Effect of moderate exercise training on T-helper cell subpopulations in elderly people

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ABSTRACT

CD28 molecule expression on the surface of T cells plays a critical role in up-regulation of various cytokines synthesis and T-helper (Th) cell proliferation and differentiation. However, aging induces a decrease in CD28 expression and unbalance of Th1/Th2, leading to impairment of Th-cell mediated immune function. The purpose of this study was to assess the effects of moderate exercise training on CD28 expression and the balance of Th1/Th2 cells in elderly people. Forty-eight elderly subjects were assigned to an exercise training group (EXC: 13 males, 15 females; aged 61–76) or a non-exercise control group (CON: 7 males, 13 females; aged 62–79). Subjects in EXC participated in exercise sessions 5-days a week for 6 months. Meanwhile, subjects in CON maintained their normal physical activity levels during the study period. Blood samples were collected before and after the training period. Samples were measured for the number of leukocytes and lymphocytes, as well as for $CD3^+$, $CD4^+$, $CD28^+CD4^+$, IFN-γ^+CD4^+, IL-4^+CD4^+ cells. The number of leukocytes, lymphocytes, and $CD3^+$ cells did not change after 6 months in both EXC and CON. The number of $CD4^+$ and $CD28^+CD4^+$ cells significantly increased after the training in EXC ($P < 0.05$), while CON did not show significant changes. In the EXC group, IFN-γ^+CD4^+ cell numbers were significantly higher following the training ($P < 0.05$), but the number of IL-4^+CD4^+ cells was not changed. In the CON group, there

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were no significant alterations in IFN-γ$^+$CD4$^+$ and IL-4$^+$CD4$^+$ cell numbers. In conclusion, moderate exercise training in the elderly is associated with improvement of expression of CD28 on Th cells and Th1/Th2 balances. Therefore, exercise training could up-regulate Th cell-mediated immune functions and be helpful for a decrease in the risk of infections and autoimmune diseases in elderly people.

Keywords: CD28; Th1; Th2; aging; exercise

INTRODUCTION

Human immune function undergoes distinctly adverse changes with aging that might be explained by decreased function of, or diminished regulation of, the immune system (34). This immune senescence potentially leads to an increased susceptibility to infectious diseases, malignancy, and autoimmune disorders in elderly individuals (25).

During this immune senescence, as the thymus involutes, T cells, which have a central role in cellular immune function, show the largest age-related alterations in distribution and function (3, 11, 38). One of the most important alterations in T cell profiles with aging is declined expression of CD28 (40). CD28 is a homodimeric immunoglobulin super-family protein expressed on the surface of T cells (21). Ligation of CD28 with its cognate receptor on antigen-presenting cells is both necessary and sufficient, concomitantly with T cell receptor (TCR) signaling, to induce the production of interleukin-2 (IL-2) and the expression of the IL-2 receptor (IL-2R), leading to T cell proliferation (9, 15). Thus, CD28 expression and/or function in T cells with aging can significantly affect overall immune function. In fact, T cells lacking CD28 are detected in patients with autoimmune diseases such as rheumatoid arthritis and HIV-1 infection (10). Also, CD28-deficient mice are susceptible to infection with Pneumocystis (5). Absence of the CD28 expression may be a contributing factor to the increased incidence of infections and autoimmune diseases in elderly people.

A certain sub-population of T cells such as the T helper (Th) cell also shows notable alteration with aging. The features of this alteration are characterized by the decreased absolute number of circulating Th cells (39) and by functional changes, including decreased expression of CD28 (40), decreased production of Th1 cytokines (IL-2 and interferon (IFN)-γ), but increased production of Th2 cytokines (IL-4), leading to a shift towards a dominance of Th2 cytokine response (1, 13, 30, 33). CD28 plays an essential role in the commitment of Th cells toward Th1 or Th2 cells. Signaling through CD28 stimulates production of cytokines in Th cells (7, 21). Thus, age-related alterations in cytokine production may be influenced in part by down-regulation of CD28 expression. It has been suggested that the dysregulation in Th1/Th2 balance may contribute to an increased rate of infections in elderly people (31). Therefore, there may be important implications for elderly individuals in regard to improvement of CD28 expression and Th1 cytokines production that is linked to the optimization of the Th1/Th2 balance.

