Exercise training-induced adaptations of immune response are mediated by β-adrenergic receptors in aged but not young mice

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Exercise training-induced adaptations of immune response are mediated by β-adrenergic receptors in aged but not young mice. J Appl Physiol 96: 1312–1322, 2004. First published December 12, 2003; 10.1152/japplphysiol.00792.2003.—β-Adrenergic blockade was used to determine whether the exercise training-induced adaptations of immune response to viral infection were mediated by catecholamines in young and old mice. Young (2 mo) and older (16 mo) male BALB/c mice were randomly assigned to an exercise or control group, and half of the mice in each group received the β-adrenergic receptor antagonist nadolol. After 8 wk of moderate exercise training, mice were challenged with herpes simplex virus (HSV) 24 h postexercise. The results showed that exercise treatment increased anti-HSV IgM antibody, enhanced IL-10, and altered the kinetics of IFN-γ and IL-2 production in young and old mice. Unique to older mice, exercise decreased mitogen-induced proliferation, increased splenocytes, and tended to decrease memory cells (CD44 hi). In contrast, exercise increased mitogen-induced proliferation but decreased the number of splenic lymphocyte and CD4+ cells in young mice. β-Adrenergic blockade blunted the exercise-induced changes in anti-HSV IgM, IL-2, IFNγ, and mitogen-induced proliferation in old but not young mice. The findings suggest that some of the immunomodulatory effects of chronic exercise are mediated via β-adrenergic receptors and that the role of β-adrenergic receptors is age dependent.

SEVERAL LINES OF EVIDENCE suggest that moderate exercise may enhance immune responsiveness and improve resistance to infection. The results from cross-sectional comparisons between exercise-trained and inactive persons suggest that exercise may increase natural killer (NK) cell activity, mitogen-induced lymphocyte proliferation, and IL-2 and IFN-γ production (29, 36) and may improve antibody response to influenza vaccination (20). With respect to animal models, moderate exercise training in mice and rats has been shown to improve secondary antibody response (23), augment the production of IL-2 and IFN-γ to viral challenge (19), and increase survival rate in response to viral or bacterial challenge (4, 9).

Neuroendocrine-immune communication is thought to, in part, mediate the exercise-induced alteration of immune responsiveness. Cells of the immune system have receptors for numerous neuroendocrine factors, and cellular function can be modulated by these neuroendocrine factors (24). In addition to the neuroendocrine receptors expressed on immune cells, the central nervous system may communicate with the immune system by innervation of lymphoid tissues. Lymphoid organs such as the spleen and lymph nodes are directly innervated by the sympathetic nervous system, and sympathetic nervous system activation can alter immune response (26). Physical exercise can be categorized as a “stressor,” which is accompanied by increased concentrations of catecholamines, corticosteroids, growth hormone, β-endorphin, prolactin, estrogen, etc., as well as sympathetic activation (11, 32). Therefore, any modulation of immune function resulting from exercise may be mediated by the altered concentrations of one or more neuroendocrine factors.

Adaptations in the exercise-induced release of hormones and tissue sensitivity to neuroendocrine factors occur with long-term exercise training. For example, the sensitivity of lymphocyte β-adrenergic receptors is altered after a period of increased exercise training (35). Consequently, the neuroendocrine-mediated alterations of immune responsiveness may also demonstrate adaptations to chronic exercise training.

