Comprehensive Welfare Vol.3 (2006)

Article

Chronic Exercise Associated with Enhanced Macrophage and Lymphocyte Cytokine Production in Young Mice

Haruo Sugiura[1], Hiroko Sugiura[2], Maki Ueya[3], Etsuo Ueya[4]

Abstract

This study was designed to evaluate the effects of voluntary running exercise on cytokine production of macrophage and lymphocyte. Twelve weeks old female BALB/c inbred mice were divided into two groups: a group has given voluntary running exercise (exercise group) and a non-exercise group (control group). Exercise consisted of spontaneous running in wheels 3 days per week for 8 weeks. The levels of nitric oxide and interleukin (IL)-1β production by lipopolysaccharide-stimulated peritoneal macrophages from the exercise group were significantly higher than those in the control group. Concanavalin A (Con A)-induced cell proliferation of the spleen in the exercise group was significantly higher than that in the control group. IL-2 and interferon-γ productions stimulated by Con A in the exercise group were significantly increased compared with the control group, but not IL-4 production. These results might suggest that 8-week voluntary running exercise effectively enhanced macrophage and lymphocyte functions in mice.

Key Words: exercise, macrophage, splenic lymphocyte, cytokine, mouse

The immune functions are known play an important role in host defense mechanisms. There have been many reports on the effects of exercise on immune functions, e.g., macrophage and lymphocyte functions[1][2]. Recent studies indicate that moderate intensity exercise enhances immune functions, but intense exercise training depresses immune functions[3][4][5][6][7]. In connection with the effect of chronic exercise training on immune functions, the results of animal experiments showed that forced and voluntary exercise in mice for 5 to 9 weeks enhanced not only *in vivo* but also *in vitro* cytotoxicity[5][6]. In addition, endurance exercise training was reported to increase the phagocytosis activity of macrophages and proliferative response of T cells to mitogens[8][9][10]. Therefore, it can be considered that chronic exercise training may improve the immune functions.

Macrophages, lymphocytes (T and B cells), dendritic and Langerhans' cells and natural killer cells are important cells for the immune system. Macrophages play a major role in inflammation, repair, humoral and cellular immunity and metabolic and neoplastic disease processes. Cytokines, being messenger molecules of the immune system, modulate natural immunity. These include interleukin (IL)-1β, IL-2, IL-4 and interferon (IFN)-γ. IFNs protect against viral infections and have an anti-tumor effect and activate macrophages, whereas IL-1 initiate non-specific inflammatory responses. Certain cytokines IL-2, IL-4, IL-5, IL-12 and tumor growth factor-β regulate lymphocyte growth, activation and differentiation[11][12]. Thus, cytokines have multiple biological functions.

It is known that exercise can modulate cytokine production[13]. In this regard, it has been reported in the studies of acute exercise involving humans, that pro-inflammatory cytokines such as, IL-1β, IL-6 and TNF-α were increased immediately after exercise[14][15]. With regard to the cytokine production from lymphocytes such as IL-2, IL-4 and IFN-γ, the results are different by exercise intensity and timing of blood sampling[16][17][18]. On the other hand, concerning the chronic exercise training, forced running exercise was reported to enhance in vitro production of T-cell growth factor stimulated

1） Laboratory of Exercise Physiology and Health Education, Gifu Pharmaceutical University
2） Department of Community and Mental Health Nursing, Gifu University School of Medicine
3） Department of Physiotherapy, Aichi Medical Treatment Academy
4） Faculty of Comprehensive Welfare, Urawa University

by Con A in the five days exercised rats[19]. Drenth et al.[20] reported that the ex vivo LPS-stimulated production of IL-1β in whole blood culture in athletes at rest was significantly higher than that in sedentary controls. In lymphocytes, the IL-2, IFN-γ and IL-4 production of the active elderly subjects were significantly of higher rates than the age-matched sedentary subjects, as reported in a review article by Venjatraman and Fernandes[21]. In contrast, 15-week of forced running exercise was showed to reduce the IL-2 production significantly in splenic lymphocytes of young (8 weeks of age) rats[22]. Thus, the results on cytokine production of macrophages and/or lymphocytes have been inconsistent. Moreover, the hypothesis of the possible impact of chronic exercise training on the cytokine production of macrophages and lymphocytes has not been well documented.

