Effects of Long-Term Running on Lymphocyte Subpopulations

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KAWADA, E., KUBOTA, K., KURABAYASHI, H., TAMURA, K., TAMURA, J. and SHIRAKURA, T. *Effects of Long-Term Running on Lymphocyte Subpopulations.* Tohoku J. Exp. Med., 1992, 167 (4), 273-277 — Thirteen healthy members (11 males and 2 females; mean age 19.8 years) of an athletic club underwent a long-term exercise program (five 8-km runnings per week for 40 weeks) as regular training for their club. The circulating lymphocyte number, T lymphocyte percentage, and OKT4/OKT8 ratio did not change through the 40-week training period. However, suppressor/inducer (Leu3a+Leu8+) and suppressor T cells (Leu2a+Leu15+) significantly increased. In contrast, helper (Leu3a+Leu8-) and cytotoxic T cells (Leu2a+Leu15-) significantly decreased. On the other hand, there was no change in three males (mean age 18.7 years) without the long-term exercise program. These results suggest that long-term running may induce some change in lymphocyte subpopulations. ——— long-term running; lymphocyte subpopulations

It is generally believed that physical exercise prompts non-specific host defense and immune functions, leading to increased resistance to infections (Asgeirson and Bellanti 1987). There have been, however, interesting reports that highly trained athletes are rather susceptible to infectious diseases (Jokl 1977). Studies in the acute or short-term effects of various exercises on the immune function have not give informations enough to understand the relationship between exercise and immunity, because the variables altered by such exercise returned to their pre-exercise levels in a short period of time after cessation of the exercises. While changes in natural killer cell activity by chronic or long-term exercises have been reported (Watson et al. 1986; Pedersen et al. 1989), little is known about those in lymphocyte subpopulations (Nehlsen-Cannarella 1991).

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METHODS

Subjects and exercise training

Thirteen healthy members (11 males and 2 females; 19.8 ± 1.3 years [mean $\pm$ s.d.]) who newly joined an athletic club of Gunma University, participated in this study. The study protocol had been approved by the Human Subjects Committee of Gunma University and all subjects provided written consent. An ordinary training program of the club was a 8-km running on level ground per day from Monday to Friday and no running on Saturday and Sunday. They took their usual student life except running. The schedule was repeated for 40 consecutive weeks. Blood samples were obtained at 9 a.m. on a fasting condition on the Monday of 1 week before starting the schedule, and of 17 weeks (before running of the day) and 41 weeks after starting the schedule.

Three male students without running training (18.7 ± 1.0 years) were also included in this study as control.

Lymphocyte numbers and subpopulations

The leucocyte numbers were examined by using Toa Microcellcounter (model CC-180; Toa, Tokyo). For calculation of lymphocytes. 200 leucocytes were classified on blood smears. Mononuclear cells were collected from the blood samples and lymphocyte subpopulations were analyzed by single- and double-color methods using a flow cytometer (FACScan; Becton-Dickinson, Mountain View, CA, USA) (Kubota et al. 1987). Monoclonal antibodies used in this study were OKT3 (CD3), OKT4 (CD4) and OKT8 (CD8) (Ortho Pharmaceutical, Raritan, NJ, USA), and Leu3a (CD4), Leu2a (CD8), Leu8 and Leu15 (CD11b) (Becton-Dickinson). In this study, Leu3a+Leu8+ cells, Leu3a+Leu8- cells, Leu2a+Leu15+ cells, and Leu2a+Leu15- cells were treated as suppressor/inducer T (Damle et al. 1984), helper T (Gatenby et al. 1982), suppressor T (Landay et al. 1983; Clement et al. 1984b), and cytotoxic T (Clement et al. 1984a), respectively.

Statistical analysis

Student's *t*-test was used.

RESULTS

There were no significant differences among the circulating lymphocyte numbers before, and 16 and 40 weeks after starting the running training. Neither the percentage of T cells nor OKT4/OKT8 ratio changed through the 40-week running training (Table 1).

