Exercise suppresses macrophage antigen presentation

M. A. CEDDIA AND J. A. WOODS
Physical Fitness Research Laboratory, Department of Kinesiology,
University of Illinois, Urbana, Illinois 61801

Ceddia, M. A., and J. A. Woods. Exercise suppresses macrophage antigen presentation. J. Appl. Physiol. 87(6): 2253–2258, 1999.—This study determined the effects of exercise on the ability of macrophages (Mφ) to present antigen to T cells. Pathogen-free male Balb/c mice (8 ± 2 wk of age) were randomly assigned to either home cage control, moderate exercise (Mod; 18 m/min, 5% grade, 0.5 h/day), exhaustive exercise (Exh, 18–30 m/min, 3 h/day), or treadmill control groups. The mice underwent treatments for 4 days during peritoneal thioglycolate inflammation. Peritoneal Mφ were harvested, purified, and incubated with chicken ovalbumin (C-OVA; 0–10 mg/ml) for 18 h. Mφ were then cocultured with C-OVA-specific T cells for 48 h, and the supernatants were analyzed via ELISA for interleukin-2 as an indication of Mφ antigen presentation (AP). Exh exhibited suppressed (25–34%) Mφ AP across a wide range of C-OVA doses when measured immediately, 3, and 24 h postexercise. In contrast, Mod had reduced Mφ AP only at 3 h postexercise. Mφ AP was also lower in the treadmill control (4–27%) compared with the home cage control group, but was significantly higher than Exh. The reduction in Mφ AP was not due to exercise-induced differences in Mφ number, percentage, or expression of intercellular adhesion molecule-1, B7–2, or major histocompatibility complex II, molecules important in AP. In conclusion, our data lend evidence that may help explain the increased incidence of infection observed after prolonged exhaustive exercise or overtraining.

immunity; stress; T lymphocytes; interleukin-2; mice; intercellular adhesion molecule-1

MACROPHAGES (Mφ) 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 within the body and are involved in the initiation of immune responses by acting as inflammatory and antigen-presenting cells (APCs) (1). In the antigen presentation (AP) process, Mφ engulf microorganisms into phagosomes, which then fuse with acidic lysosomes containing a variety of proteases that digest and process foreign proteins into antigenic peptides (17). These peptides then associate with major histocompatibility complex (MHC) II and are translocated to the Mφ cell surface where they interact with CD4+ T lymphocytes (1). The interactions between Mφ and T cells lead to the generation of antigen-specific T-cell clones that will eventually recognize and eradicate the invading microorganism, providing long-lasting immunity. The ability of the Mφ to present antigen is crucial to immune function, and, when this ability is compromised, there is an increased risk of morbidity and mortality due to infection (5, 23).

Several studies have independently examined the effects of exercise on components of Mφ AP, but none has considered the process as a whole. These studies, utilizing various modes of exhaustive exercise, have shown to increase peritoneal Mφ chemotaxis toward antigenic stimuli (11, 22), increase phagocytosis of opsonized Candida albicans (7, 10, 18, 22), alter metabolic and lysosomal enzyme activity (10), and increase microbicidal activity as measured by nitroblue tetrazolium reduction (6). In contrast, we demonstrated that exhaustive, but not moderate, exercise reduced bacterially stimulated expression of MHC II on the surface of peritoneal Mφ (28). It was hypothesized that this reduction in MHC II may lead to a reduction in Mφ AP ability. An additional purpose was to determine whether any exercise-induced changes in Mφ AP were due to differences in Mφ number or expression of various surface molecules involved in Mφ–T-cell interactions.

METHODS

Animals. A murine model was employed to test the research objectives proposed in this study. This model was selected to satisfy the need for experimental manipulation and control and to obtain fully differentiated tissue Mφ that are difficult to obtain from human donors. Balb/cByJ inbred male mice (8 ± 2 wk of age) were used in this study because of the MHC compatibility (I-Ak) with the T-cell hybridoma and our previous experience with this strain. Mice were housed in a specific-pathogen-free animal containment facility three to five per cage (12 × 17 × 28 cm) on a 12:12-h light-dark cycle (0600–1800 light) at 23°C. Mice were provided autoclaved food (8640 Harlan Teklad 22–5, Harlan, Madison, WI) and water ad libitum. All experiments were performed at the beginning of the light cycle (0600–0900). The animal treatments were approved by the Laboratory Animal Care Advisory Committee at the University of Illinois at Urbana-Champaign and were within National Institutes of Health guidelines.

