease in the relative weight of the thymus and spleen in behaviorally passive rats as compared to nonstressed specimens (by 1.14 and 1.21 times, respectively, p<0.05; Table 1). The relative weight of the thymus and spleen in active rats also tended to decrease under acute stress conditions (by 1.22 and 1.25 times, respectively). However, these changes were not statistically significant. The relative weight of the adrenal glands in rats with various behavioral characteristics practically did not change after stress exposure. The observed involution of immunocompetent organs is consistent with the H. Selye concept about consequences of biological stress in mammals [14].

Intraperitoneal injection of pro-inflammatory cytokine IL-1β was followed by a significant decrease in the relative weight of the thymus in nonstressed active and passive rats (by 1.61 [p<0.01] and 1.26 times, respectively, compared to animals of the physiological saline group; Table 1). Treatment with IL-1β was accompanied by a decrease in the relative weight of the adrenal glands in behaviorally passive specimens as compared to physiological saline-treated animals (by 1.47 times, p<0.05). The relative weight of the spleen in rats with different behavioral activity tended to decrease after administration of IL-1β (statistically insignificant).

Changes in the relative weight of the thymus, adrenal glands, and spleen in nonstressed rats of the IL-4 group were similar to those observed in IL-1β-treated animals (Table 1). Treatment with IL-4 was followed by a decrease in the relative weight of the thymus and spleen in nonstressed passive rats (by 1.24 times compared to animals receiving physiological saline, p<0.05). Active specimens did not exhibit significant changes in the weight of study organs under these conditions.

TABLE 1. Relative Weights of the Thymus, Adrenal Glands, and Spleen in Control and Stressed Rats with Different Activity in the Open Field after Injection of Physiological Saline, IL-1β, and IL-4 (mg/100 g body weight, *M±m*)

Organ, parameter		Active rats (*n*=40)		Passive rats (*n*=40)	
		control	stress	control	stress
Thymus	physiological saline	178.74±17.75	146.98±11.98	177.69±8.45	155.91±7.00*
	IL-1β	142.19±11.25^{x}	135.62±17.16	110.14±5.89^{++}	146.50±16.76*
	IL-4	158.55±10.57	144.94±16.66	143.83±8.28^{oo+}	157.48±10.92
Adrenal glands	physiological saline	9.91±1.12	10.85±0.36	11.65±1.13	11.67±1.08
	IL-1β	8.80±0.53	8.54±0.83^{+}	7.95±0.46^{+}	9.69±0.89
	IL-4	8.48±0.58	8.20±0.89^{+}	9.31±1.00	8.60±0.43^{+}
Spleen	physiological saline	645.72±67.96	516.51±53.80	668.51±30.76	553.46±43.15*
	IL-1β	577.25±41.75	515.77±48.02	577.34±57.46	534.01±33.43
	IL-4	586.01±52.72	602.45±55.17	539.32±52.04^{+}	684.47±42.66^{*+o}

Note. $^{*}p<0.05$ compared to nonstressed rats; $^{+}p<0.05$ and $^{++}p<0.01$ compared to rats receiving physiological saline; $^{x}p<0.05$ compared to passive rats; $^{o}p<0.05$ and $^{oo}p<0.01$ compared to rats of the IL-1β group.

Therefore, treatment with pro-inflammatory cytokine IL-1β and anti-inflammatory cytokine IL-4 was followed by unidirectional changes in the relative weight of the thymus, adrenal glands, and spleen in nonstressed rats. The decrease of the weight of these organs after cytokine injection in behaviorally passive animals was more pronounced than in active specimens.

The decrease in the relative weight of study organs in IL-treated rats can be related to several reasons. The cytokines have a regulatory effect on cell migration in mammalian tissues. IL-4 is involved in the regulation of migration of eosinophils, monocytes, and lymphocytes [3]. IL-1β induces a directed migration of Langerhans cells from the epithelial tissue to lymph nodes [15]. IL-1β has a modulatory effect on smooth muscle cell migration in the skin [9].

Cytokines regulate normal process of programmed cell death. For example, pro-inflammatory cytokine IL-1β induces apoptosis in hepatocytes, cholangiocytes, and endotheliocytes of the hepatic ducts [8] and Langerhans islets [10]. The systemic effect of IL-1β contributes to zinc deficiency in the body. It is followed by thymus involution due to a decrease in thymulin production and increased apoptosis in $CD4^{+}CD8^{+}$ cells. IL-1β-induced apoptosis is probably accompanied by an increase in corticosterone concentration in blood serum [4].

