Chronic exercise increases macrophage-mediated tumor cytolysis in young and old mice

Q. Lu, M. A. Ceddia, E. A. Price, S.-M. Ye, and J. A. Woods

Chronic exercise increases macrophage-mediated tumor cytolysis in young and old mice. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R482–R489, 1999.—In this study, we determined the effects of age and chronic treadmill running (16 wk; 5 days/wk; 45 min/day; 18–22 m/min) on resident peritoneal macrophage responsiveness to interferon-γ (IFN-γ) and lipopolysaccharide (LPS) in young (6 mo) and aged (22 mo) male BALB/cBy mice by measuring cytolytic ability and production of reactive nitrogen products. Macrophages (≥90% Mac-1++) were incubated with various concentrations of IFN-γ and LPS for 24 h. After washing, P815 tumor cells were utilized as targets in a 16-h 90Cr release assay. We found that aging resulted in a significant reduction in the ability of macrophages to respond to the highest doses of IFN-γ and LPS and kill P815 cells (46 ± 4 vs. 34 ± 2% in young and old mice, respectively). Exercise training significantly increased macrophage cytolysis in both age groups (66 ± 7 vs. 44 ± 2% in young and old mice, respectively); this effect was larger in the young mice. Macrophages from young exercised mice also produced significantly (50–60%) more NO2−; there was a tendency for higher NO2− in old exercised mice. The inducible nitric oxide synthase (iNOS) inhibitor N6-monomethyl-L-arginine (L-NMMA) significantly reduced macrophage cytolyis and NO2− production and completely abrogated exercise-induced increases in these measures. RT-PCR analysis revealed significantly higher iNOS mRNA levels in macrophages obtained from the exercised mice and significantly lower iNOS mRNA in old compared with young mice. We conclude that aging reduces and exercise training increases the capacity of resident peritoneal macrophages to respond to IFN-γ and LPS and increases tumor cytolyis. Enhanced iNOS gene expression and NO2− production are likely the contributing mechanisms of the exercise-induced enhancement of cytolysis in young mice. While L-NMMA did block the exercise-induced increase in cytolyis, exercise did not increase NO2− or iNOS gene expression in the old mice, indicating perhaps the contribution of other cytolytic mechanisms in old mice.

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MACROPHAGES are a first line of defense against microbial invaders and malignancies by nature of their phagocytic, cytotoxic, and intracellular killing capacities. They are ubiquitously located throughout the body and are involved in the initiation of immune responses by acting as inflammatory and antigen-presenting cells (1). Once established in the tissues, macrophages can exist in a number of functional states dependent on the milieu of stimulatory and inhibitory signals. In the peritoneum, resident macrophages are cells that have low functional activity (i.e., quiescent or resting). Interferon-γ (IFN-γ), a cytokine produced by activated T cells and natural killer cells, primes macrophages for antitumor and microbicidal activity by increasing their sensitivity to lipopolysaccharide (LPS) and upregulates reactive oxygen and nitrogen production and Fc receptor and major histocompatibility complex II expression (1, 16). In addition to the priming signal, optimal macrophage activation for complex functions like antitumor and microbicidal activity requires the presence of another signal (i.e., trigger signal). For instance, lipopolysaccharide can trigger full tumoricidal and bactericidal activity, and phorbol myristic acid or opsonized zymosan can trigger increased levels of superoxide O2− and H2O2 production (1, 16).

It was previously thought that macrophage function was not dramatically altered in old animals and humans (3, 20, 41). However, a critical shortcoming of many of these early experiments was that none of them specifically studied the response of macrophages to defined activation signals such as IFN-γ, LPS, or Propionibacterium acnes (previously called Corynebacterium parvum). Emerging evidence indicates that macrophage responsiveness to these classical activating signals decreases with advancing age in both humans and animals (2, 4, 9, 17, 23, 29, 36). Macrophage hyporesponsiveness is believed to be a contributing factor in the increased cancer and infectious disease incidence rates seen in the aged (10, 26). Because the aged population is expected to double by the year 2010, it is imperative that we explore preventative and restorative treatment modalities in an attempt to improve and extend the quality of life in aged individuals and lessen the financial burden on our health care system.

