Effect of Interleukin-1β on Functional Activity of Lymphoid Structures in the Gastrointestinal Tract of Rats during Acute Emotional Stress

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We studied the effect of interleukin-1β on functional activity of lymphoid structures in the gastrointestinal tract of rats with various behavioral parameters during stress of simultaneous immobilization and electrocutaneous stimulation. Morphofunctional characteristics of lymphoid tissue were estimated by studying elimination of intraperitoneally injected Chinese ink particles into the mesenteric lymph nodes and wall of the jejunum. Intraperitoneal injection of interleukin-1β (5 μg/kg, 10^8 U/mg) was accompanied by accumulation of Chinese ink in the mesenteric lymph nodes of unstressed passive and active rats. The observed changes reflect an immunostimulatory effect of this cytokine. Acute stress was followed by an increase in the number of ink particles in the mesenteric lymph nodes and wall of the jejunum in behaviorally active rats. Under these conditions, the number of ink particles was elevated only in the mesenteric lymph nodes of passive specimens. As differentiated from passive animals, pretreatment of active rats with interleukin-1β before acute stress was followed by the increased elimination of Chinese ink (antigenic material) from the abdominal cavity to the lymph nodes and through the wall of the jejunum. These data illustrate specific features of immune mechanisms for the stress response in mammals with various behavioral characteristics.

Key Words: *interleukin-1β; emotional stress; passive and active rats; lymphoid structures of the gastrointestinal tract*

The problem of emotional stress and stress-induced psychosomatic disorders is of considerable significance. A large body of evidence indicates that psychoemotional stress is accompanied by immune dysfunction in mammals [10]. Much attention is paid to studying the local cellular and humoral reactions in the gastrointestinal tract [3,5,6]. Published data show that functional activity of immune structures in the gastrointestinal tract of animals is altered during stress. Experiments on mice with behavioral depression revealed that stress is followed by severe deficiency of the local humoral response and decrease in the production of specific antibodies in the mucous membrane of the gastrointestinal tract under conditions of antigenic stimulation. The relationship exists between stress exposure, cytokine secretion, and humoral immunity *in vivo* [9].

Significant differences were found in the individual resistance of mammals to emotional stress [7,8]. Previous studies showed that behavioral activity in the open-field test serves as a reliable prognostic criterion for the resistance of rats to stress [2]. Behaviorally active animals are prognostically more resistant to negative consequences of stress exposure than passive specimens.

Immunomodulatory substances (*e.g.*, cytokines) hold much promise for directional increase in the individual resistance to stress. Much attention is paid to

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the superfamily of interleukin-1 (IL-1). IL-1 performs at least 50 biological functions in the organism. The cells of nearly all organs and tissues serve as the target for this compound [1]. The presence of cytokine receptors on neurons and glial cells of the hypothalamus indicates that these compounds have a neuroimmunomodulatory effect in the central nervous system [11,12]. Our previous experiments showed that acute emotional stress is accompanied by specific changes in plasma IL-1β concentration in rats with active and passive behavioral patterns in the open-filed test [4]. These data illustrate specific role of IL-1β in the stress response of animals various individual-and-typological characteristics.

Here we studied the effect of IL-1β on functional activity of lymphoid structures in the jejunum and mesenteric lymph nodes of rats with various behavioral parameters during acute emotional stress.

MATERIALS AND METHODS

Experiments were performed on 40 male Wistar rats weighing 220.0±5.2 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 rats were housed in cages (5 specimens per cage) at 20-22°C and artificial light/dark cycle (8.00-20.00, lightness; 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 for 3 min [2]. 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.

The open-filed test allowed us to reveal 20 active rats (prognostically resistant to stress) and 20 passive rats (prognostically predisposed to stress). Passive and active animals differed by the index of activity (0.2-0.6 and 4.5-6.0, respectively). Behaviorally active and passive rats were divided into 8 groups of 5 specimens each (Table 1). IL-1β in a dose of 5 μg/kg (activity 10^8 U/mg) was dissolved in 1 ml physiological saline. Saline (1 ml) or IL-1β was injected intraperitoneally to animals 1 h before stress exposure. Control (unstressed) rats received an injection of saline or IL-1β 2 h before decapitation. Human recombinant IL-1β was obtained from the State Research Institute of Highly Pure Biopreparations (Federal Medical and Biological Agency of Russia).