Exercise protocol. The exercise protocol consisted of treadmill running. This mode of exercise was chosen because exercise intensity and duration can be experimentally manipulated and quantified (unlike voluntary wheels or swimming). Mice (3–5 per group in ≥3 experiments) were randomly assigned to one of the following four groups: home cage control (HCC), moderate exercise (Mod), exhaustive exercise (Exh), or treadmill control (TC). The HCC group served as temporal controls and remained sedentary in their cages during the 4-day treatment period. The TC group served as isolation and environmental stress controls and were exposed to handling, treadmill noise, and vibration and were deprived of food during the exhaustive exercise bouts but did not exercise. This group was killed immediately after treadmill exposure and was included to control for extraneous stressors.
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(i.e., handling, noise, and vibrations) that may have been associated with exhaustive treadmill exercise. The Mod group exercised for 30 min at 18 m/min, 5% grade, and the Exh group exercised for 2.5–3 h at gradually increasing speeds (18–40 m/min), 5% grade. In past studies, moderate exercise has been defined as brief (usually 15–60 min) bouts of treadmill running at 50–75% maximum O2 consumption or ~15–22 m/min (25). Electric shock or prodding was never used in these experiments, as the mice ran well without extrinsic motivation. The animals exercised for 4 consecutive days during the time necessary for thioglycolate (TG) to recruit Mφ to the peritoneal cavity. TG was injected intraperitoneally (1 ml/mouse) on day 1 immediately after the first exercise session. TG was employed as a Mφ-eliciting agent for two reasons: 1) it resembled an inflammation, making it possible to study the effects of exercise on an inflammatory response, and 2) it provided increased numbers of Mφ necessary to perform the AP and other assays.

Tissue collection and processing. Immediately or 3 or 24 h after the final exercise session (day 4), the mice were killed by rapid CO2 asphyxiation and weighed, and the tissues were extracted and processed. The peritoneal cavity was aseptically lavaged with 10 ml of RPMI-1640 (GIBCO, Grand Island, NY) containing 1 U/ml of sterile heparin to obtain peritoneal exudate cells (PECs). PECs from three to five mice were pooled in each experiment to obtain enough cells for analysis, and each experiment was performed multiple (~3) times. The PECs were washed (190 g, 5 min, 4°C) twice, counted, and stained with trypan blue (>95% viable). The cells were adjusted to a concentration of 2 × 10^6 cells/ml in RPMI containing 5% heat-inactivated, low-endotoxin (<0.01 ng/ml) fetal bovine serum (FBS; Sigma Chemical, St. Louis, MO), 10^{-3} M 2-mercaptoethanol, penicillin (100 U/ml), streptomycin (100 U/ml), and glutamine (20 mM) for use in the Mφ AP and flow cytometric analysis.

Mφ AP assay. The Mφ AP protocol was adapted from previously published works (13, 16). Briefly, 4 × 10^5 PECs per well were plated onto 96-well flat-bottom microtiter plates and incubated at 37°C, 5% CO2, and 95% humidity for 3 h to allow the Mφ to adhere to the plate. After the incubation, the plates were washed four times with RPMI to remove all nonadherent cells, and the supernatants were harvested and stored at −80°C until determination of interleukin-2 (IL-2). Based on initial dose-response studies, we chose antigen concentrations that reflected suboptimal (2.5 mg/ml) and optimal (10 mg/ml) AP.

