Swim training suppresses tumor growth in mice

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1Department of Environmental, Biological and Health Sciences, University Center of Belo Horizonte, Belo Horizonte; Departments of 2Physiology and Biophysics, 3Morphology, 4Biochemistry and Immunology, and 5Pathology, Institute of Biological Sciences, and 6Exercise Physiology Laboratory, Federal University of Minas Gerais, Belo Horizonte, Brazil; and 7Department of Circulation and Medical Imaging, Norwegian University of Science and Technology, Trondheim; and 8Department of Cardiology, St. Olav Hospital, Trondheim, Norway

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Almeida PW, Gomes-Filho A, Ferreira AJ, Rodrigues CE, Dias-Peixoto MF, Russo RC, Teixeira MM, Cassali GD, Ferreira E, Santos IC, Garcia AM, Silami-Garcia E, Wisløff U, Pussieldi GA. Swim training suppresses tumor growth in mice. J Appl Physiol 107: 261–265, 2009. First published May 28, 2009; doi:10.1152/japplphysiol.00249.2009.—The present study was designed to determine the effects of physical training on the development of cancer induced by the injection of Ehrlich tumor cells in mice. Male Swiss mice were subjected to a swim training protocol (5 days/wk for 6 wk, 1 h at 50% of maximal capacity-trained groups) or remained sedentary in their cages (sedentary groups). The inoculation of Ehrlich tumor cells was performed at the end of the fourth week, and animals were killed after 6 wk of training. Heart and solid tumor weights were recorded, and tumor volumes were calculated. Portions of the tumors were used for the evaluation of macrophages and neutrophil accumulation or fixed in neutral 10% buffered formalin for histological analysis. The tumor volume and weight were, respectively, ~270% and 280% greater in sedentary mice than in trained mice. Macrophage infiltration in the tumor tissue was significantly lower in trained mice (0.65 ± 0.16 vs. 1.78 ± 0.43 macrophages × 103 in the sedentary group). Moreover, neutrophil accumulation in tumors was slightly reduced after exercise training, and the amount of tumor cells was reduced in trained mice. Exercise capacity was substantially increased in trained mice, as determined by a 440% increase in the exercise time at 50% of maximal capacity. In summary, swim training retarded the development of Ehrlich tumors in mice, accompanied by a reduction in macrophage infiltration and neutrophil accumulation. These findings provide conceptual support for clinical observations that controlled physical activities may be a therapeutically important approach to preventing cancer progression and may improve the outcome of cancer treatment.

cancer; macrophage; neutrophil; physical training

MANY STUDIES have associated sedentarism and low levels of aerobic capacity with the pathogenesis of several types of cancer (1, 11). Epidemiologic data analysis has revealed a reduced risk for some kind of cancers with increasing levels of physical activity (5, 7, 8, 17, 25). The strongest evidence has been observed in colorectal and postmenopausal breast cancer, and there may be a correlation in prostate, endometrial, and lung cancer as well (8). It has been estimated that 25% of cancer cases worldwide are associated with overweightness or obesity and a sedentary lifestyle (9). These lifestyle patterns may increase the risk of cancer through a number of different mechanisms, including increased estrogen and testosterone levels, hyperinsulinemia and insulin resistance, increased inflammatory processes, and depressed immune function. Randomized clinical trials have shown that physical activity can change biomarkers for the risk of cancer (1, 22), and it has been suggested that the worldwide trend toward decreasing levels of physical activity may lead to an increased incidence of cancer. Thus, lifestyle changes by individuals and populations may have a huge impact on the incidence of different types of cancer.

Clinical trials addressing the effects of physical training on carcinogenesis should be analyzed carefully, since there are weaknesses in these studies regarding the self-reporting of physical activity and lack of correlations with other parameters. Using animal models, however, it is possible to control the type, amount, and intensity of exercise necessary to influence carcinogenesis (27). The combination of experimental cancer models and exercise training is likely to result in highly translatable preclinical findings that may advance this important topic (27). The present study used a well-controlled physical training protocol to determine whether exercise training before cancer cell inoculation influences tumor growth and leukocyte infiltration in mice.