Simultaneous immobilization and electrocutaneous stimulation of rats served as the model of acute emotional stress [2,4]. The animals were placed in individual plastic cages for 1 h (length 16.5 cm, inner diameter 5.5 cm). Metal needle electrodes were inserted into the skin of the back to deliver stochastic electrocutaneous stimulation with alternating current (pulse duration 1 msec, voltage 4-6 V, frequency 50 Hz). The strength of stimulation was selected individually by the vocalization threshold during electrostimulation. The duration of one session of electrostimulation was 30 or 60 sec. The animals were subjected to 12 sessions of 30-sec stimulation and 5 sessions of 60-sec stimulation over 1 h of stress exposure. The interval between repeated sessions of electrical stimulation was 90-180 sec. Control (unstressed) rats were maintained in home cages during this period. Stressed rats and control animals were decapitated immediately after the experiment.

Vital treatment with a solution of Chinese ink is accompanied by delivery of its particles into small vessels, which provides a clear microscopic picture. Morphofunctional characteristics of lymphoid tissue in the small intestine and mesenteric lymph nodes were studied using Chinese ink. Chinese ink (250 mg) was ground and dissolved in 5 ml 0.9% physiological saline immediately before the experiment. This solution was heated and filtered. The solution of Chinese ink (2 ml) was injected intraperitoneally 1 h before stress (1-h immobilization and electrocutaneous stimulation) or 2 h before decapitation (control).

Segments of the jejunum (length ≈1 cm) and mesenteric lymph nodes (median node of *Lymphonodi jejunales*) were taken from each rat immediately after decapitation and fixed in 10% formalin. Histological samples were prepared routinely and stained with eosin. Ink particles over the entire surface of a histological section were counted using a MBI-3 binocular microscope (ocular ×10, objective ×40). The area of sections was measured with a Stefanov grid (880 μ^2).

The results were analyzed by nonparametric Mann—Whitney test. The data are expressed as mean values.

RESULTS

Single particles of Chinese ink were found in the mesenteric lymph nodes of unstressed rats. The number of these particles practically did not differ in behaviorally passive and active specimens (Table 1, Fig. 1, *a*, *b*). The number of ink particles in the wall of the jejunum in behaviorally passive animals was 8-fold higher than in active rats ($p<0.01$; Fig. 2, *a*, *b*).

