Effect of Exercise on Endotoxin Shock with Special Reference to Changes in Concentration of Cytokines

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Abstract The effects of exercise on susceptibility to endotoxin shock and the serum levels of interleukin-1 (IL-1) and interleukin-6 (IL-6) were studied in mice primed with *Propionibacterium acnes* (*P. acnes*, 1.0 mg). Endotoxin shock was induced by injection of lipopolysaccharide (LPS, 0.2 mg) 7 d after the priming injection of *P. acnes*. The exercise-loaded mice performed voluntary exercise for 120 min on a running-wheel and LPS was injected into the tail vein immediately after the end of exercise. Two of the nine animals in the exercised group survived, whereas all the mice in the control group died. The mean survival time after LPS injection in the exercised group (10.75 ± 4.51 h) was significantly longer than that in the control group (3.3 ± 1.34 h). The serum concentrations of IL-1 ($2,883 \pm 1,542$ U/ml) and IL-6 (966 ± 619 ng/ml) in the exercised group, sampled 2 h after LPS challenge, were significantly lower than those in the control group ($6,571 \pm 2,766$ U/ml and $2,428 \pm 1,228$ ng/ml, respectively). It is suggested that the exercise-induced inhibition of endotoxin shock may be caused, at least partly, by the decreased production of IL-1 and IL-6.

Key words: endotoxin shock, exercise, interleukin-1, interleukin-6.

Many investigators have reported that exercise alters the immune system and acute-phase responses during infection and inflammation. These studies have suggested that interleukin-1 (IL-1) causes acute-phase responses after severe exercise [1–4], and that interleukin-6 (IL-6) [5] is produced in response to physiological stresses, including exercise [6, 7]. We reported previously that mice which had

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been pre-injected with *Propionibacterium acnes* (*P. acnes*) became more susceptible to lipopolysaccharide (LPS)-induced shock with increased release of IL-1 and tumor necrosis factor (TNF) [8]. These two cytokines are known to accelerate the secretion of IL-6 by target cells as a part of the acute-phase reaction [5]. In *P. acnes*-primed mice, there was also a positive correlation between the serum level of IL-6 and the mortality rate when the animals were infused intravenously with IL-1 and TNF [8]. Since a significant increase in the serum level of IL-6 is usually observed in animals with endotoxin shock, an increased release of IL-6 is considered to reflect the severity of the shock. Thus, the serum concentration of IL-6 can be used as a prognostic parameter in clinical situations [8].

In this paper, we report the effects of voluntary exercise on the susceptibility to endotoxin shock in mice and the relationship between the degree of shock and changes in the serum concentrations of IL-1 and IL-6 in a murine endotoxin shock model.

MATERIALS AND METHODS

Animals. Female ICR mice, aged 5 weeks (10–12 g body weight), were purchased from Shizuoka Animal Center (Shizuoka, Japan). They were housed four per cage and were fed ad libitum with MF mouse food (Oriental Kohbo Co., Osaka, Japan) and water. The lighting conditions consisted of 12-h light period (0600–1800 h) and 12 h of darkness (1800–0600 h). All experiments described here were performed in accordance with guidelines for the Care and Use of Laboratory Animals in Hyogo College of Medicine.

Lymphocyte culture medium. The cell culture medium consisted of RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum (FCS), penicillin (50 µg/ml), streptomycin (100 µg/ml), L-glutamine (2 mM), and 2-mercaptoethanol (5×10^{-5} M).

Cytokines. Recombinant human IL-1 β (specific activity: 2×10^7 U/mg) and human TNF α (sp. act.: 3×10^6 U/mg) were a kind gift from Dainippon Pharmaceutical Co., Ltd. (Osaka, Japan). Recombinant human IL-6 (sp. act.: 5×10^7 U/mg) was kindly provided by Dr. T. Kishimoto (Osaka University, Osaka, Japan).

Bacteria and reagents. *P. acnes* (ATCC11827) was selected from a single colony on solid brain-heart infusion agar and cultured for between 2 and 4 d in the liquid brain-heart infusion medium (Difco Laboratories, Detroit, MI). It was then harvested by centrifugation (5,000 rpm for 15 min), washed and heat-killed (4 h at 60°C). LPS derived from *Escherichia coli* was purchased from Difco Laboratories.