Although several investigators have demonstrated a correlation between hormone or neuropeptide levels and the immune response to exercise, fewer studies have employed a pharmacological manipulation to directly examine the role of neuroendocrine factors in mediating the alteration of immune responsiveness to acute exercise. And far fewer studies have attempted to evaluate the immunomodulatory role of hormones or neuropeptides during chronic exercise training. The limited data suggest that endogenous opioids may mediate the training-induced enhancement of secondary antibody response (15) and that central opioid receptors may play a role in the enhancement of in vivo NK cell cytotoxicity (13). In contrast, the splenic nerve did not appear to influence exercise-induced adaptations in NK cell cytotoxicity (12). Although other endocrine factors, such as corticosteroids, catecholamines, growth hormone, prolactin, etc., are often suggested as modulators of immunity, the role of these factors in mediating the effects of exercise training have not been systematically evaluated. In addition, cells of the immune system express varying amount of receptors for hormones and neuropeptides; therefore, it is possible that the immunomodulatory effects of exercise may vary by cell type. For example, T helper type 1 (Th1) cells express β2-adrenergic receptors, whereas T helper type 2 (Th2) cells do not (33). Given this differential expression of receptors, it is possible that any immunomodulatory adaptation to the catecholamines released during exercise may occur in Th1 but not Th2 cells, and, therefore, we evaluated both Th1 (IL-2, IFN-γ) and Th2 (IL-10) cytokines. Given the lack of data on the immunomodulatory role of neuroendocrine factors during chronic exercise training and the present knowledge regarding β2-adrenergic receptor expression in Th1 and Th2 cell types, the purpose of this study was to examine the role of cate-
echolamines as potential mediators of the exercise-induced alterations in cytokine and antibody production and antigen- or mitogen-induced cell proliferation.

MATERIALS AND METHODS

Animals. Male BALB/cJNIA mice were purchased from Charles River Laboratories and obtained from the National Institute of Aging colony. Young mice (2 mo of age on arrival and 4 mo of age at the time of death) and older mice (16 mo of age on arrival and 18 mo of age at the time of death) were allowed to acclimate to housing conditions for 1 wk before experimental manipulation on a 12-h light-dark cycle. All animal procedures were approved by the Iowa State University Committee on Animal Care. After the first week, all mice were acclimated to treadmill running for ~10–15 min/day for 3 days at a speed of 8 m/min. The nonselective β-adrenergic receptor antagonist nadolol was chosen due to its limited ability to cross the blood brain barrier and its long half-life (20–24 h) (3, 17). The effectiveness of nadolol in blocking the exercise-induced effect of elevated catecholamines was tested by measuring free fatty acid (FFA) plasma levels in response to exercise and drug treatment. Mice at each age were randomly assigned to one of the following eight groups: young mice, control condition, saline (young-con-saline; n = 12); young mice, exercise condition, saline (young-ex-saline; n = 12); young mice, exercise condition, nadolol (young-ex-nadolol; n = 12); young mice, exercise condition, saline (young-con-nadolol; n = 12); older mice, control condition, saline (older-con-saline; n = 12); older mice, control condition, nadolol (older-con-nadolol; n = 12); older mice, exercise condition, saline (older-ex-saline; n = 12); and older mice, exercise condition, nadolol (older-ex-nadolol; n = 12). Exercise mice ran on a treadmill under lighted conditions at the end of a 12-h dark cycle. 5 days/wk for 8 wk at a gradually increasing speed and duration. Duration was gradually increased during weeks 1–4; from weeks 5–8, mice ran 40–45 min/day. Speed was gradually increased as well, starting at 8 m/min during week 1 and gradually progressing to 18 m/min. Although we did not measure muscle enzymes to evaluate the effectiveness of this exercise training protocol, another study that used a very similar exercise protocol (18 m/min for 45 min/day for 30 min before each exercise session or control treatment. Nadolol-treated mice received an intraperitoneal injection of nadolol (5 mg/kg) 15–30 min before each exercise or control treatment. Electric shock was never used at any time during the study to encourage or induce exercise, because mice responded well to the gradual increase in speed and duration of running. Control mice remained in their cages, and the control mouse cages were transported to the same location as the treadmill. At this location, the mice were handled by the same investigator who handled the exercise mice and were then returned to their cages, where they remained for the duration of treadmill running. This was done in an attempt to expose the control mice to similar conditions (noise of the treadmill, transport, handling, etc.) without actual physical exercise.