In the present study, we have investigated whether 8-week voluntary running exercise modifies (enhances) immune function, especially macrophage function, splenic lymphocyte proliferation and cytokine production, in mice. Macrophage function was evaluated by measuring production of nitric oxide (NO) and IL-1β by peritoneal macrophages as an indicator. Mitogenic response and production of IL-2, IFN-γ and IL-4 of spleen cells were examined for assessment of lymphocyte function.

MATERIALS and METHODS

Chemicals

RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum (FBS), 25 mM HEPES buffer, 100 units/ml penicillin, 0.1 mg/ml streptomycin, 1 mM L-glutamine and 0.1 M 2-mercaptoethanol was prepared by Gibco BRL (Gibco Laboratories Life Technologies, Inc., New York, USA); the pH was adjusted to 7.4 with $NaHCO_3$. This medium was filtered sterilely with a 0.22-μm filter (Millex-GV; Millipore, Bedford, MA). Lipopolysaccharide (LPS, *E. coli* 050: B5), concanavalin A (Con A) and other common chemicals for the NO and mitogenicity assay were purchased from Sigma (Sigma Chemical Co., St. Louis, USA).

Animals

The experimental protocol, animal care and treatment were approved by the committee for animal studies at Gifu Pharmaceutical University.

A total of 34 female BALB/c inbred mice, 11 weeks of age (weighing 18 to 20 g), obtained from Japan SLC Inc. (Hamamatsu City, Japan), were used for this experiment. They were housed, four to five per cage, with pellet food (CE-2: Nihon Clea, Japan) and water *ad libitum*, in an animal room under a 12 h light-dark cycle at a temperature of 22±1℃ and a humidity of 60 ± 5%. After a week acclimation, they were used for the experiment.

Exercise program

Thirty-four mice were divided into two groups: a group given voluntary exercise (exercise group, N=17) and a non-exercise group (control group, N=17). Each mouse of exercise group was kept in an individual cage equipped with the voluntarily revolving wheel (circumference 62.8 cm, Shinano Co. Ltd., Tokyo, Japan). A wheel was attached to one side of a mouse cage (21.5 × 8.5 × 8.5 cm^3), to which the mouse had free access. The animals were allowed to run voluntarily in the wheel from 8:00 p.m. to 8:00 a.m. of the following day. The distance that the animals ran was measured each day by a counter attached to the wheel and was expressed as m/day. In order to remove the adverse effects of the long-term individual housing (isolation stress) on the growth in mice[23], the duration of voluntary exercise was 3 days per week (every other day) for 8 weeks. The control mice were housed a standard mice cage (24 × 17 × 12 cm^3). Like the exercise group, the control animals were housed in separate cages (without the voluntarily revolving wheel) every second day. At the end of the 8-week voluntary exercise period, the mice were sacrificed by bleeding 48 h after the last exercise under ether anesthesia for the following experiments. Twenty mice were used for isolation of peritoneal macrophages and 14 for preparation of splenic lymphocytes.

Before the main experiments, the thymus and the muscle of the anterior tibialis in all groups were weighted.