In recent years, the effect of exercise on human immune function has received considerable attention. Previous evidence suggested that moderate exercise training could increase the absolute numbers of T cells and Th cells in elderly

humans (19) and the concentration of cytokines, including IL-2 and IFN-γ in older mice (16, 17). Since co-stimulation through CD28 enhances production of IL-2 and IFN-γ in T cells activated by antigens and/or mitogens, there is a real possibility that exercise may have an impact on CD28 expression. To date, there has been only one published report about the effect of exercise training on the expression of CD28 in healthy elderly subjects. Raso et al. (29) reported that 12 months of moderate resistance training undertaken by healthy elderly people did not alter the number of CD28 expressing Th cells or other immune parameters, such as distribution of T cell subsets and expression of IL-2R on T and Th cells. The expression of IL-2R on T cells in elderly subjects significantly increased following 10 months of moderate endurance training, whereas 10 months of flexibility and resistance training did not alter IL-2R expression (18). Moreover, 12 months of moderate combined (endurance and resistance) training significantly increased the absolute number of T cells and Th cells in elderly subjects (19). These results indicated that long term endurance exercise interventions improved T cell responses among elderly people.

Further, there have been several studies that examined the effects of exercise training on the level of Th1 and/or Th2 cytokines in peripheral blood (16–18), skeletal muscle (28) and lungs (22). It has been also reported that exhaustive exercise affects Th1 and Th2 cytokine producing cells in young athletes (20). However, there is only one study showing a relation between physical activity and peripheral Th1 and Th2 cells in elderly people. Using a cross-sectional design, Ogawa et al. (26) showed that exercise-trained (moderate endurance training) elderly subjects had higher IFN-γ^+CD4^+ (Th1) cell numbers compared with their exercise-untrained peers, but that their IL-4^+CD4^+ (Th2) cells showed no difference. However, there has been no longitudinal study of the effect of exercise intervention on Th1 and Th2 cells in the elderly.

The goal of the present study was to determine the effects of 6 months of moderate combined (endurance and resistance) training on CD28 expression and Th1/Th2 balance of Th cells in elderly subjects. We hypothesized that moderate exercise training undertaken by elderly subjects would increase the number of CD28 expressing Th cells and Th1 cells, but would not alter the Th2 cells.

METHODS

Subjects.

Healthy, sedentary, elderly subjects who lived independently in Japan were recruited through municipal advertisements into two groups: exercise training group (EXC; 13 males, 15 females; aged 61–76) and non-exercise control group (CON; 7 males, 13 females; aged 62–79). Potential subjects were given a detailed explanation of the risks, stress, and potential benefits of the study before they signed an informed consent form. Based on the results of medical examinations within 6 months prior to the study and self-reported medical histories, the following exclusion criteria were determined for all subjects: hormone replacements, acute illness from infection within the preceding 3 months, metabolic disorders, and major surgery during the preceding 6 months. In addition, all subjects had to have passed a complete medical examination within the past year and received

written permission from a sports doctor to be included in the study. No subjects had been treated with any drugs that are known to affect immune function. EXC subjects participated in an exercise program for a period of 6 months. We asked CON subjects not to participate in any formal exercise but just to continue their daily activities. All participants took part in the study for 6 months. This study, which conforms to the principles outlined in the Declaration of Helsinki, was approved by the Ethic Committees of the Institute of Health and Sport Sciences and the Institute of Clinical Medicine of the University of Tsukuba.