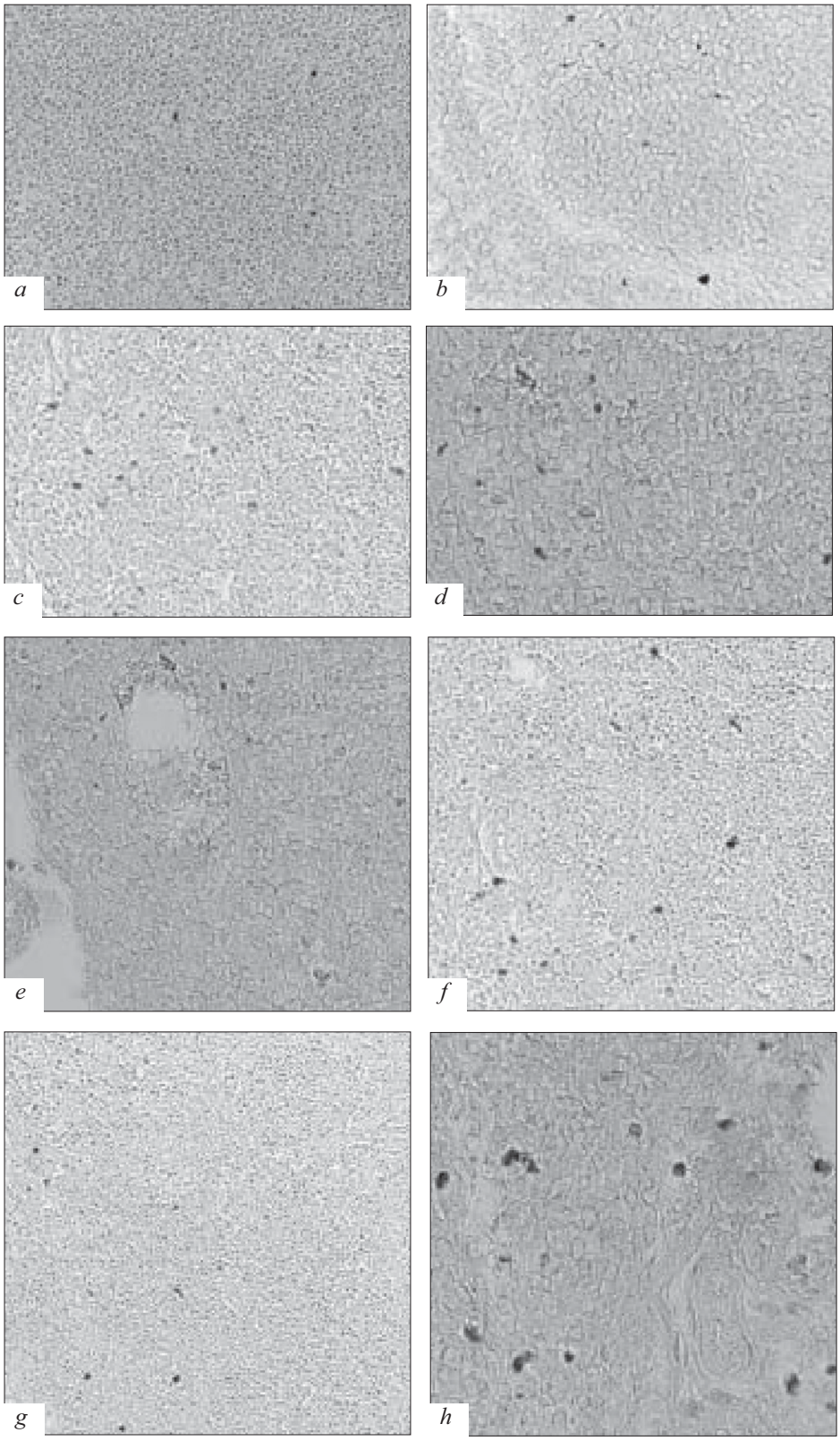

Fig. 1. Chinese ink particles between cell structures of the mesenteric lymph node in rats of various experimental groups. Here and in Fig. 2: (*a*) unstressed active rats, physiological saline; (*b*) unstressed passive rats, physiological saline; (*c*) unstressed active rats, IL-1β; (*d*) unstressed passive rats, IL-1β; (*e*) stressed active rats, physiological saline; (*f*) stressed passive rats, physiological saline; (*g*) stressed active rats, IL-1β; (*h*) stressed passive rats, IL-1β. Eosin staining, ×1600.