The T hybridoma cells (AO-40.10AG1) were created and kindly provided by Dr. Philippa Marrack, National Jewish Hospital and Research Center, Denver, CO. Although the hybridoma will grow without any stimulation, it will not produce IL-2 without the presentation of C-OVA by an APC such as a Mφ (16). Therefore, IL-2 production in this in vitro system is directly proportional to Mφ AP. The T-cell hybridoma line was maintained in media consisting of RPMI-1640 with 10% FBS, 10^{-5} M 2-mercaptoethanol, and 100 U/ml of penicillin and streptomycin, and L-glutamine (20 mM) at 37°C with 5% humidified CO2. The cells were seeded at a density of 1 × 10^5 cells/ml and were passed every 3 days. Cells were used in all experiments on the third day of growth. Frozen lots were rederived monthly, and all experiments used cells that had grown for the same amount of time to ensure accurate and reliable results.

IL-2 ELISA. An IL-2 ELISA was developed by using an IL-2 anticytokine capture antibody (Ab; Pharmingen, San Diego, CA) and a biotinylated IL-2 anticytokine detection Ab (Pharmingen). Briefly, the capture Ab was diluted to 2 µg/ml in coating buffer, and 50 µl/well were added to the ELISA plates and incubated at 4°C overnight. The plates were blocked with PBS containing 10% FBS to reduce nonspecific binding. Serial dilutions of IL-2 standards (0–2,000 pg/ml; Sigma Chemical) and the Mφ AP supernatants were added to the appropriate wells and incubated overnight at 4°C. After the second incubation, 100 µl of 1 µg/ml detection Ab were added, and the plates were incubated at room temperature for 45 min. After this incubation, 100 µl of 2.5 µg/ml strepavidin-peroxidase (Sigma Chemical) were added, and the plates were incubated at room temperature for 30 min. Finally, 100 µl of 2,2'-azino-bis (3-ethylbenz-thiazoline-6-sulfonic acid) (Sigma Chemical) substrate were added, and the plates were allowed to develop at room temperature for 60 min. Color change was quantified by light absorbency on a microplate reader at 405 nm.

Determination of Mφ number. To determine whether exercise-induced changes in Mφ AP were due to changes in Mφ numbers in the culture wells, the number of Mφ in parallel cultures was assessed by staining postadherent cells removed with a Teflon cell scraper. The PECs and postadherent samples were analyzed by flow cytometry with FITC-conjugated monoclonal Ab against Mac-3 (clone M3/84; Pharmingen), a surface glycoprotein found on mature TG-elicited Mφ but not on lymphocytes, monocytes, or neutrophils (14). The cells were centrifuged and resuspended in RPMI with 5% FBS at 1 × 10^6 cells/ml and then incubated on ice for 5 min. FITC-conjugated anti-Mac-3 and the FITC-conjugated isotypic control Ab were diluted to 2 µg per 1 × 10^6 cells in PBS with 2% neonatal calf serum, added to appropriately labeled tubes, and incubated for 45 min at 4°C. Cells containing only PBS with neonatal calf serum were used as a control for autofluorescence. The cells were washed twice with PBS (0.01% sodium azide) and resuspended in 4% paraformaldehyde, and 1 × 10^6 cells were analyzed via flow cytometry. The gating for percent positive and mean fluorescence was set and adjusted by using the isotypic control and autofluorescence.

Expression of intercellular adhesion molecule-1 (ICAM-1) (ICAM-1), B7–2, and MHC II. To determine whether exercise-induced changes in Mφ AP were due to differences in Mφ accessory molecule expression, the percentage and mean fluorescence intensity (MFI) signal of ICAM-1, B7–2, and MHC II were assessed by staining both the pre- and postadherent PECs with immunofluorescent monoclonal Ab. These three accessory molecule expression, the percentage and mean fluorescence intensity (MFI) signal of ICAM-1, B7–2, and MHC II were assessed by staining both the pre- and postadherent PECs with immunofluorescent monoconal Ab. These three accessory molecules were analyzed because of their documented importance in Mφ AP (1, 15, 20) and because they (i.e., MHC II and ICAM-1) have been shown to be affected by exercise (3, 28). Two-color analysis was performed on postadherent Mφ cultured with suboptimal and optimal doses of C-OVA for 18 h. The Mφ were stained by using FITC-anti-Mac-3 and either anti-ICAM-1, anti-B7–2, or anti-MHC II Ab conjugated to R-phycoerythrin for analysis. Appropriate isotypic controls and autofluorescence were run with each sample.