Isolation of Macrophages

All procedures were conducted under aseptic conditions. Peritoneal exudate cells (PEC) were obtained from each mouse. Mice were sacrificed by bleeding under ether anesthesia, following a method previously described[10]. The abdomen was cleaned with 70% ethanol, the abdominal skin was carefully dissected without opening the peritoneum, and 5

ml of Hanks' medium adjusted to pH 7.4 was injected intraperitoneally. The abdomen was massaged and 90 - 95% of the injected volume was recovered. The peritoneal resting macrophages in the PEC suspension were isolated by the cell adhesion method. The PEC were suspended in RPMI 1640 medium containing 10% heat-inactivated FBS and incubated in culture plate (Corning Laboratory Sciences Co., New York, USA) for 2 hours at 37℃ in a 5% CO_2 incubator. After removing non-adherent cells by washing the plate with Hanks' medium, the adherent cells were harvested from the bottom using cell scraper and resuspended in 10% FBS-RPMI 1640 medium. The cells were used in *in vitro* assays for enzyme activities, production of nitric oxide (NO) and interleukin-1beta (IL-1β) as resident peritoneal macrophage at a concentration of 2×10^6 cells/ml. Cell viability checked by the trypan blue dye exclusion test were >95%.

Preparation of Splenic Lymphocytes

All procedures were conducted under aseptic conditions. Mice were sacrificed by bleeding under ether anesthesia and the single cell suspension was prepared by pressing spleen between two slide glasses. The cell suspensions were passed through a 200-gauge stainless steel sieve and then let stand to remove tissue fragments. Erythrocytes from the spleen cell suspension were lysed with 0.83% NH_4Cl. The cell suspensions were centrifuged (600 × g for 10 min), resuspended gently in FBS-RPMI 1640. The cells were used in *in vitro* assays for proliferative responses, production of IL-2, IFN-γ and IL-4 as splenic lymphocytes at a concentration of 4×10^6 cells/ml. Cell viability checked by the trypan blue dye exclusion test were >95%.

Production of NO and IL-1β

Macrophages (2×10^6 cells) were cultured at 37℃ with 5% CO_2 in humidified air for 24 h with LPS (10 μg/ml). The accumulation of NO_2^- (as measured by the metabolite NO_2^-) in culture supernatants was measured using the assay system described by Ding et al.[24]. Briefly, at the end of cell culture, 100 μl of supernatant were removed from each well onto empty 96-well plate. After the addition of 100 μl Griess reagent (1:1, v/v, *N*-1-naphthylethylene diamine dihydrochloride 0.1% in H_2O, sulfanilamide 1% in 5% H_3PO_4) to each well at room temperature for 10 min. The absorbance at 550 nm was measured using the microplate reader (Nalge Nunc International Co., Ltd., Immuno mini NJ-2300, Osaka, Japan). NO was determined by using sodium nitrite as a standard (range of 0-100 μM). IL-1β activity in culture supernatants was measured by enzyme-linked immunosorbent assay (ELISA) using commercial kits for mouse IL-1β (Endogen, Inc., Woburn, USA). The samples were frozen and stored at -80℃ until use. The sensitivity of IL-1β assay was <3pg/ml.

Mitogenicity Assay

The cell suspension (50 μl) and Con A (10 μg/ml) in 10% FBS-RPMI 1640 were added to a well of 96-well flat-bottomed microplate (Corning Laboratory Sciences Co., New York, USA) and incubated for 72 h in 5% CO_2 at 37℃ . The proliferation of spleen cells was assayed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT). Six hours before the end of the incubation, 10 μl of 0.5% MTT dissolved in Ca^{2+} and Mg^{2+} -free phosphate buffered saline (PBS; pH 7.4) was added to the well. After the incubation, 150 μl of 0.04N HCl-isopropanol was added to each well. Then, optical density at 570 nm was measured by the microtiter plate reader. The experiments were done in triplicate sets. Stimulation index (S. I.) was calculated using the following equation.

S. I. = mean optical density of the cells stimulated with Con A / mean optical density of the cells not stimulated with Con A

This assay method has been reported to yield results similar to those obtained from traditional ^{3}H-thymidine incorporation method[25][26].