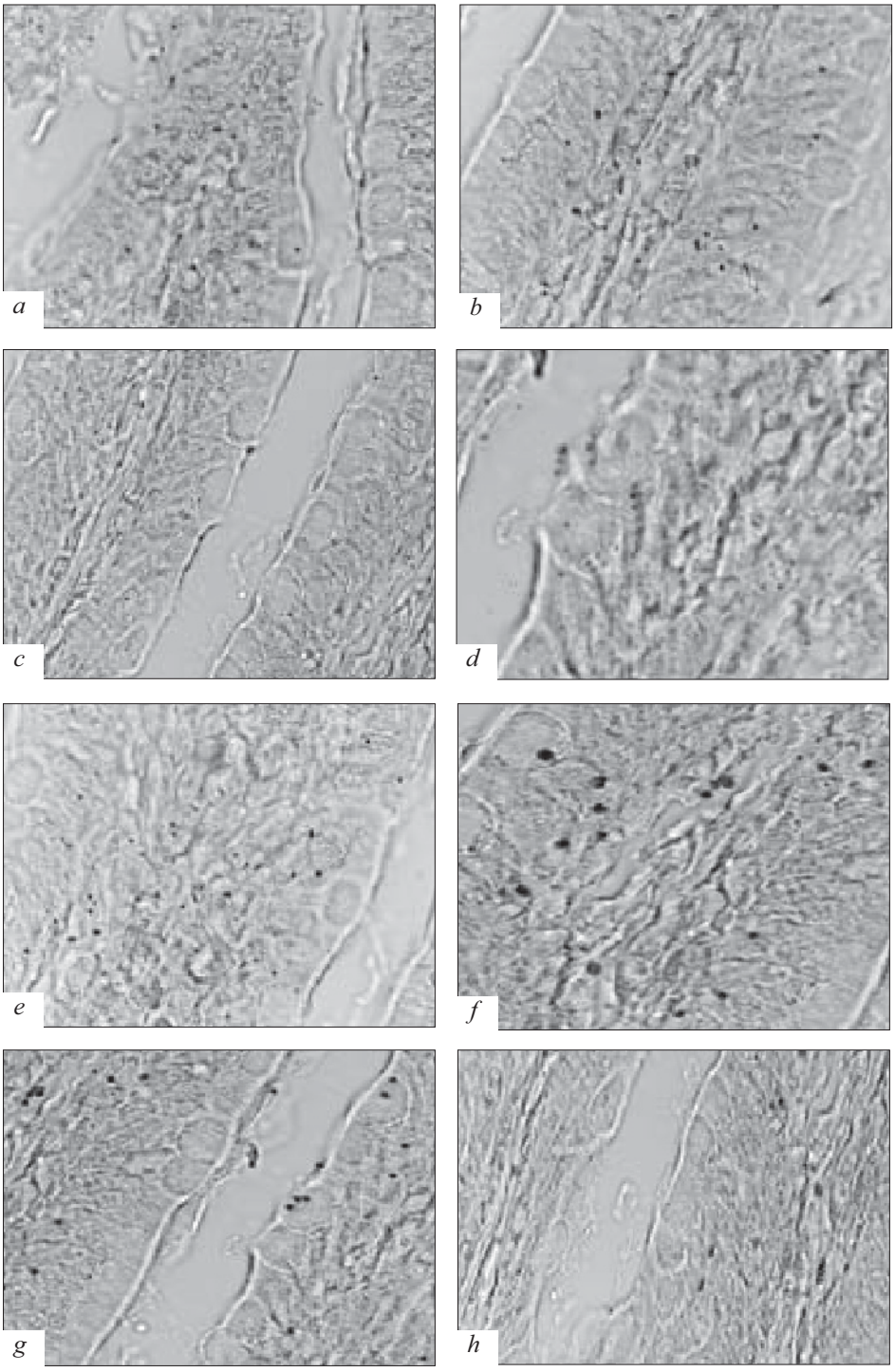

Fig. 2. Chinese ink particles in the villi of the jejunum in rats of different experimental groups.

Intraperitoneal injection of IL-β was accompanied by accumulation of Chinese ink in the mesenteric lymph nodes of unstressed passive and active rats (Table 1, Fig. 1, *c*, *d*). After injection of IL-1β the number of ink particles in the lymph nodes of these animals was higher than in saline-receiving rats (by 5.5 and 4.3 times, respectively, $p<0.05$). As differentiated from passive rats, administration of IL-1β to unstressed active animals was accompanied by the increased elimination of ink through the wall of the jejunum (by 3 times, $p<0.05$ compared to specimens of the saline group; Fig. 2, *c*, *d*).