Measurement of daily physical activity.
We used an electrical pedometer (Kenz Lifecorder; Suzuken Co. Ltd., Nagoya, Japan) in order to assess the daily physical activity in elderly subjects. With respect to this electrical pedometer, previous study showed accuracy for the assessment of counting steps (32). Participants were instructed to wear an electrical pedometer for 14 consecutive days during all waking hours, except during bathing before (PRE) and after (POST) the 6-month study period. Participants were instructed to go about their normal lives unrestricted and were asked not to look at the electrical pedometer to see how many steps they had taken each day. Electrical pedometer placement was standardized on the belt or waistband, according to the manufacturer's recommendation.

Measurement of double-product break-point.
A double-product break-point (DPBP), which is the point of accelerating double product (DP = heart rate, HR × systolic blood pressure, SBP), has been shown to have strong positive correlations with the lactate and ventilatory thresholds (27). As the method to measure DPBP is non-invasive and involves no excessive strain, it is thought to be a useful index to monitor the intensity of endurance exercise in elderly people. In this study, the DPBP was measured at PRE and POST, according to the procedures of a previous study (27). Subjects sat and rested for at least 5 min, and then they took a cycle-ergometer (232CXL, COMBI WELLNESS, Tokyo, Japan) ramp loading exercise test. This test consisted of 4 min of cycling at 20 W, followed by a ramp slope at 10 W every min. The test was stopped when the subjects reached 75% of their predicted HR max (220 – age bpm). Their DP with HR and brachial arterial SBP were measured and recorded every 15 s via an automated sphygmomanometer (CM-4001, Kyokko, Tokyo, Japan). The DP was calculated from the mean HR and SBP and then plotted against the work rate. The DPBP was determined visually as the point at which a clear and sustained increase of the DP slope occurred.

Physical fitness tests.
Subjects took six physical fitness tests at PRE and POST, as described in "Physical Fitness Test" by Japan Ministry of Education, Culture, Sports, Science and Technology (35). The test measures six characteristics: isometric grip strength (based on readings from a handgrip dynamometer), muscle endurance (based on how many sit-ups the subject could do in 30 s), balance (based on how long the subject, with open eyes, can stand on one leg), flexibility (based on a sit-and-reach exercise), agility (based on the time a subject takes to walk over a 10-m obstacle course), and endurance (based on a 6-min walking exercise).

Exercise program.
Subjects in the EXC group participated in exercise sessions 5-days a week for 6 months. They were supervised by experienced instructors, who conducted the tests and also were responsible for measuring their HR. The training program involved stretching for warm-up, endurance training, resistance training, and stretching for cool-down. The endurance training was a cycle-ergometer exercise (30 min) at 80% work rate of the DPBP. The resistance training that requires their muscles to work against gravity by moving their own weight up and down. This training comprised three sets of seven exercises (squat, trunk-curl, back-extension, leg-extension, hip-extension, leg-curl, and calf-raise) without using any weights (10 repetitions). Subjects in the CON group did not participate in the exercise sessions; during the study they simply maintained their normal levels of physical activity.

Blood collection.
Blood samples were obtained in the morning (between 8:30 and 9:30) both PRE and POST. Subjects refrained from any exercise for at least 24 hours before blood sampling. Subjects came to our experimental laboratory without taking breakfast. Samples were collected in vacutainers containing sodium EDTA. We quantified total leukocytes and lymphocytes from whole blood samples by using a multichannel hemocyte analysis system (SE-9000, Sysmex, Hyogo, Japan).

Determination of lymphocyte sub-populations.
We used a whole-blood staining method (19) to label the lymphocytes with fluorescent-dye. The surface antibodies used for subset identification were $CD3^+$ for T cells, $CD4^+$ for Th cells, and $CD28^+CD4^+$ for $CD28^+$Th cells. Cell surfaces were stained with three monoclonal antibodies: CD3 (FITC, clone: UCHT1, DakoCytomation, Glostrup, Denmark), CD4 (APC, clone: 13B8.2, Immunotech, Marseille, France), and CD28 (FITC, clone: CD28.2, BD Biosciences, San Jose, USA). The mouse IgG1 antibody (clone: DAK-GO1, DakoCytomation) was used as an isotypic control.