MATERIALS AND METHODS

Animals. Male Swiss mice weighing 35 ± 1.5 g (7 wk old) were used in this study. Animals were housed under controlled temperature and humidity with a 12:12-h light-dark schedule and free access to food and water. Animals were distributed into the following groups: healthy sedentary (HS) mice (n = 11); cancer sedentary (CS) mice (n = 8–11); healthy exercise-trained (HT) mice, which were divided into two subgroups [50% (HT50; n = 8–11) and 80% of maximal workload (HT80; n = 8)]; and cancer exercise-trained (CT) mice, which were divided into two subgroups [50% (CT50; n = 7–11) and 80% of maximal workload (CT80; n = 7–9)]. An additional group of eight mice was used for tumor cell replication. All experimental protocols were performed in compliance with guidelines for the humane use of laboratory animals from our institute and were approved by local authorities.

Physical training protocol. The exercise training was performed in swimming pools with controlled temperature (31 ± 1°C) for 1 h/day, 5 days/wk, over 6 wk. After the first week, mice were submitted to a progressive load test, which consisted of an increasing workload corresponding to 2% of body weight added every 3 min until exhaustion. The exercise intensity of the following endurance training was

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set at 50% or 80% of maximal weight obtained in the progressive test. The maximal weight carried by the animal in the progressive load test was converted to a percentage of the animal’s body weight. Thus, every week, the mice were weighed and, using the previously calculated percentage value, a new maximal load was obtained and the 50% or 80% workload was determined. With this procedure, we eliminated the need for performing a progressive load test on a weekly basis.

Preparation and inoculation of tumor cells. Three milliliters of ascitic fluid were taken from inoculated mice using disposable syringes and needles. This fluid was centrifuged (3,000 rpm) for 3 min; the supernatant was discarded, and cells were resuspended in saline solution. This procedure was repeated three times, and a dense, clear liquid was obtained, corresponding to a cell suspension with a minimum of fibrins and erythrocytes. The cell suspension (20 μl) was added to 1,980 μl of saline solution. The cell viability test was performed using 100 μl of this diluted suspension and 100 μl of 0.1% trypan blue dye. Cells that turned blue due to the dye were considered nonviable, whereas clear cells were considered viable. All material exhibited cell viability of >90% (6). Cell counts were performed in a Neubauer chamber. Ehrlich lung tumor cells (2 × 10^6) cells in 0.05 ml) were inoculated subcutaneously in the dorsal area of animals in the CS and CT groups at the end of the fourth week.

Tumor analysis. After 14 days, animals were anesthetized with a mixture of 10% ketamine and 2% xylazine (4:1; 0.1 ml/100 g ip) and killed by cervical dislocation, and the blood, heart, and solid tumor were collected. To calculate the cardiac mass index, the heart was weighed and normalized by body weight minus tumor weight. The Ehrlich tumor volume was determined using the following formula:

\[ \text{tumor volume} = \text{width} \times \text{length} \times 0.52 \] (15). Solid tumors were weighed and normalized by body weight.

Portions of the tumors were frozen at −80°C for the subsequent analysis of N-acetylglucosaminidase (NAG) and myeloperoxidase (MPO) activity or fixed in neutral 10% buffered formalin. Macrophage accumulation in the tumor was determined by assaying NAG activity. Briefly, 100 mg of the tumor were homogenized in 1.9 ml of 0.1 M NaCl, 0.02 M Na_3PO_4, and 0.015 M Na_2EDTA buffer (pH 7.2). Samples were centrifuged at 12,000 g for 10 min at 4°C, and the supernatant was discarded. The pellet was resuspended in a 1.9 ml solution of Triton X-100 diluted in 0.9% NaCl (saline). The supernatant was discarded. The pellet was resuspended in a 1.9 ml solution of Triton X-100 diluted in 0.1 M citric acid + 155 ml of 0.1 M Na_3HPO_4; pH 4.5) was added to the reactions at a final concentration of 0.676 g/ml. The product was generated by the addition of glycine buffer (equal amounts of the following solutions: 0.8 M glycine, 0.8 M NaCl, and 0.8 M NaOH). Absorbance was read at 400 nm.