Viral infection and mouse response to infection. Twenty-four hours after the last exercise or control session, all mice were infected with 2.5 × 10^3 plaque-forming units (PFU) of herpes simplex virus (HSV)-1 (Patton strain) through an intranasal route at a dose corresponding to 10% lethality. The intranasal route was chosen to mimic the typical route of viral entry in an upper respiratory infection. Immediately before infection, all mice were weighed, and body weight was measured throughout the infection. Mice were briefly exposed to CO_2 and infected via an intranasal route with 50 μl of HSV-1 in sterile saline. Virus was propagated and prepared as previously described (21). After infection, all mice were returned to their cages for 10 days. Mice remained in their cages and did not exercise during the 10 days postinfection. Intranasal administration of the virus results in symptoms characteristic of pneumonia, and the histopathology has been characterized (1). Symptoms of illness, which included redness around eyes, nose, or mouth, hunched back, lack of movement, and ruffled fur, were monitored twice daily. Body weight was measured every 2 days. None of the mice died from the infection in the 10-day postinfection period. At the time of death, all mice were examined for tumors. None of the mice had visible tumors, so all mice, therefore, were included in the study. The virus used in these experiments was tested for the ability to produce ex vivo immune responses. At the time virus was grown, five mice were infected with HSV-1 and four mice were challenged with saline. Mice were euthanized 7 days postinfection. Spleen cells were challenged with inactivated virus in vitro to establish whether the present batch of virus can induce antigen-specific responses.

Tissue collection. Ten days after infection, mice were decapitated and trunk blood was collected. Spleens were removed and dissociated in Hanks’ balanced salt solution (GIBCO, Grand Island, NY) by using a lab blender (Tekmar, Cincinnati, OH). Cell suspensions were passed through sterile nylon mesh to remove clumps of tissue. Cells were counted in sterile saline containing 1% Hematall (Sigma Chemical, St. Louis, MO) by using a Coulter counter (Coulter Electronics, Hialeah, FL).

In vitro HSV-specific stimulation of spleen cells. Spleen cells from each mouse were washed twice and resuspended in supplemented RPMI media [RPMI-1640 supplemented with 5% FBS, 100 U/ml penicillin, 100 μg/ml streptomycin sulfate, 2 mM l-glutamine, 0.075% (wt/vol) NaHCO_3, 20 mM HEPES, and 5 × 10^−5 M 2-mercaptoethanol; all from GIBCO]. Cells were adjusted to 5 × 10^6 cells/ml and 1 ml was added to each well of a Falcon 24-well tissue culture plate (Becton-Dickinson Labware) containing 1 ml/well ultraviolet-inactivated HSV-1 in supplemented RPMI media. The concentration of inactivated HSV-1 would yield 2.5 × 10^7 PFU per well (5 PFU per cell) when active. Control wells also contained 5 × 10^6 cells/ml and 1 ml of media without inactivated HSV-1. In pilot experiments, a range of cell concentrations (1 × 10^5 to 1 × 10^7) and PFU/cell (1–10 PFU/cell) were tested, and the optimal amounts were used in subsequent experiments (5 × 10^6 cells/ml and 5 PFU/cell).

ELISA for serum anti-HSV antibody. Serum IgM and IgG anti-HSV antibodies were detected by ELISA. ELISA plates (Immulon, Alexandria, VA) were coated overnight at 4°C with HSV-1 diluted in carbonate buffering buffer (pH 9.6) at a concentration of 2.3 × 10^7 PFU/ml and were blocked with PBS-0.1% BSA. Plates were washed three times with PBS/0.05% Tween between each step. Serum was diluted and added; plates were incubated overnight at 4°C. Alkaline phosphatase-conjugated goat anti-mouse IgM or IgG (Cappel Reaserch Products, Durham, NC) was added and then incubated overnight at 4°C. Finally, substrate (p-nitrophenyl phosphate, Sigma Chemical) was added. Absorbance was read at 405 nm by using an automated plate reader (Bio-Rad, Hercules, CA).

Cytokine assays. Cytokine assays were performed by ELISA on supernatants from HSV-stimulated spleen cells. Supernatants for cytokine determination were collected from spleen cells at 24, 48, and 72 h of culture. IL-2, IFN-γ, and IL-10 levels in supernatants induced by HSV were determined by an ELISA using monoclonal antibodies and the protocol supplied by PharMingen (San Diego, CA).

