# Modulatory Influence of Rarely Repeated Immobilization Episodes on the Interleukin-1β-Dependent Reaction of Blood Leukocytes and Hepatoprotective Effect of Restraint Stress

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Repeated episodes of 1-h restraint stress were accompanied by a decrease in the sensitivity of blood leukocytes and cytochrome P450-dependent monooxygenases of the liver to recombinant IL-1β. These changes are associated with the anti-inflammatory hepatoprotective effect of chronic stress.

**Key Words:** *stress; interleukin-1β; leukocytes; cytochrome P450*

Proinflammatory cytokine interleukin-1β has a pleiotropic effect on blood leukocytes. Direct or indirect effects of this cytokine is manifested in activation of granulocytopoiesis, monocytopoiesis, and lymphopoiesis, regulation of leukocyte redistribution between various compartments of the blood system, migration of leukocyte effector cells into the inflammatory site, and increase in phagocytic activity of neutrophilic granulocytes [2]. Previous studies showed that repeated episodes of 1-h restraint stress are followed by reduction of leukocyte infiltration in the liver (despite a decrease in blood level of the anti-inflammatory cytokine IL-4) [3]. The concentrations of proinflammatory cytokines IL-1 and TNF did not decrease under these conditions. The reduced sensitivity of leukocytes to proinflammatory cytokines and decrease in the concentration of anti-inflammatory cytokines probably contribute to the proinflammatory effect of stress exposure.

Our study was designed to test this hypothesis.

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## MATERIALS AND METHODS

Experiments were performed on 32 male and female outbred rats. The average effective dose ($ED_{50}$) of human recombinant IL-1β (Russian State Research Center, Institute of Highly Pure Biopreparations) for leukocytosis was estimated by the Litchfield–Wilcoxon method (6.5 μg/kg). The animals were divided into 4 groups. Group 1 animals served as the control. Group 2 animals were exposed to rarely repeated episodes of 1-h immobilization (RRIM). Repeated immobilization was produced by 1-h fixation of animals in the supine position on a plywood plate. The intervals between stress episodes were 72 h. The procedure was repeated 4 times. Group 3 animals received an intraperitoneal injection of human recombinant IL-1β (Betaleukin) in a dose of 6.5 μg/kg. Group 4 animals were injected with Betaleukin 24 h after the last episode of repeated stress. The animals were intraperitoneally immunized with sheep erythrocytes ($10^7$ cells/g) 24 h after the last episode of stress exposure. The antigen in the challenge dose ($10^7$ sheep erythrocytes per 1 g body weight) was injected into the hindlimb pad on day 4 after immunization. An equivalent volume of isotonic NaCl was administered into the contralateral limb. The reaction

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was evaluated volumetrically 24 h after an injection of sheep erythrocytes in the challenge dose [1]. The animals were euthanized with diethyl ether 24 h after Betaleukin injection. The leukogram and myelogram were estimated in smears. The smears were fixed in methanol and stained by the Romanovsky–Giemsa method. Monooxygenases activities were measured fluorometrically with the submitochondrial fraction of liver homogenates (S12-fraction) [5]. Activities of 7-ethoxyresorufin-O-deethylase (EROD activity; CYP1A1-dependent monooxygenation) and 7-benzyloxyresorufin-O-debenzylase (BROD activity; CYP2B1/2-dependent monooxygenation) were measured [6].

The results were analyzed by Mann–Whitney *U* test, Wald–Wolfowitz *WW* test, and Newman–Keuls (ANOVA, Statistica 6.0 software).

## RESULTS

An injection of Betaleukin to stressed and nonstressed animals had a strong effect on blood leukocytes (Table 1). Leukocytosis was observed in animals of the IL-1 group. The total number of leukocytes was elevated by 34%. The count of band neutrophils, segmented cells, and circulating monocytes increased by 2.24 ($P$=0.019$U$), 2.63 ($P$=0.019$U$), and 6 times ($P$=0.0019$U$), respectively, compared to the control. Only the number of lymphocytes did not change in specimens of the IL-1 group. Preliminary stress exposures 3-fold decreased the number of circulating band neutrophils ($P$=0.033$U$) and segmented neutrophils ($P$=0.033$U$). The count of monocytes decreased by 4.5 times ($P$=0.044NK). Opposite changes were observed in the amount of circulating lymphocytes. The number of blood lymphocytes in animals of the stress+IL-1 group increased by 83% as compared to specimens of the IL-1 group ($P$=0.033$U$). No differences were found in the total number of leukocytes in animals of the IL-1 group and stress+IL-1 group. Bone marrow hypoplasia in nonstressed animals was revealed 24 hours after injection of the cytokine. The count of bone marrow nucleated cells was reduced by 40% ($P$=0.02$U$). The development of hypoplasia was mainly related to mobilization of bone marrow neutrophils. The number of these cells in treated animals was 2.5-fold lower than in control specimens ($P$=0.004$U$). Moreover, the animals of this group were characterized by a 3-fold decrease in the count of bone marrow monocytes ($P$=0.037$U$). IL-1 administration after stress exposures was accompanied by a significant increase in the number of bone marrow neutrophils. These data indicate that preliminary episodes of stress exposure prevent the IL-1-dependent mobilization of bone marrow neutrophils into the circulation. The inhibitory effect of RRIM on the cytokine-dependent reaction of blood lymphocytes is probably related to