Neutrophil accumulation in the tumor was measured by assaying the MPO activity. Tissue was assayed for MPO activity by measuring the change in optical density at 450 nm using tetramethylbenzidine as the substrate (2). Results are expressed as neutrophil infiltration. An index unit denotes the MPO activity in 10^5 casein-elicted murine peritoneal neutrophils processed in the same way.

Histological analysis. Tumor fragments were fixed in neutral 10% buffered formalin (pH 7.2) for 24 h at room temperature. After fixation, tissues were dehydrated through graded alcohol solutions, embedded in paraffin, and serially sectioned at 6 μm. Sections were stained with hematoxylin and eosin for histological analysis. Areas of neoplasia, necrosis, and normal tissue were evaluated in the tissue sections.

Statistical analysis. All data are expressed as means ± SE. Statistical significance was estimated using one-way ANOVA followed by the Newman-Keuls post hoc test for body weight, heart weight-to-body weight ratio, tumor volume, and tumor weight analyses. Paired and unpaired Student’s t-tests were used to compare the data from the progressive load test as well as macrophage infiltration and neutrophil accumulation, respectively. The level of significance was set at P ≤ 0.05. All data were tabulated and analyzed using the Statistical Package for the Social Sciences (version 14.0).

RESULTS

No significant differences in body weight gain were found in any of the groups throughout the study period (HS group: 19.2 ± 2.0 g, CS group: 25.1 ± 3.0 g, HT50 group: 19.8 ± 3.0 g; HT80 group: 22.9 ± 1.4 g; CT50 group: 13.6 ± 1.6 g; and CT80 group: 20.6 ± 1.0 g).

The swim training protocol did not induce cardiac hypertrophy after 6 wk of training at 50% of maximal workload. However, when the exercise intensity was increased to 80% of maximal workload, there was a significant increase in heart weight, suggesting that the development of cardiac hypertrophy is dependent on workload (Fig. 1A). No significant differences in heart weights were found between HT and CT mice either at 50% or 80% of maximal workload. Although swim training at 50% of maximal workload did not cause cardiac hypertrophy in healthy mice, physical activity increased the time to exhaustion of these animals by 476% (Fig. 1B). Thus, the lower exercise intensity was efficient in increasing physical capacity without causing cardiac hypertrophy.

Interestingly, swim training at 50% of maximal workload (but not at 80%) significantly reduced the weight of the Ehrlich tumors (0.55 ± 0.10 vs. 0.18 ± 0.05 mg/g in CT50 mice; Fig. 2A). A similar result was found regarding tumor volume (0.48 ±
To further confirm the antitumorogenic effect of swim training, histological sections of tumors were evaluated from sedentary mice and mice trained at 50% of maximal workload. The presence of tumor cells (active cells) was markedly reduced in trained mice (Fig. 4). In contrast, the necrotic area was larger in these animals, suggesting that swim training reduces the proliferation of cancer cells and increases their death.

DISCUSSION

The major findings of the present study are that moderate swim training markedly reduced the growth of Ehrlich tumors in mice and suppressed macrophage infiltration and neutrophil accumulation in tumor tissue. These results are in agreement with previous experimental and epidemiological studies (4, 5, 7, 11, 13, 14, 19, 21, 24, 25). An inverse relationship has been found between exercise training and tumor growth in subjects who performed physical activity before cancer diagnosis (14). However, another study (30) failed to demonstrate any beneficial effects from physical training on tumor development and
progression. Interestingly, even a tumorigenic effect of exercise training has been reported (10, 28). The reason for these discrepancies may be related to the species evaluated and, more likely, may be dependent on the type and intensity of the exercise. Indeed, the present study found that only the moderate exercise protocol (50% of maximal load) suppressed tumor growth. The increase in the exercise regimen to 80% of maximal load did not have any effect on tumor development. This may be of clinical relevance when developing an exercise intervention for cancer survivors. Thus, the analysis of the results regarding physical exercise and cancer development and, more importantly, the establishment of exercise intervention protocols for cancer survivors should take into account the type and intensity of physical exercise.