Production of Interleukin- 2 (IL-2), Interferon- gamma (IFN-γ) and Interleukin- 4 (IL-4)

The 100 μl of spleen cell suspension at a concentration of 4×10^6 cells/ml were incubated at 37℃ with 5% CO_2 in humidified air for 48 h with Con A (10 μg/ml). IL-2, IFN-γ and IL-4 activities in culture supernatants were determined by using mouse IL-2, IFN-γ and IL-4 ELISA kit (Endogen, Inc., Woburn, MA, USA), respectively. The samples were frozen and stored at -80℃ until use. The sensitivity of IL-2, IFN-γ and IL-4 assay were <3pg/ml, <10pg/ml and <5pg/ml, respectively.

Statistical analysis

All data were presented as mean ± S.E. A primary observation showed that the results of experiments were

distributed normally. Hence, the parametric test was applied for detecting the significant differences between the exercise group and the control group. Data were analyzed using two-tailed Student's *t* test. Where applicable (data of running distance), one-way analysis of variance (*ANOVA*) was applied to test the null hypothesis of no difference in the mean values. The multiple comparison tests were conducted using the Scheffé method. Significance levels were set at $P < 0.05$.

RESULS

1. General observation

Mean body weight, relative muscle, thymus weights and food intake in each group are shown in **Table 1**. After 8 weeks, there were no significant differences between the two groups in the body, relative thymus weights. These results demonstrate that the 8-week voluntary running exercise did not invoke a maladaptive stress response leading to body weight and thymic involution that has been seen with more stressful exercise protocols in rodents[27]. The relative muscle weight and food intake in the exercise group were significantly greater than that in the control group ($P < 0.01$).

2. Running distance

Fig. 1 shows the running distance per day in the exercise group. During the first 2 weeks in the running exercise increased the running to between 8,553 m/day (SE 378) and 9,649 m/day (SE 271). After that the running distance per day decreased progressively and the running distance range from 7,194 m/day (SE 498) to 9,565 m/day (SE 271). When *ANOVA* applied to the results of the running distance among different ages, no significant differences could be detected ($P > 0.05$).

3. Production of NO and IL-1β

Fig. 2 and **3** shown the levels of NO (as measured by the metabolite NO_2^-) production by LPS-stimulated peritoneal macrophages from the exercise group showed an 1.3-fold higher than that in the control group ($P < 0.01$), and IL-1β production of peritoneal macrophages stimulated by LPS in the exercise group showed an 1.5-fold higher than that in the control group ($P < 0.05$). Voluntary exercise did not enhance spontaneous NO and IL-1β productions by unstimulated peritoneal macrophages.

4. Splenic lymphocyte proliferation

The results of splenic lymphocyte proliferation stimulate by Con A are presented in **Fig. 4**. The ability of spleen lymphocytes from exercise group to blastogenesis in response to stimulation with Con A was significantly higher compared with cells from the control group ($P < 0.01$). Voluntary running exercise did not enhance Con A-unstimulated splenic lymphocyte proliferation in mice (data not shown).

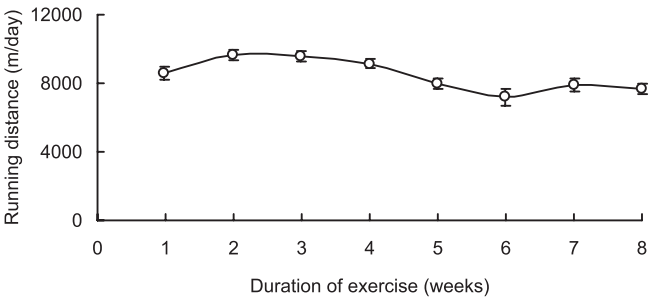

Fig. 1 Daily running distance in the voluntary-exercise group. Each value represents the mean ± SE.