Table 2 gives results of lymphocyte subpopulations by the double-color

TABLE 1. *Circulating lymphocyte numbers and subpopulations by the single-color method*

Time of samples obtained	Numbers of lymphocytes ($\times 10^9/\mu\text{l}$)	T cells (OKT3+) (%)	OKT4/OKT8 ratio
Before running	2.2 ± 0.7^a	63.3 ± 9.0	0.94 ± 0.27
After 16 weeks	2.3 ± 0.5	64.6 ± 9.3	0.92 ± 0.26
After 40 weeks	2.4 ± 0.6	67.0 ± 7.7	0.93 ± 0.29

^aMean $\pm$ s.d. ($n = 13$).

TABLE 2. *Lymphocyte subpopulations by the double-color method*

Time of samples obtained	Suppressor inducer T cells (Leu3a+Leu8+) (%)	Suppressor T cells (Leu2a+Leu15+) (%)	Helper T cells (Leu3a+Leu8-) (%)	Cytotoxic T cells (Leu2a+Leu15-) (%)
Before running	9.7 $\pm$ 2.5 ^a	3.2 $\pm$ 1.1	19.5 $\pm$ 5.6	29.0 $\pm$ 5.4
After 16 weeks	11.1 $\pm$ 1.8	5.3 $\pm$ 1.9**	20.6 $\pm$ 5.4	30.5 $\pm$ 6.1
After 40 weeks	15.5 $\pm$ 3.6**	10.7 $\pm$ 4.0**	13.8 $\pm$ 5.4*	22.5 $\pm$ 6.3*

^aMean $\pm$ S.D. ($n=13$).** $p < 0.005$ when compared with the value before running.* $p < 0.025$ when compared with the value before running.

method. The percentages of suppressor/inducer (Leu3a+Leu8+) and suppressor T cells (Leu2a+Leu15+) increased by the 40-week running training, while those of helper (Leu3a+Leu8-) and cytotoxic T cells (Leu2a+Leu15-) decreased. These changes were statistically significant. In contrast, no significant change was observed in the control students (data not shown).

DISCUSSION

In this study, we have shown that a long-term running training decreased helper and cytotoxic T cells, whereas it increased suppressor and suppressor/inducer T cells. However, it is not clear from this study whether the increase in suppressor T cells was a secondary phenomenon due to the increase in suppressor/inducer T cells.

As to the acute effects of various types of short-term exercises on lymphocyte subpopulations, several groups indicated in common a decrease in OKT4+ (CD4+) T cells but an increase in OKT8+(CD8+) T cells. Resulting in a reduction of CD4/CD8 ratio (Hedfords et al. 1983; Edwards et al. 1984; Berk et al. 1986; Oshida et al. 1988; Ricken et al. 1990). In contrast, Nieman et al. (1989) reported decrease both in the percentage and in the number of suppressor T cells in the recovery phase of a 3 hr endurance running in experienced marathoners. There are, on the other hand, few reports describing chronic effects of long-term exercises of lymphocyte subpopulations. Nehlsen-Cannarella et al. (1991) examined the effect of moderate exercise training (five 45-min sessions per week, brisk walking at 60% heart rate reserve for 15 weeks) in sedentary, mildly obese women and found no change in the numbers of CD4+ and CD8+ T cells. As the exercise programs and subjects' ages were different, it is difficult to compare the results of Nehlsen-Cannarella et al. (1991) with our data. Although lymphocyte subpopulations are generally thought to change within a year, no

change was recognized in the three control students in this study.

It was mentioned that short-term exercise leads to elevated natural killer cell activity (Edwards et al. 1984; Berk et al. 1986). Watson et al. (1986) reported increased number on T cells, and decreased natural killer cell activity, by chronic aerobic training (five 40-50 min exercise per week for 15 weeks). Pedersen et al. (1989) described elevated natural killer cell activity and CD16+ cells (natural killer cells) in highly trained racing cyclists in comparison with untrained controls. Unfortunately, natural killer cell activity was not tested in this study.

Taken together, it may be suggested that chronic repeated exercise gives rise to any effects on immunologic functions. However, we have observed normalization of abnormal CD4/CD8 ratio through routine rehabilitation programs of several months in patients suffering from neurologic impairment due to stroke (H. Kurabayashi et al. unpublished data). There is a case where mild physical exercise or conditioning is beneficial in improving immunologic abnormalities. Thus, it should be clarified whether alterations of immunologic functions induced by chronic exercise are in fact associated with clinically important susceptibility to infectious diseases before any conclusion can be reached.

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