We have shown that, in young mice, short-term (3–7 days) exercise can increase macrophage antitumor activity (cytostasis) by 40–70%, mediated in part by increased macrophage production of tumor necrosis factor-α (TNF-α) and nitric oxide (NO) and perhaps by increased sensitivity to IFN-γ (39, 40). Others have documented exercise-induced increases in macrophage chemotaxis, adherence, respiratory burst, cytokine production, and phagocytic activities after a single bout of exercise (7, 13–15, 24, 30, 31). Unfortunately, no studies exist on the effects of chronic exercise training on macrophage function in the young or old. Therefore the purpose of this study was to determine if chronic exercise can increase macrophage function and responsiveness in young and old mice. We tested this hypoth-
osis by chronically exercising young and senescent BALB/cByJ Nia mice for a 16-wk period and by measuring the ability of harvested resident macrophages to perform in vitro tumor cytolyis in response to IFN-γ and LPS.

**MATERIALS AND METHODS**

Animals. A murine model was adopted to test the research objectives in this study due to the need for experimental manipulation, control, and obtainment of tissues. Specific pathogen-free (SPF) inbred male BALB/cByJ Nia mice aged 2 (young) and 18 (old) mo were purchased from the National Institute on Aging (Charles River) and used in all experiments. This particular strain’s median (50% survival) life span is 25 mo. Animals were excluded if they exhibited signs and symptoms of illness (i.e., ruffled fur, lethargy, dramatic loss of body weight, swollen eyes, or visible tumors) in the month before death, or upon death if they had visible tumors or splenomegaly. Approximately 2 of every 10 aged animals were dropped from the study because of the above criteria, and exercise training had no impact on this rate. Mice were acclimated to our SPF facility for at least 10 days before any experimentation and housed in microisolated shoe box cages in facilities maintained at a temperature of 23°C. Running experiments were conducted in this facility to maintain a clean environment throughout the study. All mice were kept in a 12:12-h light-dark cycle (0700–1900) and given autoclaved food (8640 Harlan Teklad 22–5, Harlan, Madison, WI) and water ad libitum. Food intake was monitored daily in a subset of mice from each group. Mice were killed by CO2 asphyxiation, and all animal treatments were approved by the Laboratory Animal Care Advisory Committee at the University of Illinois at Urbana-Champaign and within the guidelines set by the National Institutes of Health for the care and use of laboratory animals.

Exercise protocol. Exercise bouts took place between 1000 and 1200 just at the end of the dark cycle. Our exercise protocol consisted of treadmill running. We chose this method of exercise because exercise intensity and duration can be experimentally manipulated and quantified (unlike voluntary wheels or swimming), and this is of paramount importance for our ultimate goal of defining optimal exercise dosage. In addition, old animals will not run voluntarily when given access to a wheel. Our control animals (young home cage control (Y-HCC), old home cage control (O-HCC)) were exposed to handling, noise, and environment identical to those of the exercising animals. In past studies, we (39, 40) and others (28) have defined moderate exercise as brief (usually 15–60 min) bouts of treadmill running at 50–75% maximum O2 consumption (V02max) or ≈ 15–22 min (15). In this study, mice were acclimated such that, by the second week of training they were running at 75% V02max (i.e., 15–22 min/m). Mice were run at this intensity for 45 min/day, 5 days/wk for 16 wk (young exercised (Y-Exc), old exercised (O-Exc) without negative reinforcement by electrical shock. Citrate synthase activity was determined in the soleus muscle as described by Serere (35) to document an aerobic training effect.

Tissue collection, processing, and reagents. Mice were killed 24 h after the last exercise bout to minimize the influence of the last bout of exercise. Resident peritoneal macrophages were aseptically harvested by lavage with 10 ml of RPMI 1640 containing 10 U/ml heparin. These cells were counted, checked for viability in trypan blue, and used in in vitro assays for antitumor cytolyis and production of NO2. Macrophage culture supernatants were collected and frozen at −80°C for later analysis of NO2. All assays and tissue culture were performed in RPMI 1640 (GIBCO, Grand Island, NY) containing penicillin (100 U/ml), streptomycin (100 µg/ml), glutamine (20 mM), and low-endotoxin (<0.01 ng/ml) fetal bovine serum (Sigma, St. Louis, MO). The P815 target was purchased from American Type Culture Collection. LPS (E. coli 0111:B4) and other common chemicals for the NO2 assay were purchased from Sigma.