There is abundant speculation regarding the mechanisms underlying the inhibitory effect of exercise training on tumor growth (for a review, see Ref. 18). The findings of the present study clearly demonstrate that moderate swim training decreases macrophage infiltration and neutrophil accumulation in tumor tissue. Macrophages play several roles in tumor angiogenesis. The relationship between macrophage infiltration and tumor growth is well established and is thought to occur in most kinds of tumors (16, 20, 26). In one clinical study (29), patients with a low degree of macrophage infiltration had a significantly better disease-free survival prognosis than those with a high degree of macrophage infiltration. Decreased macrophage infiltration has been associated with a local decrease in VEGF production and consequent angiogenesis (29). Indeed, in the tumor microenvironment, tumor-associated macrophages (TAMs) secrete growth and proangiogenic factors and matrix-degrading enzymes and may provoke significant, anergy-inducing effects (20, 26). Together, these TAM functions may stimulate tumor growth, tumor spreading, and disease progression, which makes these macrophages potential targets for novel therapies for controlling cancer (26). Therefore, the inhibition of macrophage infiltration by moderate exercise provides an explanation for the beneficial effects of moderate exercise on tumor growth. It is important to note that this effect is related to VEGF expression in the tumor, since it has been reported that physical training increases the expression and level of VEGF in skeletal muscles (12). Corroborating this possibility, the histological data of the present study revealed that the amount of tumor cells was markedly reduced in trained mice and that the necrotic area was increased in these animals as well. However, no experimental protocol was performed to evaluate the blood supply or angiogenesis process in the tumor. This is a clear limitation of the present study, since other biological processes, such as apoptosis, can cause similar effects. Thus, future experiments are obviously needed to confirm or refute this possibility.

The mechanisms by which moderate exercise modulated macrophage infiltration and consequent tumor growth were not investigated here. However, it is clear that exercise has a major effect on systemic low-level inflammation (for a review, see Ref. 3) and tumor-associated inflammation, as shown here. While acute physical exercise is associated with a systemic cytokine response comparable with levels observed during severe infection, the cytokine cascade differs importantly from the classic acute phase response in infectious systems, i.e., in concentric exercise without muscle damage, the increase in TNF-α and IL-1β, if present, is minute (3). In chronic exercise, an increase in IL-1 receptor antagonist, IL-10, and soluble TNF-α receptors occurs and seems to explain the effects of chronic exercise on inflammation. Moreover, IL-6 production has been suggested as an important factor in mediating the effects of exercise on systemic low-grade inflammation (3). Anti-inflammatory molecules can potentially decrease the production of the chemokine CCL2, which is a key regulator of TAM recruitment in tumors (26). In keeping with these data, mice subjected to voluntary wheel running present a reduced number of splenic T and B lymphocytes (23). Further studies are needed to pinpoint the exact mechanisms by which exercise modulates macrophage infiltration in our model of tumor growth.

To account for the stress of the experience in water, CS mice were placed in water without training. Despite the difficulty of keeping the mice in the water without swimming, this maneuver did not induce any significant changes in CS animals. Moreover, when mice were subjected to intense training (80% of maximal workload), and despite the stress of the water, no beneficial effects of exercise were observed. It therefore appears that moderate training (50% of maximal workload), rather than water stress, accounts for the protective results obtained.

Both exercise and tumors may have an impact on diet intake and energy balance in animals. We did not perform any detailed evaluation of diet or energy balance in the present study. However, there were no significant changes in body weight throughout the experiments. Moreover, the combination of intensive exercise and tumor did not affect tumor growth, whereas moderate exercise did. Intense exercise would certainly have a greater impact on energy balance than moderate exercise, suggesting that the results observed were not affected by metabolic alterations.

In summary, the results of the present study demonstrate that swim training inhibited the development of Ehrlich tumors in mice, which was accompanied by a reduction in macrophage infiltration and neutrophil accumulation. This study advances the understanding of the beneficial effects of physical training on cancer outcomes, providing conceptual support for clinical observations that physical activity may be a therapeutically important approach to preventing and limiting cancer progression. Well-controlled physical protocols should be designed and tested to validate these results in humans.

REFERENCES