Flow cytometry. We used flow cytometry to calculate the percentage of CD4+ and CD44hi (memory population) cells in the spleens of mice. The antibodies PE anti-CD44 and FITC anti-CD4 were used. CD44hi cells (memory population) were identified on the basis of two distinct peaks found in the young control population with approximately the 75% brightest staining cells being CD44hi. The gates were held constant across all samples analyzed.

Mitogen-induced proliferation. Spleen cells were adjusted to 5 × 10^6 cells/ml and 100 μl were added to each well of a Falcon 96-well tissue culture plate. Phytohemagglutinin (PHA; 5 μg/ml), concanavalin A (ConA; 10 μg/ml), or media alone was added to triplicate wells containing cells from each mouse. Plates were incubated at 37°C in
The mouse responses to infection are significantly lower than the preinfection measure, with the exception of young exercised mice. Table 2 shows that the cytokine responses measured are specific to HSV-1 infection. Cytokine responses to in vitro restimulation with inactivated HSV-1 were compared between infected and mock-infected mice. IL-2, IFN-γ, and IL-10 were significantly higher in infected mice, suggesting that the cytokine response is specific to HSV-1 infection. Table 3 demonstrates the recall response to viral challenge in the mice used in the present exercise experiment. Cells obtained from all of the mice in this experiment were cultured with inactivated virus or media alone. Wells containing HSV-1 produced significantly higher amounts of cytokine in the supernatant than wells containing media alone.

**Effect of nadolol on catecholamines during exercise.** In preliminary experiments, the effectiveness of β-adrenergic blocking agents was tested by measuring FFA plasma levels in exercised mice (Table 4). The exercise-induced increase in plasma FFA is primarily mediated via β-adrenergic stimulation of lipolysis within adipocytes (38). As demonstrated in Table 4, the administration of propranolol (10 mg/kg) before the third day of a 3-h exercise session blunted plasma FFA, similar to our previous findings (21). Also shown in Fig. 4 are the results of young mice trained for 4 wk. Nadolol (5 mg/kg) or saline was administered 30 min before a 30-min exercise session at 18 m/min. Again, the physiological effect of elevated plasma FFA due to the exercise-induced release of catecholamines is blunted by nadolol. Although this effect was not measured after 8 wk of exercise, the exercise speed and/or duration continued to increase from week 4 to week 8.

**Anti-HSV antibody.** The effect of exercise and drug treatment on anti-HSV IgM varied by age (significant age × group interaction, P < 0.05). In young mice, exercise significantly increased antibody titer (P = 0.042) and treatment with the drug nadolol had no effect (Fig. 1A). This suggests that the exercise-induced increase in antibody titer is not mediated via β-adrenergic receptors in young mice. In contrast, in aged mice, exercise treatment increased the anti-HSV IgM titer in saline treated mice only (Fig. 1B), whereas the exercise mice treated with nadolol did not exhibit an increase in antibody titer (significant group × drug interaction, P < 0.05), suggesting that β-adrenergic receptor activation mediates the exercise-induced enhancement of antibody production in aged mice at day 10 postinfection. Anti-HSV IgG was measured but was not measured below a level that we were able to detect at 10 days postinfection.

**IFN-γ (HSV-specific production).** The effect of exercise and age was not consistent across the in vitro incubation periods (significant in vitro time × age × group interaction, P < 0.05, and significant age × group interaction, P < 0.05), and, therefore, each age group was analyzed separately. With respect to the young mice, exercise significantly increased the production of IFN-γ (main effect of group, P = 0.02), and the effect of exercise tended to be greater in mice receiving nadolol and at the earlier time points in vitro (24 and 48 h) (Fig. 2A).

**Table 1. Body weight before and after infection**

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Weight Preinfection, g</th>
<th>Body Weight 10 days Postinfection, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young—control</td>
<td>30.98 ± 1.3</td>
<td>31.18 ± 1.5</td>
</tr>
<tr>
<td>Young—exercise</td>
<td>33.84 ± 1.8</td>
<td>33.24 ± 1.6*</td>
</tr>
<tr>
<td>Old—control</td>
<td>32.53 ± 3.8</td>
<td>33.25 ± 4.1*</td>
</tr>
<tr>
<td>Old—exercise</td>
<td>33.53 ± 2.0</td>
<td>34.00 ± 2.5</td>
</tr>
</tbody>
</table>

Values are means ± SD. *Significantly different from preinfection weight (P < 0.05).