Macrophage-mediated tumor cytolyis. To test macrophage-mediated tumor cytolyis and responsiveness to activation signals, we employed a 51Cr release assay system (19, 32). This assay has been used to elucidate the two-signal model of macrophage activation (32). Resident peritoneal exudate cells were seeded at a density of 2 × 105 cells/ml, and 0.2 ml/well was added to sterile polystyrene 96-well flat-bottomed microtiter plates. The plates were then incubated for 2 h in 5% CO2 at 37°C to allow macrophages to adhere. Plates were then vigorously washed with warm media to remove contaminating nonadherent cells and then incubated in the presence or absence of varying concentrations of IFN-γ and LPS for 24 h. After the 24-h activation period and extensive washing, radiolabeled P815 tumor targets were added (104 cells/well) and distributed uniformly by centrifuging the plate at 55 g for 5 min, resulting in an initial effector-to-target ratio of 20:1. These tumor targets were chosen on the basis of previous documentation of susceptibility to macrophage cytolytic activity (19). Briefly, target cells were labeled for 1.5 h at 37°C with 100 μCi of 51Cr-labeled sodium chromate (specific activity 300–500 mCi/mg; ICN Biomedicals, Costa Mesa, CA) per 105 target cells and were extensively washed to remove free radiolabel before addition to macrophages. Each measure was done in triplicate wells. After 16 h of incubation at 37°C, 5% CO2, the uppermost 0.1 ml of supernatant was removed and assayed for radioactivity in a scintillation counter. Results are expressed as percent specific 51Cr release (percent cytolysis) and were calculated as

%lyse = \frac{\text{experimental cpm} - \text{spontaneous cpm}}{\text{total cpm} - \text{spontaneous cpm}} \times 100

where cpm refers to counts per minute of 51Cr. Spontaneous release was determined from untreated (i.e., no IFN-γ or LPS) macrophage monolayers incubated with labeled targets. Total release was determined by addition of 10% Triton X-100. In some experiments, 0.5 mM of Nω-monomethyl-L-arginine (L-NMMA; CalBiochem, La Jolla, CA), a competitive inhibitor of inducible nitric oxide synthase (iNOS), was added to macrophage-P815 cocultures during the IFN-γ-LPS incubation to determine the contribution of nitric oxide (NO) to tumor cytolysis.

Macrophage NO2 production. Macrophage supernatants were collected after a 24-h incubation with 100 U/ml IFN-γ and 1,000 ng/ml LPS and assayed for NO2 by the commonly used Gries reaction according to the microassay by Ding et al. (8). Absorbance was measured at 550 nm in an ELISA plate reader. NO2 concentration was determined by using a sodium nitrite standard curve and expressed as total NO2 (in µM) per 2 × 106 cells initially plated. In some experiments, 0.5 mM of L-NMMA was used to block NO production.

RNA extraction and iNOS RT-PCR. Total cellular RNA was extracted from primary peritoneal macrophages using TriReagent (Sigma). Macrophages were washed and lysed by adding TriReagent to each petri dish. After complete dissociation of nucleoprotein complexes, RNA was isolated according to the chloroform-isopropanol-alcohol protocol (5). RNA concentration was determined by measuring spectrophotometric absorbency (A260/280) at a range of dilutions (U-2010 Spectrophotometer, Hitachi, San Jose, CA). The integrity of each
sample was verified by agarose gel electrophoresis and visualization of the 18S and 28S bands with ethidium bromide (EB) staining.

RT-PCR was performed as previously described (34) with some modifications. Two micrograms of total RNA were reverse transcribed in a 25-µl volume containing 10 mM of deoxynucleoside triphosphate (dNTP) (Promega, Madison, WI), 2 µM random hexamer (Pharmacia LKB Biotechnology, Piscataway, NJ), 200 U Moloney murine leukemia virus reverse transcriptase (GIBCO BRL), 20 mM Tris-HCl (pH 8.4), 50 mM KCl, and 1.5 mM MgCl₂. The reverse transcriptase was inactivated by heating at 95°C for 5 min after incubating at 37°C for 60 min. The PCR was carried out in a 100-µl volume containing 5 µl template cDNA, 2 pmol of iNOS primers (Table 1), and 2 pmol glyceroldehyde-3-phosphate dehydrogenase (GAPDH) primers (Table 1) in 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 mM MgCl₂, 1 U of Taq DNA polymerase (all from GIBCO BRL), 0.2 mM dNTP, and sterile distilled water. Amplification was initiated by 5 min of denaturation at 94°C for one cycle and was followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 2 min. After the last cycle of amplification, the samples were incubated in 7°C for 10 min and then held at 4°C. A log-linear dose-response curve was determined for each set of primers to determine the number of amplification cycles.