**TABLE 1**. Effect of RRIM and IL-1 on Some Parameters of Blood Leukocytes and Severity of Liver Necrosis ($M\pm m$)

| Parameter | Group | | | |
|---|---|---|---|---|
| | 1 (control) | 2 (RRIM) | 3 (IL-1) | 4 ( RRIM+IL-1) |
| Hepatic monocytes/macrophages, cells/mm$^2$ | 3.04±0.55 | 1.9±0.1 | 4.43±0.49 | 3.80±0.09<br>$P_{3,4}$=0.026*WW* |
| Necrotic injury of the liver, % | 0.800±0.075 | 0.780±0.075 | 7.00±0.94 | 0.670±0.049<br>$P_{3,4}$=0.019*U* |
| Circulating lymphocytes, ×10$^9$/liter | 5.43±3.30 | 5.54±1.40 | 4.9±0.7 | 7.30±0.81<br>$P_{3,4}$=0.02*U* |
| Band neutrophils, ×10$^9$/liter | 0.472±0.890 | 0.41±0.17 | 1.05±0.24<br>$P_{1,3}$=0.019*U* | 0.35±0.04<br>$P_{3,4}$=0.02*U* |
| Segmented neutrophils, ×10$^9$/liter | 0.896±2.100 | 0.72±0.03 | 2.10±0.61<br>$P_{1,3}$=0.019*U* | 0.850±0.047<br>$P_{3,4}$=0.02*U* |
| Circulating monocytes, ×10$^9$/liter | 0.466±0.033 | 0.44±0.08 | 1.40±0.28<br>$P_{1,3}$=0.019*U* | 0.31±0.05<br>$P_{3,4}$=0.04*U* |
| Bone marrow neutrophils, ×10$^6$ cells | 20.77±1.76 | 24.25±4.45 | 7.59±2.39<br>$P_{1,3}$=0.02*U* | 19.8±3.5<br>$P_{3,4}$=0.004*U* |

**Note**. Each group consists of 8 specimens.

a typical anti-inflammatory hepatotropic action under these conditions. The area of necrotic changes in the liver parenchyma was shown to increase in rats of the IL-1 group. The number of monocytes/macrophages tended to increase in these animals. The number of monocytes/macrophages and severity of liver necrosis in animals of the stress+IL-1 group were lower than in specimens of the IL-1 group. It should be emphasized that the hepatoprotective effect of chronic stress is associated not only with blood signs, but also with metabolic criteria for the reduced sensitivity to cytokine products. The majority of proinflammatory cytokines can inhibit activity of cytochrome P450 isoforms. Our results are consistent with these data. Twenty-four hours after IL-1 injection, the nonstressed animals were characterized by a decrease in CYP1A1-dependent EROD activity (from 6.52±0.24 ($n$=6) to 2.65±0.11 pmol/g tissue/min ($n$=6); $P$=0.04$U$) and CYP2B1/2-dependent BROD activity (from 58.26±5.54 ($n$=6) to 36.5±1.4 pmol/g tissue/min ($n$=6); $P$=0.006$U$). BROD activity in animals of the stress+IL-1 group was higher than in specimens of IL-1 group (47.8±4.2 ($n$=6) and 36.5±1.4 pmol/g tissue/min ($n$=6), respectively; $P$=0.044$WW$). The cytokine-dependent inhibition of cytochrome P450 isoforms is a particular case of suppressed biotransformation of xenobiotics and lipophilic endobiotics during immunization. Immunization of rats with sheep erythrocytes (SE group) was accompanied by the reduction of CYP1A1-dependent monooxygenation (from 5.4±0.2 ($n$=6) to 3.88±0.10 pmol/g tissue/min ($n$=9); $P$=0.045$U$) and slight decrease in activity of CYP2B1/2 isoform (from 65.08±1.87 ($n$=6) to 63.33±3.35 pmol/g tissue/min ($n$=10).

The immune-dependent suppression of cytochrome P450-dependent monooxygenases was completely abolished by preliminary stress exposures. As compared to animals of the SE group, specimens of the stress+SE group were characterized by the increased activities of BROD (63.33±3.35 ($n$=9) and 75.22±3.07 pmol/g tissue/min ($n$=10), respectively; $P$=0.046$U$) and EROD (3.88±0.10 ($n$=9) and 5.53±0.26 pmol/g tissue/min ($n$=10), respectively; $P$=0.037$U$) 96 h after alloantigen injection. Therefore, RRIM prevents the immune-dependent depriming of cytochrome P450-dependent monooxygenases.

Our results indicate that preliminary stress exposures reduce the sensitivity of blood leukocytes to the proinflammatory cytokine. Under *in vivo* conditions, some effects of the cytokine on blood leukocytes can be mediated by glucocorticoids. This regimen of repeated stress exposures is characterized by desensitization to glucocorticoids. These changes can affect the directionality of variations in white blood cells in animals of the stress+IL-1 group. At the same time, desensitization to glucocorticoids provokes the proinflammatory hepatotropic effects of chronic stress. However, RRIM was accompanied by anti-inflammatory changes in the liver. Moreover, glucocorticoids do not mediate the cytokine-dependent induction of cytochrome P450 isoforms (CYP1A1 and CYP2B1/2). We believe that the hepatotropic protective effects of RRIM are partly related to desensitization to proinflammatory cytokines.

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