**Table 2. In vivo test of antigen-specific response**

<table>
<thead>
<tr>
<th>Cytokine Measured</th>
<th>Virus-Infected Mice, pg/ml</th>
<th>Mock-Infected Mice, pg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-2</td>
<td>257 ± 28</td>
<td>0* (below limits of detection)</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>593 ± 117</td>
<td>33 ± 24,8*</td>
</tr>
<tr>
<td>IL-10</td>
<td>321 ± 75</td>
<td>54 ± 39*</td>
</tr>
</tbody>
</table>

Values are means ± SE. *Significantly different from virus-infected mice (P < 0.05).

**Table 3. In vitro test of antigen-specific response**

<table>
<thead>
<tr>
<th>Cytokine Measured</th>
<th>Virus-Infected Culture Wells, pg/ml</th>
<th>Media-Alone Culture Wells, pg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-2</td>
<td>457 ± 55</td>
<td>106 ± 31</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>263 ± 33</td>
<td>63 ± 18</td>
</tr>
<tr>
<td>IL-10</td>
<td>129 ± 30</td>
<td>55 ± 19</td>
</tr>
</tbody>
</table>

Values are means ± SE. All mice were challenged with HSV virus. At 10 days postinfection, spleen cells were cultured with inactivated HSV virus or media alone. The increased cytokine production in the wells containing virus demonstrates a recall response in vitro.

**Table 4. Plasma concentration of FFA as an indicator of catecholamine effect**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Saline, mM</th>
<th>Propranolol, mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.36 ± 0.02</td>
<td>0.38 ± 0.03</td>
</tr>
<tr>
<td>3 days 3 h of exercise</td>
<td>1.0 ± 0.08</td>
<td>0.62 ± 0.04*</td>
</tr>
<tr>
<td>4 wk 30 min of exercise</td>
<td>0.71 ± 0.04</td>
<td>0.44 ± 0.05*</td>
</tr>
</tbody>
</table>

Values are means ± SE. FFA, free fatty acid. *Significantly different from the same exercise condition with saline (P < 0.05).
Blocking the adrenergic receptors with nadolol did not attenuate the exercise effect, suggesting that β-adrenergic receptor activation did not mediate the exercise effect in young mice. With regard to the older mice, the effect of exercise was not consistent across time (group × time interaction, \( P < 0.05 \)) such that exercise tended to increase IFN-γ at early time points in vitro but decrease at later time points. The effect of exercise at the early time point likely involved β-adrenergic receptor activation given that the exercised mice receiving nadolol did not produce greater amounts of IFN-γ (significant drug × group interaction, \( P < 0.05 \)) (Fig. 2B).

**IL-2 (HSV-specific production).** The effect of exercise on HSV-induced IL-2 production was not consistent across ages or time periods of in vitro culture (significant in vitro time × age × group interaction, \( P < 0.05 \), and significant age × group interaction, \( P < 0.05 \)), and, therefore, the different age groups were analyzed separately. In the overall analysis, there was a trend toward greater IL-2 production in young mice compared with old mice. Exercised young mice produced greater amounts of IL-2 than control mice, and this effect was observed for both mice treated with saline and nadolol, suggesting that β-adrenergic mechanisms do not mediate the effect of exercise on IL-2 (significant main effect of exercise, \( P = 0.029 \)) (Fig. 3A). In contrast, with respect to old mice, exercise altered IL-2 production, and the effect of exercise varied over time (time × group interaction, \( P < 0.05 \)) (Fig. 3B). At early time points in vitro (24 h), exercise tended to enhance IL-2 production, but this effect was no longer present at the 48- or 72-h time points. In addition, the effect of β-adrenergic blockade varied by time of in vitro culture. At the early time point hours, nadolol treatment attenuated the enhancing effect of exercise on IL-2 production (significant drug × group interaction, \( P < 0.05 \)), but this effect was not present at later time points (48 and 72 h in vitro). IL-2 was not significantly different in the exercise mice treated with nadolol compared with the exercise mice treated with saline.