Fifteen microliters of PCR products and 123-bp ladder (GIBCO BRL) were loaded onto a 10% acrylamide gel and electrophoresed for 60 min at 100 V. Gels were stained with Tris-borate-EDTA buffer (pH 8.0) containing 0.5 µg/ml EB, and DNA was visualized on an ultraviolet light illuminator. Gels were photographed with type 55 positive/negative film (Polaroid, Cambridge, CA). The gel photographs were scanned with a computerized laser densitometer.

Percentage of adherent macrophages. To determine if exercise or age-induced changes in macrophage cytolytic function were due to changes in macrophage percentage in the culture wells, we assessed the number of adherent macrophages in parallel cultures by staining postadherent cells removed by Teflon scraping with fluoroisothyocyanate-conjugated monoclonal antibodies against Mac-3 (clone M3/84; Pharmingen, San Diego, CA), a surface glycoprotein found on mature macrophages but not on lymphocytes, monocytes, or neutrophils.

Data analysis. All data are reported as means ± SE. Significant differences between groups were determined by two-way [2 (age) × 2 (treatment)] ANOVA. Drug (i.e., L-NMMA) treatment effects were analyzed using a two-way [4 (group) × 2 (drug)] ANOVA. In instances where assumptions of normality or equal variance were violated, a conservative Geisser-Greenhouse F test was used to determine significance. Significance levels were set at P < 0.05. Student-Newman-Keuls contrast procedures were performed when significant main effects were found.

RESULTS

Descriptive data. Table 2 contains descriptive data for the seven separate experiments performed in this study. Young animals were 6 mo of age, whereas old animals were 22 mo at death. There were both age and treatment main effects but no interactions for body weight, in that body weight was significantly greater in the old groups and significantly lower in the exercise groups compared with age-matched controls. Spleen weight was significantly greater in the old compared with the young mice, and there was an interaction between age and exercise training such that spleen weight increased in the young and decreased in the old mice. There were no significant differences in thymus weight among the groups. This fact demonstrates that the exercise training did not invoke a maladaptive stress response leading to thymic involution that has been seen with more stressful exercise protocols in rodents (12). Food intake values indicated no significant differences between the different aged or exercised groups. This was important to demonstrate because caloric restriction has been shown to improve immune function and increase the life span of laboratory rodents (37). The fact that we did not see reductions in food intake demonstrates that reduced caloric intake was not related to the exercise-induced changes in immune function. As expected, citrate synthase activity increased significantly in the exercise-trained animals compared with sedentary controls.

Macrophage cytolytic activity. The ability of macrophages to lyse P815 target cells was measured by pooling peritoneal exudate cells from animals within each group (anywhere from 4–8 animals per experiment). This was necessary to obtain enough cells for analysis. At least three separate experiments were performed with similar results. Macrophages were unable to significantly lyse P815 cells (<5% killed) in the absence of IFN-γ and LPS, demonstrating that exercise training alone did not activate macrophages for tumor killing. The data in Fig. 1 represent the responses of three different priming doses of IFN-γ (1, 10, and 100 U/ml) across a wide range of LPS triggering doses (1–1,000 ng/ml). At the low priming dose (Fig. 1A), macrophage cytolytic capacity is significantly increased in the Y-Exc group compared with Y-HCC. At several LPS doses, cells from Y-Exc were better able to kill tumor cells compared with Y-HCC. Also evident is age-related hyporesponsiveness at the highest (1,000 ng/ml) LPS dose. At this low dose of IFN-γ, exercise had no effect on macrophage cytolytic ability in old mice. At the intermediate priming dose of IFN-γ (Fig. 1B), the Y-Exc group again demonstrated increased cytolytic ability compared with Y-HCC, and a small aging effect was present (Y-HCC > O-HCC at high LPS concentration). Unlike with the lower dose of IFN-γ, the O-Exc group manifested increased cytolytic ability at the highest LPS dose compared with O-HCC. This effect, however, was not as large as that demonstrated in the