Data analysis. All data are reported as means ± SE. Significant differences among groups were determined by two-way (group × dose or time) ANOVA or one-way ANOVA, depending on the variable. Significance levels were set at P < 0.05. Student-Newman-Keuls contrast procedures were performed when significant main effects existed.
RESULTS

Effects of different doses of exercise on Mφ AP. Previous studies have shown that the T-cell hybridoma (AO-40.10A G1) used in this study does not produce IL-2 unless C-OVA antigenic peptides are presented in the context of MHC II on the surface of an APC (16, 26). However, because the influence of exercise on Mφ-T-cell interactions and IL-2 production is unknown, preliminary experiments were done to determine whether exercise affected the ability of Mφ to influence T-cell IL-2 production in the absence of coculture with C-OVA. No T-cell IL-2 production was found in any group at any time in the absence of Mφ coculture with C-OVA (Fig. 1), thereby ruling out an effect of exercise on any extraneous Mφ-T-cell interactions leading to T-cell IL-2 production. In addition, experiments were performed with a related but nonidentical protein (turkey ovalbumin), and no IL-2 production was found (data not shown), thus demonstrating the specificity of the response.

Figure 1 includes raw IL-2 data from representative experiments for the purpose of illustrating the effects of different doses of exercise on Mφ AP at three different times postexercise (immediately, 3, and 24 h) across a wide range (0–10 mg/ml) of C-OVA doses. The data in the figure represent the means ± SE of duplicate culture wells. In these representative experiments, at the immediate postexercise time, both exercise groups were run in the same experiment with one HCC group (Fig. 1A), whereas at 3 and 24 h postexercise only one exercise group (Mod or Exh) was run with a HCC group (Fig. 1, B and C). Results indicated that exhaustive exercise suppressed Mφ AP across all C-OVA doses when measured immediately postexercise, whereas moderate exercise had no appreciable effect (Fig. 1A).

At 3 h postexercise, both moderate and exhaustive exercise significantly reduced Mφ-dependent T-cell IL-2 production at all C-OVA concentrations (Fig. 1B). Whereas this suppression continued at the 24-h postexercise time point in both groups, high concentrations of C-OVA overcame the suppression in the Mod, but not Exh, group (Fig. 1C).

Even though care was taken to treat the T hybridoma cells consistently across all experiments (i.e., similar...
passage and culture conditions), inherent interassay variability common in bioassays of this type prohibited the use of all of the raw IL-2 data in one statistical analysis. This is demonstrated by the differences in IL-2 production in the HCC group in the different experiments illustrated in Fig. 1. Therefore, an analysis of all experiments expressed as a percent difference from HCC [e.g., \((\text{Exh} - \text{HCC})/\text{HCC} \times 100\)] was performed for data collected at suboptimal (2.5 mg/ml; Fig. 2A) and optimal (10 mg/ml; Fig. 2B) doses of C-OVA at all postexercise times.

At the suboptimal C-OVA dose (Fig. 2A), two-way (group × time) ANOVA demonstrated a significant \(F_{(4, 46)} = 3.8, P = 0.011\) group × time interaction, indicating that the groups responded differently over time. Contrast analysis revealed that exhaustive exercise significantly suppressed (−34–38%) Mϕ AP immediately and 3 h postexercise. In contrast, there was a small (−10%) suppression in Mϕ AP immediately postexercise in Mod that was significantly less than that in Exh. Both groups exhibited significant exercise-induced suppression 3 h postexercise. There was a trend (P = 0.08) toward suppression at 24 h postexercise in the Exh and Mod groups compared with HCC. There was also a significant \(F_{(4, 44)} = 4.73, P = 0.004\) interaction effect at the optimal C-OVA dose (Fig. 2B). Contrast procedures revealed significant suppression (−24–37%) in Mϕ AP relative to HCC in the Exh group immediately and 3 and 24 h postexercise. In contrast, Mϕ AP was suppressed (45%) only at the 3-h postexercise time point in the Mod group and not immediately or 24 h postexercise. The small suppression immediately postexercise in Mod and the ability of high (i.e., 10 mg/ml) concentrations of C-OVA to overcome the suppressive effect suggest a more robust suppressive effect in Mϕ AP in response to exhaustive exercise compared with moderate exercise.