**IL-10 (HSV-specific production).** Young and old mice responded similarly with respect to the effect of exercise training on HSV-induced IL-10 production. Exercise enhanced the production of IL-10 in both young and old mice (significant main effect of group, \( P = 0.008 \)) (Fig. 4). The effect of exercise on IL-10 was observed in both saline- and nadolol-treated mice, suggesting that β-adrenergic mechanisms do not mediate the effects of exercise on IL-10 production.

**Spleen cell populations.** The total number of spleen cells and lymphocytes was altered by exercise and drug treatment, although these effects were not consistent across age groups (significant age × drug and age × group interactions, \( P < 0.05 \)). Therefore, the age groups were analyzed separately (Table 5). In young mice, exercise did not significantly alter splenocyte number, although there was a trend toward decreased cell number in exercised mice compared with control mice (\( P = 0.08 \)). However, total lymphocyte number was decreased by exercise in young mice (Table 5). In old mice, exercise and nadolol treatment increased splenocyte number (main effect of exercise, \( P = 0.027 \); main effect of nadolol, \( P = 0.029 \)), and lymphocyte number tended to increase with exercise training (\( P = 0.10 \)) (Table 5). The percentage of CD4+ cells was not altered by exercise or drug treatment (Table 5), however, the total number of CD4+ cells was decreased by exercise in young mice only (\( P = 0.008 \)) (Table 5). The percentage of “memory” cells was determined by CD44hi expression. A greater number of memory cells was observed in the old mice (main effect of age, \( P < 0.001 \)). However, the effect of exercise differed by age group (significant age × group interaction, \( P < 0.05 \)), and thus young and old mice were analyzed separately. In young mice, exercise slightly increased the percentage of memory cells (\( P = 0.018 \)) but did not alter the total number of memory cells, suggesting that another population of cells may be leaving the spleen (Figs. 5A and 6A). With respect to the old mice, exercise tended to decrease the percentage of memory cells (\( P = 0.07 \)) (Fig. 5B). In contrast, the actual number of memory cells decreased in the old exercise mice treated with saline but increased in the old exercise mice treated with nadolol (group × drug interaction, \( P < 0.05 \)) (Fig. 6B).

**PHA- and ConA-induced proliferation.** With respect to mitogen-induced proliferation, the spleen cells from young mice had a greater amount of proliferation than the cells from old mice (\( P = 0.02 \) for PHA, \( P = 0.008 \) for ConA). In response to PHA, young and old mice responded to the effect of training.
Young exercised mice had an increase in proliferation compared with young control mice, whereas old exercise mice showed a decrease in proliferation compared with old control mice. There was not a main effect of drug, although the decrease in proliferation in old exercised mice tended to be smaller in nadolol-treated exercised mice. ConA-induced proliferation was not different between exercised and control young mice, and there was no effect of drug in young mice (Fig. 7B). In contrast, ConA-induced proliferation tended to decrease in exercise-trained old mice ($P = 0.089$), and this decrease was not observed in the nadolol-treated exercise old mice, suggesting a possible role for mediation by catecholamines via $\beta$-adrenergic receptors.

**DISCUSSION**

The results from this study suggest that catecholamines may mediate exercise-training-induced alterations of immune responsiveness to viral antigen, although the role of catecholamines is complex and varies by age, immune parameter, and timing of immune measurement. The rationale for this investigation is that neuroendocrine-immune interactions are often suggested to be the mechanism mediating exercise-associated immunomodulation, yet very few studies have actually determined whether neuroendocrine factors are involved. To our knowledge, these are the first experiments to examine how catecholamines may mediate immune responsiveness during exercise training in young and aged popula-
The β-adrenergic receptor antagonist nadolol was used in these experiments to block the effect of catecholamines on β-adrenergic receptors during exercise. Our findings demonstrate that catecholamines may mediate the effect of exercise, but the role of catecholamines is age dependent (present in the old mice). The exercise-induced modulation of immunity that is attributed to catecholamines appears to be limited to antibody, IL-10, and the kinetics of Th1 cytokines. We have evaluated immune response in the spleen and blood. In preliminary studies, it was observed that anti-HSV cytokine responses in the spleen parallel the lung cytokine response, but peak levels in the spleen are observed several days later than in the lung. Although one assumes that the effect of exercise and catecholamines may be similar in the lung and spleen, this remains to be tested.