The increase in monocyte apoptosis is also observed after administration of anti-inflammatory cytokines (*e.g.*, IL-4). Some authors reported that this cytokine has anti-thymogenic properties [4,13]. Experiments on transgenic animals showed that IL-4-induced involution of the thymus was associated with suppression of thymocyte differentiation and atrophy of the gland [4].

Published data suggest that the observed decrease in the weight of study immunocompetent organs after treatment with IL-1β and IL-4 is related to increased migration and apoptosis of cells.

Then we studied the effect of IL-1β and IL-4 on stress-marker organs in rats during immobilization with simultaneous electrocutaneous stimulation (Table 1). As distinct from physiological saline-treated animals, poststress involution of the thymus and spleen was not observed in animals treated with the cytokines. The relative weight of immunocompetent organs in behaviorally active animals receiving IL-1β or IL-4 before stress exposure did not differ from that in nonstressed specimens. The exposure of passive rats to acute stress after pretreatment with cytokines was followed by a significant increase in the weight of the thymus (by 1.33 times in stressed animals receiving IL-1β, $p<0.05$) and spleen (by 1.27 times in stressed animals receiving IL-4, $p<0.05$).

Hence, a pro-inflammatory cytokine IL-1β and anti-inflammatory cytokine IL-4 have similar effects on the functional state of stress-marker organs in rats. Pretreatment with study cytokines prevents involution of the thymus and spleen in animals during restraint stress with simultaneous electrocutaneous stimulation. IL-1β and IL-4 have a greater effect in passive rats than in active specimens, which reflects the specific features of immune mechanisms in animals with different emotional reactivity.

We are grateful to Prof. A. S. Simbirtsev (State Research Institute of Highly Pure Biopreparations,

Federal Medical and Biological Agency of Russia, St. Petersburg) for kindly provided recombinant IL-1β.

This work was supported by the grant of the President of Russian Federation for Support of Leading Scientific Schools of Russian Federation (grant No. NSh-3232.2008.4).

REFERENCES

1. I. A. Borshchenko, A. V. Baskov, A. G. Korshunov, and F. S. Satanova, *Vopr. Neirokhir.*, No. 2, 28-31 (2000).
2. E. A. Ivanova, S. S. Pertsov, E. V. Koplik, and A. S. Simbirtsev, *Byull. Eksp. Biol. Med.*, **148**, No. 10, 377-382 (2009).
3. S. A. Ketlinskii and A. S. Simbirtsev, *Cytokines* [in Russian], St. Petersburg (2008).
4. E. P. Kiseleva, *Uspekhi Sovrem. Tekhnol*., **124**, No. 6, 589-601 (2004).
5. E. V. Koplik, *Vestn. Nov. Med. Tekhnol*., **9**, No. 1, 16-18 (2002).
6. S. S. Pertsov, E. V. Koplik, V. L. Stepanyuk, and A. S. Simbirtsev, *Byull. Eksp. Biol. Med.*, **148**, No. 8, 161-165 (2009).
7. K. V. Sudakov, *Emotional Stress: Theoretical and Clinical Aspects* [in Russian], Volgograd (1997).
8. *Chronic Viral Hepatitis* [in Russian], Eds. V. V. Serov and Z. G. Aprosina, Moscow (2002).
9. A. M. Carter, *Diab. Vacs. Dis. Res*., **2**, No. 3, 113-121 (2005).
10. A. Dunger, P. Augstein, S. Schmidt, and U. Fischer, *J. Autoimmun*., **9**, No. 3, 309-313 (1996).
11. A. Gadek-Michalska, A. J. Bugajski, and J. Bugajski, *J. Physiol. Pharmacol*., **59**, No. 3, 563-575 (2008).
12. J. P. Godbout and R. Glaser, *J. Neuroimmune Pharmacol*., **1**, No. 4, 421-427 (2006).
13. W. E. Paul, *J. Am. Soc. Hematol*., **77**, No. 9, 1859-1869 (1991).
14. H. Selye, *J. Clin. Endocr*., **6**, 117-230 (1946).
15. B. Wang, P. Amerio, and D. N. Sauder, *J. Leukoc. Biol*., **66**, No. 1, 33-39 (1999).