Table 1 Body, relative muscle, thymus weights, and food intake in each group

Group	n	Body wt. (g) 12 W	Body wt. (g) 20 W	Muscle wt.# (mg/g body wt.)	Thymus wt. (mg/g body wt.)	Food intake (g/day)
Control	17	20.7±0.3	23.8±0.4	1.31±0.05	1.12±0.08	2.75±0.06
Exercise	17	20.9±0.3	24.0±0.3	1.56±0.04**	1.11±0.04	3.23±0.11**

Values are mean±SE. Statistically significant difference from the control at $^{**}P < 0.01$ (two tailed unpaired t-test). #: tibialis anterior muscle

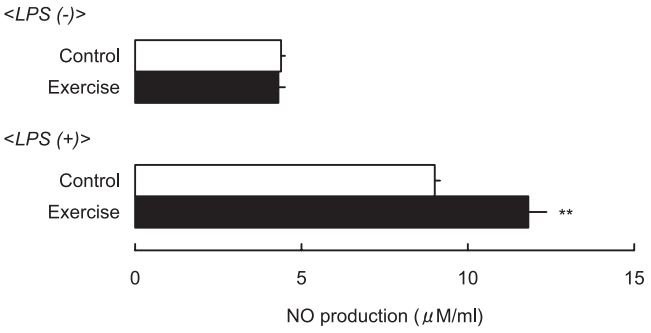

Fig. 2 Effect of voluntary running exercise on nitric oxide (NO) roduction by peritoneal macrophages in mice. Macrophages from control and voluntary exercised mice were incubated with LPS (10 μg/ml) for 24 h. The accumulation of NO in culture supernatants was measured by Griess reaent as described in METHOD. Results are expressed as mean ± SE.
**: Significantly different from the control group. $P < 0.01$ (two tailed unpaired *t*-test).

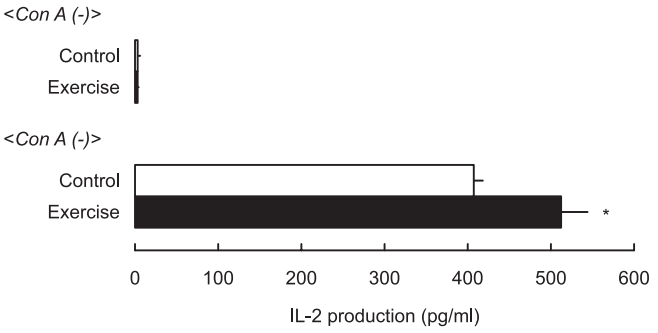

Fig. 5 Effect of voluntary running exercise on interleukin-2 (IL-2) production in splenic lymphocytes stimulated by concanavalin A (Con A) in mice. Splenic lymphocytes from control and voluntary exercised mice were incubated with Con A (10 μg/ml) for 48 h.
Production of IL-2 in culture supernatants was measured by ELISA system. Results are expressed as mean ± SE.
*: Significantly different from the control group. $P < 0.05$ (two tailed unpaired *t*-test).

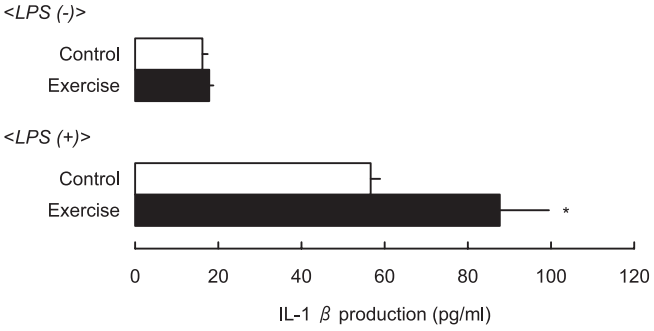

Fig. 3 Effect of voluntary running exercise on interleukin-1b (IL-1β) production by peritoneal macrophages in mice. Macrophages from control and voluntary exercised mice were incubated with LPS (10 μg/ml) for 24 h. Production of IL-1β in culture supernatants was measured by ELISA system. Results are expressed as mean ± SE.
*: Significantly different from the control group. $P < 0.05$ (two tailed unpaired *t*-test).