Table 1. Oligonucleotide primers used in RT-PCR

<table>
<thead>
<tr>
<th>cDNA</th>
<th>Sense Primer</th>
<th>Antisense Primer</th>
<th>Product, bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>INOS</td>
<td>5′-CACCACAAAGGCCACATCGGATT</td>
<td>CCGACCTGATGTTGCCATTT-3′</td>
<td>426</td>
</tr>
<tr>
<td>GAPDH</td>
<td>5′-TGCCCTCCTCACCACACCTGCAAT</td>
<td>AACACGGAAGGCCATGCCAG-3′</td>
<td>258</td>
</tr>
</tbody>
</table>

INOS, inducible nitric oxide synthase; GAPDH, glyceroldehyde-3-phosphate dehydrogenase.
young group. At the highest priming dose (Fig. 1C), both the Y-Exc and O-Exc groups killed significantly more target cells at high LPS doses compared with their age-matched sedentary counterparts, and O-HCC had reduced cytolysis compared with Y-HCC. In a separate experiment, we found that macrophage-mediated cytolysis (in young mice) was not altered by a single exercise bout performed 24 h before death (data not shown).

Macrophage NO₃⁻ production. Because chronic exercise increased tumor cytolysis, we determined if chronic exercise also increased macrophage NO production in young and old mice. NO is an important toxic effector molecule involved in macrophage cytolysis of tumor

### Table 2. Descriptive data

<table>
<thead>
<tr>
<th>Group</th>
<th>Age, mo</th>
<th>n</th>
<th>Body Wt, g</th>
<th>Spleen Wt, mg</th>
<th>Thymus Wt, mg</th>
<th>Food Intake, g·day⁻¹·mice⁻¹</th>
<th>Citrate Synthase, µM·g wet wt⁻¹·min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y-HCC</td>
<td>6</td>
<td>35</td>
<td>29.6±0.8*</td>
<td>10.9±0.5*</td>
<td>5.7±0.3</td>
<td>11.7±0.4</td>
<td>8.9±0.8†</td>
</tr>
<tr>
<td>Y-Exc</td>
<td>6</td>
<td>38</td>
<td>28.0±0.9†</td>
<td>13.1±0.7†</td>
<td>5.5±0.3</td>
<td>12.4±0.3</td>
<td>12.7±0.6*</td>
</tr>
<tr>
<td>O-HCC</td>
<td>22</td>
<td>30</td>
<td>34.3±0.5‡</td>
<td>15.5±1.0‡</td>
<td>6.3±0.3</td>
<td>11.1±0.5</td>
<td>9.5±1.1†</td>
</tr>
<tr>
<td>O-Exc</td>
<td>22</td>
<td>34</td>
<td>32.5±0.6§</td>
<td>13.9±0.9§</td>
<td>5.5±0.3</td>
<td>11.8±0.4</td>
<td>12.2±1.4†</td>
</tr>
</tbody>
</table>

All values except for age and n are means ± SE; n = no. of mice in each group. Within a column, values with different symbols are significantly different (P < 0.05) from one another. Citrate synthase activity tended (P = 0.12) to be higher in old exercised (O-Exc) vs. old home-cage control (O-HCC) mice. Y-Exc, young exercised mice; Y-HCC, young home-cage control mice.

Fig. 1. Effects of exercise training on resident peritoneal macrophage (Mφ) cytolysis of P815 tumor cells in response to low (1 U/ml, A), intermediate (10 U/ml, B), and high (100 U/ml, C) priming doses of interferon-γ (IFN-γ) across a wide range of triggering doses of lipopolysaccharide (LPS). Initial effector-to-target ratio was 20:1. Data represent means ± SD of means of ≥3 separate experiments. Y-Exc, young exercised mice; O-Exc, old exercised mice; Y-HCC, young home cage control mice; O-HCC, old home cage control mice. Significant differences (P < 0.05): *Y-Exc > Y-HCC; †Y-HCC > O-HCC; §O-Exc > O-HCC.
We found that exercise training increased macrophage NO production (as measured by the metabolite NO\textsubscript{2\textsuperscript{-}}) in young but not old mice (Fig. 2). In addition, there was a tendency for old mice to produce less NO\textsubscript{2\textsuperscript{-}} compared with young mice. Addition of the iNOS inhibitor L-NMMA significantly reduced NO\textsubscript{2\textsuperscript{-}} accumulation to a similar extent in all groups (Fig. 2).