Determination of Th1 and Th2 cells.
Cells were stimulated and the population of cytokine-producing cells was determined by flow cytometry, using the method described in a previous study (1). The surface and intracellular cytokine antibodies used for subset identification were IFN-γ^+CD4^+ for Th1 cells and IL-4^+CD4^+ for Th2 cells. Intracellular cytokines were stained using monoclonal antibodies: IFN-γ (PE, clone: B27, BD Biosciences) and IL-4 (FITC, clone: MP4-25D2, BD Biosciences). The mouse IgG1 antibody (clone: DAK-GO1, DakoCytomation) was used as an isotypic control. Whole blood samples were stimulated with phorbol 12-myristate 13-acetate (50 ng/ml) and ionomycin (500 ng/ml) for 4 h at 37°C in presence of brefeldin A (10 μg/ml). The cells were incubated with anti-human antibody: CD4 (Immunotech). The cells were fixed with a 4% formaldehyde buffer solution. The next day, cells were incubated with 100 l of buffer solution containing 0.5% saponin to make the cell membranes permeable. The cytokine antibodies were then added and incubated.

Flow cytometry analysis.
Labeled cells were analyzed by flow cytometry using a fluorescence-activated cell sorter analyzer (FACSCalibur, BD Biosciences). The usual quantity of cells scanned was 10,000 cells per sample. The data were analyzed using the CELLQuest software (BD Biosciences), to determine proportions of fluorescent-labeled lymphocytes. Absolute numbers of cells in specific cell subsets were calculated using the total number of cells multiplied by the percentage of positive cells within the subset of interest.

Statistical analysis.
All data were represented as means ± SE. For all analysis, $P < 0.05$ was considered statistically significant. Comparison between the EXC and CON groups for the baseline criterion measures was made by a Student *t*-test. ANOVA for 2 (group, EXC and CON groups) × 2 (time, PRE and POST) repeated measures was used to determine the effect of treatment during the 6 months period between each group. A Tukey-Kramer post-hoc test was performed whenever there were significant effects in ANOVA. Time effect of intervention within each group was analyzed by a Student's *t*-test.

RESULTS

Physical characteristics for the EXC and the CON group are presented in Table 1. It can be seen that the EXC group and the CON group were of similar age and body composition before the study period. Body mass and body mass index (BMI) did not change significantly during the study period in either EXC or CON.

Table 1. Descriptive data for EXC and CON groups before and after 6 months.

Characteristics	EXC (n=28)		CON (n=20)	
	PRE	POST	PRE	POST
Age (yr)	68.5 ± 0.7		69.8 ± 1.1	
Height (cm)	156.2 ± 1.6		153.7 ± 2.1	
Body weight (kg)	60.1 ± 1.7	59.8 ± 1.8	60.2 ± 2.4	60.9 ± 2.1
BMI (kg/m^2)	24.6 ± 0.5	24.4 ± 0.6	25.5 ± 0.8	25.8 ± 0.8

Values are means ± SE. EXC, exercise-trained group; CON, control group; BMI, body mass index; PRE, pre-training; POST, post-training.

With regards to physical activity, the mean value ± SE of step count per day at PRE and POST were 8161 ± 774 and 9170 ± 779 step/day in EXC, and 5827 ± 805 and 6251 ± 705 step/day in CON. Step count per day in both two groups did not change significantly after the study period. In EXC, the mean value ± SE of the work rate at DPBP before and after the study period were 1.00 ± 0.04 and 1.05 ± 0.05 W/kg. This rate did not change significantly following exercise training. With regard to physical fitness tests in EXC, the mean value ± SE of each fitness tests before and after the study period were as follows: grip strength test, 29.5 ± 1.7 and 30.7 ± 1.7 kg; sit-ups test in 30 s, 11.5 ± 1.4 and 12.8 ± 1.5 times; sit-and-

Table 2. The number of leukocyte, lymphocyte, $CD3^+$ and $CD4^+$ cells in peripheral blood of EXC (n=28) and CON (n=20) groups before and after 6 months.