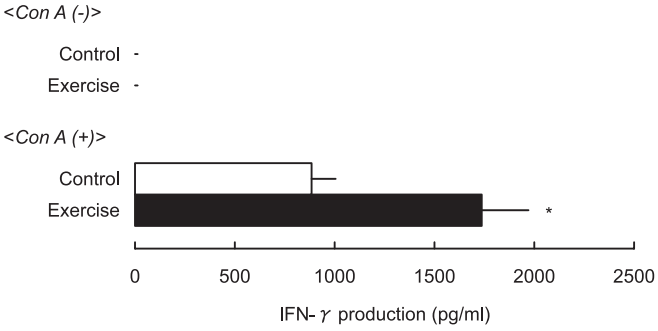

Fig. 6 Effect of voluntary running exercise on interferon-γ (IFN-γ) production in splenic lymphocytes stimulated by concanavalin A (Con A) in mice. Splenic lymphocytes from control and voluntary exercised mice were incubated with Con A (10 μg/ml) for 48 h.
Production of IFN-γ in culture supernatants was measured by ELISA system. Results are expressed as mean ± SE.
*: Significantly different from the control group. $P < 0.05$ (two tailed unpaired *t*-test).

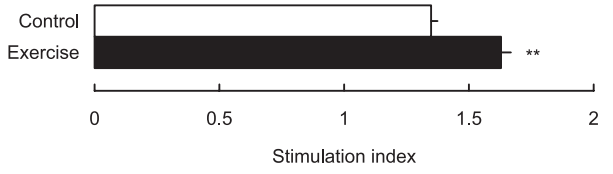

Fig. 4 Effect of voluntary running exercise on concanavalin A (Con A)-stimulated splenocytes proliferative responses in mice. Splenic lymphocytes from control and voluntary exercised mice were incubated with Con A (10 μg/ml) for 72 h. The proliferation of splenic lymphocytes was assayed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT). Results are expressed as mean ± SE.
**: Significantly different from the control group. $P < 0.01$ (two tailed unpaired *t*-test).

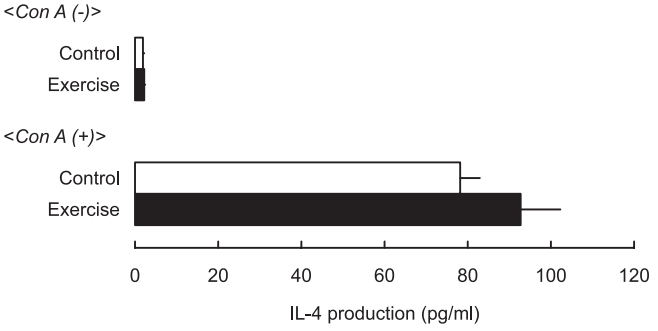

Fig. 7 Effect of voluntary running exercise on interleukin-4 (IL-4) production in splenic lymphocytes stimulated by concanavalin A (Con A) in mice. Splenic lymphocytes from control and voluntary exercised mice were incubated with Con A (10 μg/ml) for 72 h.
Production of IL-4 in culture supernatants was measured by ELISA system. Results are expressed as mean ± SE.

5. Production of IL-2, IFN-γ and IL-4

The data of IL-2, IFN-γ and IL-4 productions in splenocytes are presented in **Fig. 5**, **6** and **7**. Voluntary running exercise did not enhance Con A-unstimulated IL-2, IFN-γ and IL-4 production by splenocytes in mice. Compared to the control group, the exercise group had a significant increase of about 1.3-fold in splenic lymphocytes production of IL-2 stimulated by Con A ($P < 0.05$). Compared to the control group, the exercise group had a significant increase of about two folds in splenic lymphocytes production of IFN-γ stimulated by Con A ($P < 0.05$). IL-4 production of splenocytes stimulated by Con A in the exercise group was higher than that in the control group; however the difference was not statistically significant.

DISCUSSION

In the present study, we investigated the effects of 8-week voluntary running exercise on NO and IL-1β production capacity by peritoneal macrophages *in vitro* as an indicator of macrophage functions, and then mitogenic response, IL-2 production, IFN-γ production and IL-4 production of spleen cells *in vitro* as an indicator of lymphocyte functions in mice. The results in the current study indicate that 8-week voluntary running exercise effectively enhanced both macrophage and lymphocyte functions in mice.