Our findings are consistent with the possibility that exercise training can improve the immune response to viral infection in both young and aged populations. The immunomodulatory effects that we observed in this study may be a mechanism to explain the previously reported decrease in morbidity and/or mortality to infectious pathogens in exercise-trained animals (4, 10). Anti-HSV IgM titer was increased by exercise training in both young and older mice, which is consistent with other (16, 23), but not all (5), studies. The effect of exercise training on antibody response to exercise may vary by the timing of antibody measure, by antibody class or subclass, and/or by priming or recall response. Further studies are needed to clarify this. Our findings extend previous work in this area, which suggested that the catecholamines released during exercise may mediate the training-associated enhancement of antibody response to viral infection. Also, the kinetics of antigen-specific Th1 cytokine response was altered by exercise training. In general, in exercise-trained mice, the Th1 cytokines were elevated at an earlier time point in vitro (young and older mice) and tended to decrease more rapidly (in older mice). The change in Th1 cytokine kinetics associated with exercise training may have relevance in vivo. A strong Th1 response to viral infection may result in tissue damage, and it has been shown that prolonged elevation of IFN-γ (induced by IL-12) during pulmonary viral infection increased pulmonary inflammation, resulted in more severe tissue damage, and delayed recovery from infection (14, 22). It may be of benefit to the host to upregulate and downregulate Th1 responses quickly, potentially avoiding prolonged inflammation and tissue damage. Conversely, an elevation of anti-inflammatory cytokines such as IL-10 may prevent further tissue damage. We observed in both young and old mice that exercise training was associated with increased viral-induced IL-10 at all time points in vitro.

Fig. 3. In vitro HSV-1 stimulated IL-2 production by spleen cells from young (A) and old (B) Ex or Con, Nad- or saline-treated male BALB/cJNIA mice. Spleen cells were collected 10 days postinfection and restimulated in vitro with HSV. Supernatants were collected at 24, 48, or 72 h of in vitro culture. Values shown reflect HSV-treated wells minus media only (Con) wells. Exercise significantly increased IL-2 in young mice (*main effect of exercise, F = 0.029). The effect of exercise varied across time in the old mice (exercise × time interaction, P < 0.05). The exercise effect was blunted in Nad mice (†group × drug interaction, P < 0.05) at 24 h. Values are means ± SE.
It is possible that exercise training may alter viral-induced proinflammatory and anti-inflammatory cytokine expression in such a way as to minimize inflammation and tissue damage, and our data is consistent with this possibility. However, this intriguing hypothesis has not yet been tested to our knowledge.

An interesting finding from this investigation is that the effect of exercise on mitogen-induced responses generally differed from the effect of exercise on antigen-induced responses. In particular, exercise tended to enhance antigen-induced immune responses (antibody, IL-10, IL-2 and IFN-γ kinetics) in older mice. In contrast, exercise training was associated with decreased mitogen-induced proliferation in cell numbers (Table 5).