Cells	EXC		CON	
	PRE	POST	PRE	POST
Leukocyte (cells/μl)	5361 ± 231	5293 ± 224	5520 ± 271	5805 ± 220
Lymphocyte ((cells/μl)	1989 ± 127	2040 ± 125	1827 ± 90	1981 ± 109
$CD3^+$ cell (%)	58.7 ± 3.5	62.2 ± 93	62.1 ± 2.3	62.3 ± 2.4
$CD3^+$ (cells/μl)	1170 ± 108	280 ± 93	1141 ± 79	1246 ± 92
$CD4^+$ cell (%)	42.3 ± 3.0	46.9 ± 1.9	43.3 ± 2.0	42.7 ± 1.9
$CD4^+$ cell (cells/μl)	831 ± 70	958 ± 71*	796 ± 57	847 ± 61

Values are means ± SE. *Significant difference from PRE, $P < 0.05$

reaches test, 34.6 ± 1.8 and 40.9 ± 1.4 cm; standing on one leg with open eyes test, 60.7 ± 8.0 and 71.1 ± 8.3 s; 10-m obstacle course test, 7.81 ± 0.24 and 6.55 ± 0.21 s; 6-min walking test, 508.3 ± 12.1 and 587.1 ± 15.7 m. The EXC group did more sit-ups, more sit-and-reaches, and showed more endurance during the 6-min walking test at POST than at PRE ($P < 0.01$). Time taken for the 10-m obstacle walk was significantly reduced following exercise training ($P < 0.01$). Therefore, muscle endurance, flexibility, agility and endurance in EXC could be improved by 6 months of exercise training.

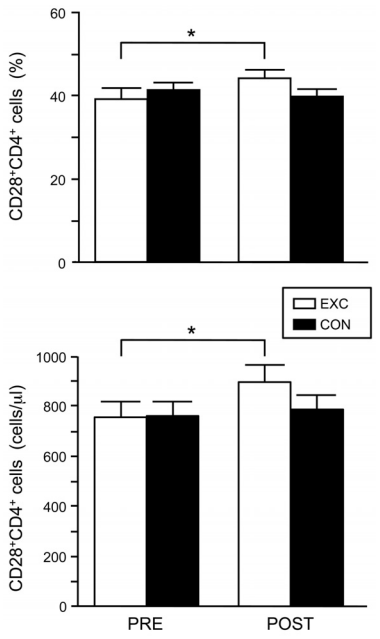

Figure 1. The percentage and absolute number of peripheral blood $CD4^+$ cells expressing CD28 before and after 6 months in EXC (n = 28) and CON (n = 20). Values are means ± SE. *Significant difference from PRE, $P < 0.05$.

As shown in Table 2, the subjects in both the EXC and the CON groups had similar leukocyte and lymphocyte numbers in whole blood before the study period. These numbers did not change significantly after the study period in either EXC or CON.

As shown in Table 2, the percentage and absolute number of $CD3^+$ and $CD4^+$ cells at PRE did not show any inter-group differences between EXC and CON. There was no significant group × time interaction in percentage and absolute number of $CD3^+$ cells and $CD4^+$ cells. The percentage and absolute number of $CD3^+$ cells did not change in either group after the study period. Within the EXC group, the absolute number of $CD4^+$ cells increased after exercise training ($P < 0.05$). Within the CON group, $CD4^+$ cells did not show significant change.

Figure 1 shows the changes in the percentage and absolute number of $CD28^+CD4^+$ cells in both EXC and CON.