Macrophages are known to play an important role in host defense mechanisms for protection from microbial invaders. When macrophages are stimulated with bacterial products, a variety of cytokines and chemicals are released to induce fundamental defense system[11]. Among them, it has generally been found that production of NO by macrophages is an important mediator of tumoricidal and microbicidal activity[28]. Murine peritoneal macrophages in culture synthesize significant quantities of NO in response to LPS[29]. The increased capacity of macrophages for NO production (as measured by the metabolite NO_2^-) was seen only when the cells were activated with LPS in the exercise group. There have been very few reports on the effect of exercise on the production of NO[30][31][32][33]. Several studies have demonstrated that acute exercise increases plasma and urinary levels of NO[30][31]. In a recent study on experimental animals, it has been reported that chronic treadmill running (45 min/day, 5 days/week for 16 weeks, running speed at 18-22 m/min) significantly increased peritoneal macrophage NO production in young (6 months) male BALB/cByJ mice[33]. Although the style of exercise is different, our data support these findings and suggest that long-term voluntary running exercise can also be augmented the production of NO in peritoneal macrophages.

Cytokines play an important role with regard to the regulation of specific and non-specific immune responses[12]. IL-1 is one of the representative cytokines secreted by macrophages to play a key role in the cytokine network, e.g., T-cell and NK cell activation[11][12]. Also, IL-1 is major proinflammatory cytokine characteristically produced at sites of inflammation by macrophages[34], and IL-1 is considered to help eliminate microbial invaders[35]. In this study, 8-week voluntary exercise significantly increased IL-1β production of peritoneal macrophages stimulated by LPS. This result may suggest that chronic exercise training prevents the organism from foreign agents through enhancement of cytokine secretion in peritoneal macrophages such as IL-1β.

T-lymphocyte mitogens such as Con A are thought to act in several subsequent steps, initially inducing IL-1 secretion in non-lymphoid cells, later stimulating T-helper cells to produce IL-2, which finally causes other T lymphocytes to proliferate after they have developed IL-2 receptors. Also, it is known that mitogen activity, which reflects an early stage in the immune response, have been measured for the first screening of the immunomodulating activity[36][37]. Several investigators have reported the effects of exercise training on mitogen-induced lymphocyte proliferation[3][4][10][22][38][39]. However, studies of mitogenic response to exercise have produced a number of contradictory conclusions. It has been reported that a single swimming session of 2 h duration significantly reduce splenic T-lymphocyte proliferation in young male Wister rats[39]. Forced running exercise training (treadmill running at 75% maximal running capacity for 1 h/day, 5 days/week, for 15 weeks) was reported suppress the proliferative response in 8 months old rats[22]. In contrast to these findings, in the present study we found increased splenic lymphocyte proliferation in voluntary exercise group. This result is in accordance with the findings of Coleman et al.[3], who demonstrated that voluntary wheel running for 8-week (12 h/day at dark onset) in rats significantly enhanced lymphocyte proliferative responses to Con A. Therefore, it seems that nonstressful chronic exercise and/or moderate exercise training, as applied in our previous[4]

and present studies, enhances the mitogenic response but intense exercise training depresses splenic lymphocyte responses.