To determine the independent effects of exercise vs. environmental stress (i.e., treadmill noise and vibration, handling, isolation) on Mϕ AP, a group of mice (TC) were exposed to the same environment (3 h of treadmill exposure) as the Exh group but did not exercise. We measured Mϕ AP in this group immediately after treadmill exposure only (Fig. 2, A and B). The TC group exhibited −4–27% reduction in Mϕ AP immediately postexposure at the suboptimal C-OVA dose, which was significantly \((P < 0.05)\) lower than that in the HCC group, but significantly \((P < 0.05)\) greater than that in the Exh group (Fig. 2A). At the optimal C-OVA dose, there was no significant suppression in Mϕ AP in the TC group (Fig. 2B). Thus the exhaustive exercise-induced suppression of Mϕ AP was significantly greater than that observed as a result of environmental stress alone. In other words, the suppression induced by exhaustive exercise could not be explained solely by environmental stress.

Effects of exercise on Mϕ number and adherence. The number and percentage of Mϕ in the culture wells were quantified, because a reduction in the number or percentage of Mϕ per well could possibly affect AP (19) and because previous studies have shown that exercise may affect the adherence capacities of Mϕ (6). No significant differences among treatment groups at any time point were found by using a one-way ANOVA in which total PEC number \([F_{(5, 58)} = 0.86, P = 0.53]\), percentage of Mac-3+ cells pre- \([F_{(5, 52)} = 0.32, P = 0.90]\) or postadherence \([F_{(5, 43)} = 0.52, P = 0.79]\), and Mϕ cell number pre- \([F_{(5, 58)} = 0.10, P = 0.43]\) or postadherence \([F_{(5, 43)} = 0.52, P = 0.79]\) were used as dependent variables (Table 1). Therefore, the reduction in AP observed in the Exh group immediately, 3, and 24 h postexercise was not a result of differences in Mϕ number or percentage.

Effects of exercise on Mϕ accessory molecule expression. Expression of ICAM-1, B7−2, and MHC II are important in Mϕ AP to T cells (1, 15). Because similar numbers and percentages of Mϕ among treatment groups were observed, but there was an exhaustive exercise-induced decrease in Mϕ AP, it was hypothesized that Mϕ expression of ICAM-1, B7−2, and MHC II might be affected by exercise. A one-way ANOVA with the use of percent positive or MFI as the dependent variable and treatment group as the independent

![Fig. 2. Percent difference from HCC in Mϕ AP at suboptimal (2.5 mg/ml) (A) and optimal (10 mg/ml) (B) C-OVA concentrations measured immediately (Im Post) and 3 and 24 h postexercise in Mod or Exh groups or in treadmill control group (TC). There were significant \(P = 0.0108\) (A); \(P = 0.0036\) (B) group × time interactions using 2-way ANOVA. *Significant \((P < 0.05)\) exercise-induced suppression of Mϕ AP vs. HCC; #significant difference from HCC and Exh; + suppressive trend \((P = 0.08)\).](http://jap.physiology.org/)
variable revealed no significant differences among the groups immediately postexercise for the percentage of Mϕ expressing ICAM-1 [F(3, 11) = 1.44, P = 0.30], B7–2 [F(3, 13) = 0.61, P = 0.62], or MHC II [F(3, 13) = 0.71, P = 0.57] (Table 2). In addition, no significant differences were found among the groups immediately postexercise for Mϕ MFI of ICAM-1 [F(3, 13) = 3.20, P = 0.08], B7-2 [F(3, 11) = 1.28, P = 0.34], or MHC II [F(3, 13) = 2.68, P = 0.10]. Therefore, the reduction in AP observed immediately postexercise was not a result of either a lower percentage of Mϕ expressing these accessory molecules or a lower amount (i.e., MFI) of these accessory molecules expressed on the Mϕ surface.