Induction of experimental endotoxin shock. The mice received an i.p. injection of inactivated *P. acnes* (1 mg wet weight per mouse), and 7 d later they were given an i.v. injection of LPS (0.2 mg) [9] or a mixture of IL-1 β (1 µg) and TNF α (1 µg). The LPS and cytokines were diluted with endotoxin-free HBSS containing 5% mouse serum and injected in a volume of 0.2 ml into a tail vein.

Course of exercise and treatment of mice. Four running-wheels, described

previously in detail by Iso and Shimai [10], were used for exercising the mice in this experiment. In order for the mice to run without electric shock, all were first trained to run with a Sidman-type avoidance schedule using a 5-s shock-shock interval and a 10-s response-shock interval, for 120 min per day on three successive days. Mice which ran without shocks were used for the study. Thus, the exercise protocol used in the present experiment is better than compulsory exercise such as running on a treadmill [11, 12]. The exercise group comprised 59 mice, and 58 mice were used as non-exercised controls (control group). The challenge with *P. acnes* was carried out 7 d after the last training period. Seven days after the challenge with *P. acnes*, the mice in the exercise group performed voluntary exercise for 120 min and were then injected with LPS immediately after exercise. The control mice were treated in the same manner but without exercise. The mean survival time was recorded for nine mice from the exercised group and ten from the control group. Blood samples were collected from 31 mice from the exercised group and 30 from the control group 2 h after LPS injection for analysis of serum cytokine levels. The influence of exogenous cytokines on 19 mice from the exercised group and 18 from the control group was assessed in terms of the mortality rate 24 h after intravenous injection of a mixture of IL-1 β (1 μ g/mouse) and TNF α (1 μ g/mouse) instead of LPS.

Plasma samples. To measure the concentrations of cytokines in the serum 2 h after exposure to LPS, randomly selected mice were anesthetized with ether, sacrificed by cervical dislocation and blood was withdrawn using the method of transjugular cannulation. Plasma was separated by centrifugation of the freshly drawn blood and stored at -20°C until it was assayed for cytokine concentration.

IL-1 assay. IL-1 activity was measured by the thymocyte co-stimulation assay described previously [13]. Briefly, samples were serially diluted with culture medium (100 μ l/well) and incubated for 48 h with a single-cell suspension of non-LPS-responsive C3H/HeN mouse thymocytes (5×10^5 cells/well/200 μ l) and concanavalin A (Con A, 2 μ g/ml) in a 96-well culture plate at 37°C under a 5% CO_2 atmosphere. Then, 0.2 μ Ci/well of [^3H]thymidine was added after 72 h of culture, and the incorporation of radioactivity into the cells during the last 16 h was measured.

IL-6 assay. IL-6 activity was measured by proliferation assay, described previously in detail [14] using the murine IL-6-dependent cell line MH-60.BSF-2, generously provided by Dr. T. Kishimoto. Briefly, samples were serially diluted with culture medium and incubated for 48 h with IL-6-dependent cells (1×10^4 cell/well/200 μ l) in a 96-well culture plate at 37°C under a 5% CO_2 atmosphere. Then, 0.2 μ Ci/well of [^3H]thymidine was added after 42 h of culture, and the incorporation of radioactivity during the last 6 h was measured.

Statistical analysis. The data are expressed as means $\pm$ SD. Statistical analysis was performed using unpaired Student's *t*-test of the χ^2 -test.

RESULTS

Survival time

The survival times of the mice that developed endotoxin shock in the exercised and control groups are compared in Fig. 1. Two of the nine mice in the exercised group survived, whereas all of the mice in the control group ($n=10$) died. The mean survival time was 10.75 ± 4.51 h ($n=7$) in the exercised group, while it was 3.3 ± 1.34 h ($n=10$) in the control group. This difference was statistically significant ($p < 0.001$).

IL-1 production

Figure 2 shows the serum IL-1 concentrations in mice 2 h after LPS injection. The IL-1 concentration in the control group increased to $6,571 \pm 2,766$ U/ml in response to LPS, whereas it rose only slightly in the exercise group ($2,883 \pm 1,542$ U/ml). There was a significant difference ($p < 0.001$) in the IL-1 level between the groups.