Effects of L-NMMA on macrophage-mediated cytolysis. Experiments were performed to determine if NO was involved in the exercise training-induced increase in macrophage cytolysis by adding L-NMMA to macrophage-P815 cocultures. As can be seen in Fig. 3, L-NMMA completely abrogated the exercise-induced increase in macrophage cytolysis of P815 cells in both young and old mice, suggesting that exercise training increased the ability of IFN-\gamma-LPS-stimulated macrophages to produce NO and that this was the mechanism responsible for the increase in cytolytic activity.

iNOS mRNA expression. RT-PCR was performed to determine if aging or exercise training affected macrophage gene expression of iNOS in the absence or presence of IFN-\gamma and LPS (Fig. 4). Constitutive iNOS mRNA expression was undetectable in all groups. IFN-\gamma and LPS treatment significantly increased iNOS gene expression in all groups. Exercise training increased IFN-\gamma- and LPS-stimulated iNOS mRNA expression in the young but not the old mice. Finally, iNOS mRNA expression was lower in the old compared with the young mice.

Quantification of adherent macrophage number. Exercise training had no effect on the number of peritoneal exudate cells (PEC) obtained by lavage. However, significantly more PECs were obtained from the aged mice compared with young mice (5.6 \pm 0.5, 4.9 \pm 0.6, 12.2 \pm 2.0, and 15.5 \pm 1.8 \times 10\textsuperscript{6}/mouse for Y-HCC, Y-Exc, O-HCC, and O-Exc, respectively). There were no differences in the percentage of plastic adherent macrophages (i.e., Mac\textsuperscript{3\textsuperscript{-}} cells) due to age or exercise (95.3 \pm 3.6, 89.4 \pm 10.5, 95.8 \pm 4.1, and 91.5 \pm 6.0% for Y-HCC, Y-Exc, O-HCC, and O-Exc, respectively). Therefore differences in macrophage tumor killing, NO\textsubscript{2\textsuperscript{-}} production, and iNOS mRNA expression due to exercise training or aging were not attributable to alterations in the number of macrophages in the culture wells.
DISCUSSION

In this report we demonstrate that chronic exercise improves macrophage function and responsiveness to activating signals in young and old mice. Our data show that the ability of peritoneal macrophages to respond to IFN-γ and LPS and kill tumor cells is depressed in elderly mice and enhanced by 4 mo of chronic treadmill exercise in both young and old mice. Macrophage production of NO is a potent lytic mechanism responsible for the killing of various tumor cells (27). We found that exercise training increased macrophage NO production (as measured by the metabolite NO$_2^-$) in young but not old mice. In both groups, the iNOS inhibitor L-NMMA completely abrogated the exercise-induced increase in tumor killing, thereby elucidating the mechanism as to how exercise training potentiates tumor cell lysis. Furthermore, there was an age-related reduction in IFN-γ- and LPS-induced expression of iNOS mRNA levels. Interestingly, exercise training increased iNOS gene expression twofold in young but not old mice.

Until recently it was thought that aging had no effect on the functions of macrophages. However, recent studies using defined activation signals (i.e., IFN-γ, LPS) have demonstrated that aging results in a dramatic reduction in tumor cell killing and effector molecule and cytokine production (2, 6, 9, 17, 23, 29, 36) in response to these stimulatory signals. For instance, Khare et al. (23) demonstrated a 40–50% reduction in the ability of macrophages from old mice to kill P815 cells after incubation with LPS (10 µg/ml) and IFN-γ (10 U/ml). These cells also produced less NO, iNOS protein, and TNF-α (23). Our results are consistent with this and other recent studies in that macrophages from aged mice had a reduced capacity to lyse P815 targets, tended to produce less NO$_2^-$, and had lower iNOS gene expression in response to cytokine stimulation.