It is accepted that cytokines are major factors involved in the regulation of immune response to antigens and infectious agents. Generally, murine helper T cells can be classified into two types according to their cytokine production. Th1-type T cells produce IL-2 and IFN-γ, and Th2-type T cells produce IL-4, IL-5, and IL-10[44]. Th1 cells upregulates mainly cell-mediated immunity and downregulates humoral immunity and Th2 cells act oppositely[41][42]. Among them, it is known that IL-2 is the important cytokine, which is related to T-cell proliferation after the mitogen stimulation[11]. Little is known about the effects of chronic exercise training on cytokine production of T-lymphocytes[10][19][22][38][43][44]. Lin et al.[44] found a significantly lower IL-2 production of spleen lymphocytes stimulated by Con A in the rats that had run a treadmill for 5 days a week for 10 weeks than in sedentary controls. Similarly, Pahlavani et al.[45] reported that swimming exercise (5 days a week for 6 months) depressed the Con A-induced IL-2 production of spleen lymphocytes in 7 months of age rats. In contrast previous studies[44][45] we found that Con A-induced IL-2 production of spleen lymphocytes in exercise group significantly higher than that in the control group. These differences could be explained, at least partly, by the difference in the exercise intensity. In the study of Pahlavani et al.[45], it might be considered that the swimming exercise may cause the animals the adverse effect, because the adrenal weight of the swimming exercised group was significantly increased. Thus, it seems that the long-term swimming exercise lowers the IL-2 production of splenic lymphocytes. It has been reported that adrenal hypertrophy appeared to be associated with an increase in the resting levels of plasma corticosterone[46]. Moreover, it is known that glucocorticoid induced thymus shrinkage, and inhibit lymphocyte proliferation and cytokine production[47][48]. Although we did not measure the plasma levels of glucocorticoid in this study, the body weight gain, the relative thymus weight were not different in the control and the exercise groups. From these results, the voluntary running exercise, as applied in our study, shows that it does not become a stress that is negative for the mice. Therefore, it is suggested that nonstressful chronic exercise enhances the IL-2 production of splenic lymphocytes. Moreover, in the present study we found that the exercise group had a significant enhancement in splenic lymphocytes productions of IFN-γ stimulated by Con A as compared to the control group, but not IL-4 production. Recently, it has been reported that continued aerobic exercise training increased the number of $CD4^+$ cells in HIV-1 seropositive individuals[49]. From the results of cytokine productions such as IL-2, IFN-γ and IL-4 observed in this study, it is suggest that 8-week voluntary running exercise may alter the balance of Th1 and Th2 in the lymphocytes and may be useful for explaining the enhancement in cell-mediated immunity function observed to the HIV infection persons following aerobic exercise training.

In conclusion, 8-week voluntary running exercise could enhance of NO production, cytokine secretion by peritoneal macrophages such as IL-1β, lymphocyte proliferation and production of IL-2 and IFN-γ by splenic lymphocytes. These results suggested that regular physical activity enhances not only macrophage function but also lymphocyte responsiveness.

ACKNOWLEDGEMENTS

This study was supported in part by a Grant-in-Aid for Scientific Research (Grant no. 10780032 and 16500418) from the Ministry of Education, Science, Sports and Culture of Japan.

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マウス腹腔マクロファージおよびＴ細胞のサイトカイン産生能に及ぼす運動の影響

杉浦　春雄（岐阜薬科大学保健体育学教室）
杉浦　浩子（岐阜大学医学部）
植屋　摩紀（愛知医療学院理学療法科）
植屋　悦男（浦和大学総合福祉学部）

要約：
本研究は、マウス腹腔マクロファージおよび脾細胞のサイトカイン産生能に及ぼす自由運動の影響について検討した。BALB/c系、雌マウス（12週齢）を運動群と対照群に区分し実験を行った。運動は、回転ケージを用いた自由運動を週3日、8週間実施した。腹腔マクロファージ機能において、リポ多糖刺激に対する一酸化窒素産生能およびインターロイキン(IL)-1β産生能は、対照群と比較して運動群が高い値を示し有意差が認められた。脾細胞機能において、concanavalin A (Con A)刺激に対する脾細胞増殖反応は、運動群が有意に高い値を示した。Con A刺激に対する脾細胞のサイトカイン産生能では、運動群のIL-2およびインターフェロン-γが対照群より有意に高い値を示したが、IL-4産生能ではみられなかった。以上の結果から、8週間の自由運動は若齢マウスの腹腔マクロファージ機能および脾細胞の機能を向上させ、免疫機能増強作用のあることが示唆された。

キーワード：運動、マクロファージ、脾リンパ球、サイトカイン、マウス