IL-6 production

The mean serum IL-6 concentrations in both groups of mice are shown in Fig. 3. After LPS injection, the mean concentration of IL-6 in the exercised group was low (966 ± 619 ng/ml), whereas it was significantly higher in the control group ($2,428 \pm 1,228$ ng/ml, $p < 0.001$).

Fig. 1. Comparison of survival time in mice which received LPS (0.2 mg, i.v.) between the exercised group and control group. Two filled circles in parentheses indicate survived mice. $\circ$, mean value, bar indicates the standard deviation. Difference between two groups $p < 0.001$.

Fig. 2. Serum concentration of IL-1 in exercise-loaded and -unloaded mice 2 h after LPS administration (0.2 mg, i.v.). $\bigcirc$, mean value, bar indicates the standard deviation. Difference between two groups, $p < 0.001$.

Fig. 3. Serum concentration of IL-6 in exercise-loaded and -unloaded mice 2 h after LPS administration (0.2 mg, i.v.). $\bigcirc$, mean value, bar indicates the standard deviation. Difference between two groups, $p < 0.001$.

Sensitivity to exogenous cytokines

The mortality rate 24 h after infusion of a mixture of IL-1 β (1 μ g/mouse) and TNF α (1 μ g/mouse) was measured in the *P. acnes*-primed mice in both the exercised and control groups (Table 1). The mortality rate was 57.9% (11 of 19)

Table 1. Number of animals which survived 24 h after the infusion of a mixture IL-1 β (1 μ g/mouse) and TNF α (1 μ g/mouse).

	Survival (%)	Death (%)	Total
Exercised group	8 (42.1)	11 (57.9)	19
Control group	4 (22.2)	14 (77.8)	18
Total	12 (32.4)	25 (67.6)	37

Figures in parentheses indicate percentage.

in the exercised group, whereas it was 77.8% (14 of 18) in the control group. This difference was not statistically significant.

DISCUSSION

The present study revealed that the mean survival time of mice after exposure to LPS was significantly longer in the exercised group than in the control group. Moreover, two of the nine mice in the exercised group survived with only slight endotoxin shock, whereas all the mice in the control group died (Fig. 1). These findings suggest that the alleviation of endotoxin shock and the prolongation of mean survival time may be related to the exercise training before LPS challenge. The severity of endotoxin shock may also be affected by increased cardiovascular function induced by exercise, such as raised cardiac output. However, the increase in cardiovascular function caused by voluntary exercise is mild, and it usually returns to the resting level within 10 min of cessation of exercise [15]. In the present experiment, the earliest onset of endotoxin shock induced by LPS injection occurred 30 min after administration. Therefore, the effect of increased cardiovascular function due to voluntary exercise on endotoxin shock is thought to be absent or minimal.

In the present study, the serum concentrations of IL-1 and IL-6 in the exercised group remained low in comparison with those of the control group (Figs. 2 and 3). This raises the possibility that the suppression of endotoxin shock in the exercise-loaded mice might be brought about by suppressing the production of IL-1 and TNF.

It is well known that exercise activates the pituitary-adrenal axis. The increase in the plasma levels of adrenocorticotrophin (ACTH) and cortisol is proportional to the increase in the exercise load [16, 17]. It has also been reported that physiological stress, including exercise, modulates the immune response and increases the serum level of cortisol [18–20]. Voluntary running for 120 min (a total calculated running distance of more than 1,500 m), as used in the present experiment, was considered to be a work-load sufficient to increase the secretion of ACTH and glucocorticoid, which has been reported to have a beneficial effect on the bones and muscles in the rat [21]. Besedovsky *et al.* demonstrated that exercise-induced increases in ACTH and glucocorticoid may suppress IL-1 produc-

tion at the mRNA level [22, 23]. It is therefore suggested that these hormones, produced during exercise, may suppress the induction of endotoxin shock through their actions of reducing the release of IL-1.

Thus, it can be said that superior resistance to endotoxin shock induced by voluntary exercise immediately before injection of LPS resulted from a decrease in the serum concentration of IL-1 as well as decreased activity of the target cells of IL-1 and TNF. These findings suggest that voluntary exercise increases the capacity of animals to withstand endotoxin shock through changes in the immune system.

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