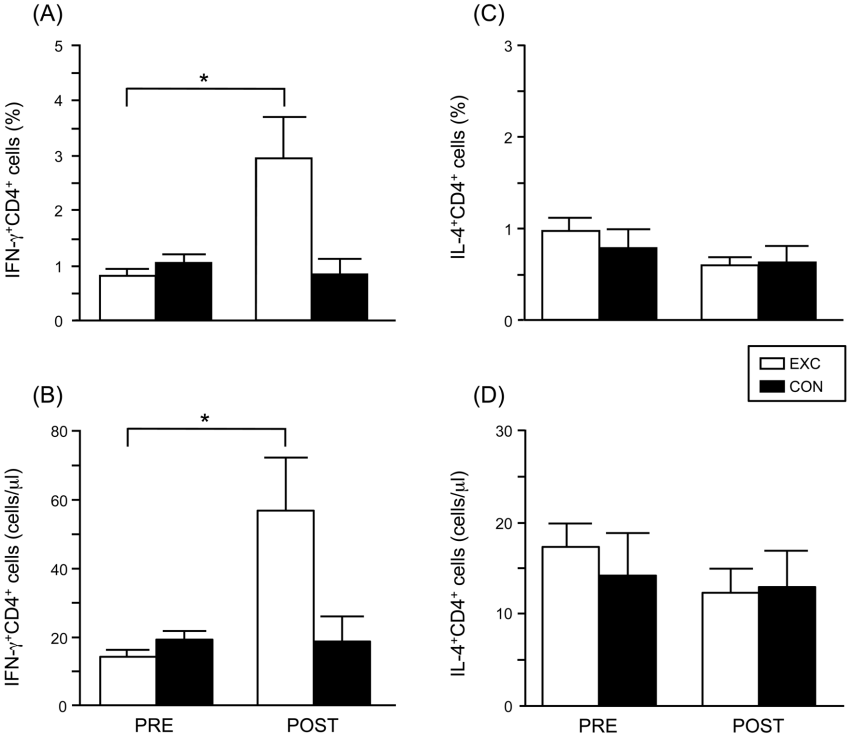

Figure 2. Differences in $CD4^+$ cells in activated peripheral blood between EXC (n = 28) and CON (n = 9) before and after 6 months. (A) The percentage of IFN-γ^+CD4^+ cells. (B) The absolute number of IFN-γ^+CD4^+ cells. (C) The percentage of IL-4^+CD4^+ cells. (D) The absolute number of IL-4^+CD4^+ cells. Values are means ± SE. *Significant difference from PRE, $P < 0.05$.

The percentage and absolute number of $CD28^+CD4^+$ cells at PRE did not show any inter-group differences between EXC and CON. There was significant group × time interaction in the percentage of $CD28^+CD4^+$ cells ($F = 6.59$, $P = 0.01$). EXC showed a significant increase in the percentage of $CD28^+CD4^+$ cells after the training ($P < 0.05$), whereas CON did not show any significant change. There was no significant group × time interaction in the absolute number of $CD28^+CD4^+$ cells. Within the EXC group, the number of $CD28^+CD4^+$ cells was significantly increased after exercise training ($P < 0.05$). Within the CON group, the number of $CD28^+CD4^+$ cells did not show any significant change.

Figure 2 shows the changes in the percentage and absolute numbers of IFN-γ^+CD4^+ (Th1) and IL-4^+CD4^+ (Th2) cells in both EXC and CON. The percentages and absolute number of IFN-γ^+CD4^+ cells at PRE were not significantly different between EXC and CON. The group × time interaction for percentage of IFN-γ^+CD4^+ cells was close to significance ($F = 3.08$, $P = 0.09$). There was no significant group × time interaction in the absolute number of IFN-γ^+CD4^+ cells. Within EXC group, the percentage and absolute number of IFN-γ^+CD4^+ cells

were significantly increased after the training ($P < 0.05$). Within the CON group, IFN-γ^+CD4^+ cells did not show significant change. The percentages and absolute number of IL-4^+CD4^+ cells at PRE were not significantly different between EXC and CON. There was not significantly group × time interaction in the percentage and absolute number of IL-4^+CD4^+ cells. Also, the percentage and absolute number of IL-4^+CD4^+ cells did not change significantly after 6 months in both EXC and CON.