Our data clearly demonstrate that 4 mo of exercise training increases peritoneal macrophage cytolytic capacity, NO$_2^-$ production, and iNOS mRNA expression in young mice. In old mice, exercise training increases cytolytic capacity at high doses of IFN-γ and LPS but has a minimal effect on NO$_2^-$ production and iNOS gene expression. The exercise-induced increase in cytolytic activity of P815 target cells in both young and old mice could be completely abolished by the addition of the iNOS inhibitor L-NMMA, indicating that exercise-induced increases in NO production were responsible for the increase in tumor killing. The discrepancy in old mice between the lack of an exercise effect on macrophage NO$_2^-$ production and iNOS gene expression and the apparent ability of L-NMMA to block the exercise-induced increase in tumor killing is difficult to reconcile. We can offer two possible explanations. First, the NO$_2^-$ production and iNOS gene expression experiments were performed on macrophages in the absence of P815 cells, whereas P815 cells were present in the L-NMMA studies. It could be that macrophage-P815 interactions are required for the manifestation of an exercise effect on NO production in aged mice. Second, NO (the molecule responsible for tumor killing) is metabolized to NO$_3^-$ and NO$_2^-$ (27). We only measured NO$_2^-$; therefore, we may not have accounted for all of the NO produced in the aged exercised group. This does not explain the lack of an effect on iNOS gene expression, however.

To our knowledge no studies have examined the effects of acute or chronic exercise on iNOS gene expression in any tissue. Several studies have demonstrated that acute exercise increases plasma and urinary levels of nitrite and/or nitrate, suggesting the production of NO (21, 22). However, this NO production is believed to be related to vasodilation with the source likely being endothelial cells of the vasculature, rich in endothelial isoform of NOS (eNOS) (27). In a recent study, exercise training has been shown to upregulate eNOS gene expression in pig coronary arteries (38).

Unfortunately, no other reports exist regarding the effects of chronic exercise on age-dysregulated macrophage function or responsiveness to defined activation signals. However, both moderate and exhaustive single bouts of exercise have been shown by several groups, and in several different species, to enhance a variety of macrophage capacities, including chemotaxis (13–15), adherence (7, 31), oxidative metabolism, and phagocytic activity (7, 13, 14, 30, 31). We (39, 40) have previously shown that both moderate and exhaustive treadmill running increases antitumor cytostatic activity of thioglycollate (TG)-elicited and P. acnes-activated murine peritoneal macrophages. This effect lasted for at least 8 h after the exercise session and was not due to altered numbers of macrophages in the assay system but was attributable, in part, to increased production of TNF-α from TG-elicited macrophages and increased NO production from P. acnes-activated macrophages. In a similar study, Lotzerich et al. (24) found that the cytostatic but not antibody-dependent cytolytic activity of murine peritoneal macrophages was enhanced after a single exhaustive running session.

We found that exercise-induced increases in tumor cytolytic capacity were greater in the young compared with the old mice. Exercise failed to augment NO$_2^-$ production or iNOS gene expression in the old mice, suggesting that young mice are more amenable to exercise-induced changes. The 4-mo exercise stimulus may not have been long enough for the old to realize the magnitude of change seen in the young. It may be that longer exercise training periods are needed in the old animals or that exercise needs to be performed at earlier ages. There is precedent in caloric restriction studies to support this contention (37), in that the beneficial effects of this experimental paradigm are most evident when caloric restriction is started in young or middle ages (2–14 mo).

In summary, we conclude that aging reduces and exercise training increases the capacity of resident peritoneal macrophages to respond to IFN-γ and LPS with increased tumor cytolysis. Enhanced iNOS gene expression and NO$_2^-$ production are likely the contributing mechanisms of the exercise-induced enhancement of cytology in young mice. Although L-NMMA did block the exercise-induced increase in cytolytic activity of murine peritoneal macrophages in young mice.
mice, exercise training did not increase macrophage NO$_2$ production or iNOS gene expression, indicating perhaps the contribution of other cytolytic mechanisms in old mice. The mechanism for this increase in responsiveness to IFN-γ and LPS was not addressed in the current study; however, these changes were not due to alterations in the number of macrophages in the culture wells. It remains to be determined whether exercise training alters macrophage IFN-γ and/or LPS receptor density, affinity, or signal transduction.

Perspectives

The realization of dysregulated immune function and increased disease incidence, morbidity, and mortality, coupled with the enormous costs of caring for afflicted aged individuals, has been the impetus for several interventions designed to prevent, delay, or restore the age-related dysregulation in immune function (11, 18, 25). Unfortunately, pharmacological, genetic, and tissue grafting or ablation techniques have been impractical and costly to develop and administer, and most are accompanied by adverse side effects. Research involving behavioral preventative or restorative therapies has been lacking. Most of the research in this area has come from studies involving moderate dietary restriction in rodents, which has been found to increase longevity and reduce the incidence of disease. This study was supported by National Institute on Aging Grant AG-13928 to J. A. Woods.

REFERENCES