**DISCUSSION**

The effects of moderate and exhaustive exercise on the ability of TG-elicted peritoneal Mϕ to present antigen to T hybridoma cells at three different postexercise time points were examined. Exhaustive exercise was found to suppress Mϕ AP across a wide range of C-OVA doses when measured immediately, 3, and 24 h postexercise. The degree of suppression was not related to the dose of C-OVA, as the percent suppression across all doses was similar (data not shown). In contrast, the effects of moderate exercise on Mϕ AP varied, depending on the time point measured and the dose of C-OVA. Unlike exhaustive exercise, moderate exercise had little effect on Mϕ AP immediately postexercise. However, moderate exercise suppressed Mϕ AP at 3 and 24 h postexercise at a suboptimal dose of C-OVA. At an optimal C-OVA dose, moderate exercise suppressed Mϕ AP only at 3 h postexercise and not immediately or 24 h postexercise. These data suggest that the suppressive effect of exhaustive exercise was more robust than that of moderate exercise.

The exhaustive exercise-induced suppression of Mϕ AP could not be entirely explained by environmental stress and handling. Our data indicated that Mϕ AP in the Exh group was significantly lower than that exhibited in the TC group, which was exposed to the treadmill for a period similar to that of the Exh group without running. Therefore, exhaustive exercise caused a significant suppression of Mϕ AP beyond that observed as a result of changes in the environmental setting.

No studies have examined the effects of exercise stress on Mϕ AP. However, other studies have demonstrated that other types of chronic stress, such as chronic ethanol consumption (19, 27), dietary protein deprivation (5, 24), chemical hypotension (9), chronic viral infection (4), human immunodeficiency virus (23), trauma (2), and old age (12), suppress AP in a variety of cell types including Mϕ, dendritic cells, and B cells. In contrast, other studies have found that an acute bout of restraint stress either enhanced (8) or had no effect (29) on Mϕ AP. Taken together, the data would indicate that multiple bouts of prolonged stress are necessary to suppress AP in a variety of APCs. Preliminary data support this contention because 1 day of exhaustive exercise (on day 4 after TG injection, data not shown) did not suppress Mϕ AP, whereas 4 consecutive days did.

The results indicated that the effect of exhaustive exercise on Mϕ AP was not due to differences in Mϕ number or adherence. Therefore, it was suspected that Mϕ expression of surface molecules important to Mϕ-T-cell interactions was altered by exercise. Indeed, it has been shown that the expression of these molecules (i.e., MHC II and ICAM-1) can be affected by exercise (3, 28). In a previous study, we documented that exhaustive,
but not moderate, exercise decreased the ability of Propionibacterium acnes to increase the expression of MHC II on peritoneal Mφ (28). In the present study, we found no exercise-induced differences in Mφ expression of ICAM-1, B7-2, or MHC II molecules immediately postexercise. Direct comparisons between the two studies are made difficult by the fact that, in the present study, we did not stimulate MHC II expression with cytokines or bacteria. Even though our data suggest that surface expression of ICAM-1, B7-2, or MHC II is not responsible for exercise-induced suppression in Mφ AP, our data do not preclude the possibility that other surface molecules (i.e., CD40 or B7-1) associated with Mφ AP were altered in response to exercise. Moreover, expression of these molecules at 3 and 24 h postexercise was not measured, and, therefore, we cannot say with confidence that they did not contribute to suppressed Mφ AP at these later times. Last, only total surface expression of MHC II, and not MHC II containing C-OVA peptide, was measured; therefore, it is possible that there could have been a difference among the groups with respect to the expression of MHC II containing C-OVA on the Mφ surface. Future studies are required to determine the intracellular mechanism(s) responsible for the exercise-induced suppression in Mφ AP.

In conclusion, our results are the first to demonstrate that exercise suppresses Mφ AP. Four consecutive days of exhaustive exercise resulted in a marked and prolonged (at least 24 h) depression in the ability of Mφ to present antigen to T cells. Moderate exercise also suppressed Mφ AP, although this effect was less pronounced. Thus it is possible that at least some of the previously reported immune suppression after repeated exhaustive exercise (21) may be due in part to a decrease in Mφ AP to T lymphocytes.

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Address for reprint requests and other correspondence: J. A. Woods, 906 S. Goodwin Ave., Univ. of Illinois, Urbana, IL 61801 (E-mail: Woods1@uiuc.edu)

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REFERENCES