DISCUSSION

The primary finding of our investigation was that 6 months of moderate combined (endurance and resistance) training increased spontaneously CD28 expressing Th cells and mitogens stimulated IFN-γ producing Th1 cells in elderly subjects. These results suggest that regular moderate exercise training can bolster Th cell-mediated immune responses and have an impact on Th1 cytokines, which contribute to the alteration of the Th1/Th2 balance in elderly people.

We focused on the CD28 molecule, which plays a critical role in orchestrating immune responses, including up-regulation of various cytokines synthesis and Th cell proliferation (21). CD28 expression on Th cells is decreased with aging (40). Thus, decreases in the level of CD28 expression contribute to degraded Th cell function, leading to an increased incidence of infections and autoimmune diseases in elderly people (5, 10, 40). So, improvement of expression of CD28 on Th cells may have important implications for the immune function of elderly individuals.

In our study, Th cells and CD28 expressing Th cells were significantly increased in elderly subjects following moderate endurance and resistance training. Raso et al. (29) reported that moderate resistance training provided no benefits to healthy elderly subjects in regard to T cell subsets, and expression of CD28 and IL-2R. However, other investigators have reported that absolute numbers of T cells and Th cells (19) and IL-2R expression on T cells (18) increased in healthy elderly subjects following moderate combined (endurance and resistance) or endurance training program. Both these studies may suggest that the effects of exercise on CD28 expression, as well as on IL-2R expression, could depend on exercise type: endurance exercise. Specifically, moderate endurance training or combined training, which includes endurance exercise, could up-regulate CD28 expression on Th cells in elderly people.

The molecular mechanisms underlying the up-regulation of CD28 expression through exercise training have been unclear. Possible mechanisms might be reactive oxygen species and pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α). Age-related increases of oxidative stress and TNF-α level down-regulate CD28 expression (8, 23). Previous studies suggested that regular exercise training could reduce oxidative stress and TNF-α levels (4, 37). It is therefore possible that exercise-induced decrease of chronic oxidative stress and inflammation could be linked to up-regulation of CD28 expression in elderly people.

CD28 signaling induces the production of IL-2 and expression of IL-2R, leading to Th cell activation and proliferation (9, 15). The expression of IL-2R (CD25), which has been used as a marker of T cell activation along with CD28,

on T cells significantly increased following 10 months of moderate endurance training in healthy elderly people (18). IL-2 production was also increased following endurance training in older mice (16, 17). In our study, the number of CD28 expressing Th cells was significantly increased in elderly subjects following moderate exercise training. Therefore, exercise-induced increase of CD28 expression could be linked to up-regulated IL-2 production and IL-2R expression. In our study, we did not determine IL-2 and IL-2R, which reflect T cell activity. Further studies are needed to determine these parameters, along with the expression of CD28, so that the process of exercise-induced T cell activation can be examined closely.

Ligation of CD28 is linked to up-regulation of IFN-γ and IL-2 production (9, 13, 15, 21). Thus, impaired expression of CD28 with aging may down-regulate these immune competences. It has been well documented that moderate endurance training in older mice can bolster production of IFN-γ and IL-2 in response to mitogens and viral challenges (16, 17). Our results also indicated that the number of IFN-γ producing Th cells in response to mitogens significantly increased following moderate exercise training. Th1 cytokines such as IFN-γ and IL-2 drive T cell mediated immune responses, which are essential to eliminate many viruses. Aging is associated with deficits in Th1 cytokine productions (1, 13, 30). Also, increased susceptibility to influenza in elderly people may be related to an impairment of influenza-specific T cell responses (6). Moderate exercise training could increase CD28 expression, leading to the bolstering of T-cell mediated antiviral immunity in elderly individuals. It could help counter the age-associated decline in the potential of Th cells to produce Th1 cytokines such as IFN-γ and IL-2.