Lymph nodes are the most abundant structures of the peripheral immune system in mammals. A protec-

tive function of the lymph nodes is related to deactivation of an antigenic material. The increased accumulation of Chinese ink in the mesenteric lymph nodes of IL-1β-receiving rats probably reflects activation of immune processes under the influence of this cytokine. Our results are consistent with published data that IL-1β is one of the major mediators for the local inflammatory reaction and acute phase response of an organism [1]. IL-1β stimulates a variety of defense reactions that are directed towards prevention of infection dissemination, elimination of foreign microorganisms, and recovery of the integrity in damaged tissues.

Simultaneous immobilization and electrocutaneous stimulation were followed by a significant increase in the elimination of Chinese ink from the abdominal cavity (Table 1). Stress exposure in behaviorally passive animals was accompanied by a selective increase in the number of ink particles in the mesenteric lymph nodes (by 14.5 times, $p<0.01$ compared to unstressed specimens; Fig. 1, *f*), but not in the wall of the jejunum (Fig. 2, *f*). The number of Chinese ink particles in the mesenteric lymph nodes and wall of the jejunum in active rats was increased after acute stress (by 29 [$p<0.001$] and 5.2 times [$p<0.05$], respectively, compared to unstressed specimens; Fig. 1, *e*, 2, *e*).

Pretreatment with IL-1β before stress had the most significant effect on Chinese ink elimination from the abdominal cavity of behaviorally activity rats (Table 1). These animals were characterized by a decrease in the number of ink particles in the mesenteric lymph nodes (by 7.9 times, $p<0.01$ compared to active rats of the saline group; Fig. 1, *g*). However, the number of Chinese ink particles in the wall of the jejunum in active rats significantly increased after stress exposure and injection of IL-1β (by 7.6 times, $p<0.01$ compared to unstressed specimens; Fig. 2, *g*). The number of ink particles in these rats was 4.4-fold higher than in animals receiving saline ($p<0.05$). In passive rats the number of ink particles remained unchanged in the mesenteric lymph nodes (Fig. 1, *h*), but decreased in the wall of the jejunum (by 3 times, $p<0.05$ compared to unstressed animals; Fig. 2, *h*).

Our results suggest that administration of IL-1β before acute stress in behaviorally active rats not only contributes to rapid elimination of Chinese ink from the abdominal cavity to the mesenteric lymph nodes, but also decreases the latency of the immune response in lymphoid structures of the lymph nodes to ink particles (antigenic material). These changes are accompanied by a decrease in the number of Chinese ink particles in the mesenteric lymph nodes and increase in the elimination of ink through the wall of the small intestine.

We conclude that emotional stress is accompanied by various functional changes in lymphoid tissues of the jejunum and mesenteric lymph nodes in rats with different behavioral profiles in the open field. Under normal conditions, IL-1β has an immunostimulatory effect on behaviorally active and passive animals. During acute stress, the effect of IL-1β on functional activity of lymphoid structures of the gastrointestinal tract is most pronounced in active rats (prognostically resistant to stress). These data illustrate the significance of an individual approach to studying the immune mechanisms of emotional stress and correction of mammalian resistance to the same stress exposure.

TABLE 1. Number of Chinese Ink Particles in Rats of Different Experimental Groups (per 10 mm² Histological Section)

Group	Passive rats (*n*=20)		Active rats (*n*=20)	
	PS	IL-1β	PS	IL-1β
	Jejunum			
Control	4.8	4.5	0.6^{xx}	1.8^{+x}
Stress	5.2	1.5^{*+}	3.1^{*x}	13.7^{***+xx}
	Mesenteric lymph node			
Control	0.2	1.1^{+}	0.3	1.3^{+}
Stress	2.9**	3.0*	8.7^{***x}	1.1^{++x}

Note. PS, physiological saline. *$p<0.05$, **$p<0.01$, and ***$p<0.001$ compared to control (unstressed) rats; $^{+}p<0.05$ and $^{++}p<0.01$ compared to rats receiving saline; $^{x}p<0.05$ and $^{xx}p<0.01$ compared to passive rats.

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