The impact of age on Th1/Th2 cytokines production has been examined in an effort to elucidate the possible mechanisms that underlie age-associated alterations in human immune function. Previous investigators suggested that aging induces a shift towards Th2 cytokine dominance (1, 27, 33). Suppressor of cytokine signaling 3 (SOCS3) protein in Th cells acts as negative regulator of CD28-mediated IL-2 production and IL-12 signaling which induces IFN-γ secretion (14, 24). SOCS3 protein is increased with aging (12, 36). This enhancement of SOCS3 as well as age-related decline of CD28 expression could down-regulate Th1 cytokines activity, leading to Th2 predominance (12, 36). In our study, moderate exercise training resulted in the following: the number of IFN-γ producing Th (Th1) cells increased in parallel with CD28 expressing Th cells, while the number of IL-4 producing Th (Th2) cells remained constant. These results support data from a previous cross-sectional study of elderly subjects, which revealed that IFN-γ producing Th cells were significantly higher in endurance-trained elderly subjects than in untrained peers and that there was no significant difference in IL-4 producing Th cells (26). One possible mechanism of that exercise-induced immune response that includes an increase of Th1 cells but no change in Th2 cells may be related to SOCS3 protein. However, no relationship has been elucidated between SOCS3 in Th cells and exercise training. Further studies need to examine this relationship. Other possible mechanism of that may be related to catecholamines. Kohut et al. (17) suggested that the repeated increase in circulating catecholamines that occurs with each bout of exercise may have a great impact on Th1 cells that produce IL-2 and IFN-γ, con-

sidering that Th1 cells express β_2-adrenergic receptors, whereas Th2 cells do not.

There could be several potential mechanisms underlying the exercise training-induced enhancement of CD28 expression and Th1 cell dominance that may be intricately intertwined with one another. Additional research is required to fully elucidate the contribution of potential mechanisms to changed CD28 expression and Th1/Th2 balance in response to moderate exercise training undertaken by elderly subjects. If these mechanisms were clearly understood, more effective health-related programming could be established to enhance the immune function in elderly people.

The present study has the following study limitations. First, elderly subjects were not randomly assigned to groups. In this study, subjects were, in part, recruited from elderly people who belonged to each community group, so it was hard to assign them randomly to exercise or non-exercise control groups. Further studies need to have subjects in the control group engage in sham-training such as mild flexibility and calisthenics under low-intensity and low-frequency. Second, there was a relatively small sample size that limited our power to do analysis on immune parameters. It is related to the stringent inclusion criteria and the difficulty of finding healthy, non-frail and sedentary elderly subjects who are willing and unable to enroll in any other formal exercise program during 6 months. Third, the numbers of male and female subjects were not equally represented in exercise and control groups. Although the influence of gender difference on CD28 expression in response to exercise in elderly people is unclear, a previous study reported that female people had higher absolute number of $CD4^+$ cells compared with male (2). Future studies need to examine the effects of gender and aging on immune parameters including CD28 expression and Th1/Th2 in response to exercise.

In conclusion, we demonstrated that 6 months of moderate endurance and resistance training for healthy elderly subjects significantly enhanced CD28 expressing Th cells and Th1 cells but no change in Th2 cells. Regular moderate exercise training may enhance CD28 expression, leading to up-regulated cytokines activity and Th cell proliferation and differentiation. Also, moderate exercise training could have great impact on Th1 cytokines to change the Th1/Th2 balance. These findings can help prevent infections and autoimmune diseases in elderly people, as well as improve their immune function as they age.

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