**Table 5. Cell numbers**

<table>
<thead>
<tr>
<th>Group</th>
<th>Spleen Cell No.*</th>
<th>Lymphocyte No.†</th>
<th>CD4+ Cells, %</th>
<th>CD4+ Cell No.‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young con/sal</td>
<td>7.6±0.52</td>
<td>4.4±0.54</td>
<td>21.4±1.9</td>
<td>8.9±1.0</td>
</tr>
<tr>
<td>Young con/nad</td>
<td>7.1±0.62</td>
<td>4.5±0.72</td>
<td>23.8±1.6</td>
<td>11.0±2.0</td>
</tr>
<tr>
<td>Young ex/sal</td>
<td>6.3±0.66</td>
<td>3.3±0.61</td>
<td>19.4±3.2</td>
<td>6.3±1.3</td>
</tr>
<tr>
<td>Young ex/nad</td>
<td>5.7±0.86</td>
<td>3.3±0.66</td>
<td>19.3±1.6</td>
<td>6.3±0.76</td>
</tr>
<tr>
<td>Old con/sal</td>
<td>4.4±1.11</td>
<td>2.3±0.66</td>
<td>23.7±2.5</td>
<td>4.5±0.77</td>
</tr>
<tr>
<td>Old con/nad</td>
<td>5.6±1.32</td>
<td>2.8±0.72</td>
<td>19.9±1.8</td>
<td>4.4±0.82</td>
</tr>
<tr>
<td>Old ex/sal</td>
<td>5.7±0.92</td>
<td>3.2±0.1</td>
<td>17.8±1.6</td>
<td>3.7±0.78</td>
</tr>
<tr>
<td>Old ex/nad</td>
<td>12.2±2.3</td>
<td>5.0±1.1</td>
<td>17.2±1.8</td>
<td>8.1±2.5</td>
</tr>
</tbody>
</table>

Values are means ± SE. con, Control; sal, saline; nad, nadolol; ex, exercise.

*Spleen cells are 1 × 10⁷; †Cells are 1 × 10⁷; ‡Cells are 1 × 10⁶.

(24, 48, and 72 h). It is possible that exercise training may alter viral-induced proinflammatory and anti-inflammatory cytokine expression in such a way as to minimize inflammation and tissue damage, and our data is consistent with this possibility. However, this intriguing hypothesis has not yet been tested to our knowledge.

An interesting finding from this investigation is that the effect of exercise on mitogen-induced responses generally differed from the effect of exercise on antigen-induced responses. In particular, exercise tended to enhance antigen-induced immune responses (antibody, IL-10, IL-2 and IFN-γ kinetics) in older mice. In contrast, exercise training was associated with decreased mitogen-induced proliferation in cell numbers (Table 5).
older mice. Young exercised mice showed enhanced responses to antigen (antibody and cytokines). Our observation of an exercise-induced enhancement of mitogen-induced proliferation in young mice is consistent with other studies using mice rather than rats (8, 28, 31, 37). Perhaps most interesting is our finding that the effects of exercise training on antigen-induced immune responses are different, particularly in aged mice. Although many studies have used mitogens as a convenient method of evaluating immune function, it is possible that some beneficial effects of exercise training in terms of better protection from infection have been overlooked. To determine the role of exercise in improving resistance to infection, the physiological relevance of antigens as immune stimuli rather than mitogens should be considered, as suggested by our findings.

Changes in total spleen cell number were also observed as a result of exercise training. Interestingly, in young mice, exercise training was associated with fewer splenocytes, similar to other studies (8), whereas exercised old mice had a slight increase in the total number of splenocytes. No changes in splenic CD4+ cell percentage were found as a result of exercise, but the total number of splenic CD4+ cells decreased in the exercised young mice. With regard to the number of CD44+ (memory cells), both the number and percentage of CD44+ cells as well as the percentage of CD4+CD44+ tended to decrease in saline-treated exercised old mice, but, interestingly, the number of CD44+ cells increased in the nadolol-treated exercised old mice, suggesting that catecholamines may have some role in the departure of memory cells from the spleen. Although we did not assess the CD8+ population, another study (39) recently reported that exercise training decreased the number of both CD4+ and CD8+ splenic memory cells in aged (18 mo) BALB/c mice. The age-related decrease in the memory-to-naive cell ratio is thought to contribute to the poor response to novel antigens that has been observed among older populations. If exercise training can shift the memory-to-naive cell ratio back toward a young phenotype, then perhaps the immune response to new antigens would be enhanced. Further studies are needed to determine whether these changes in cell populations may confer greater protection to infectious agents.

Fig. 5. Percentage of CD44+ (memory) lymphocytes in young (A) and old (B) Ex or Con, Nad or saline-treated male BALB/cNJIA mice. In young mice, exercise increased the percentage of CD44+ cells (P = 0.018), whereas old Ex mice showed a trend (+P = 0.07) toward a lower percentage of CD44+ cells in saline-treated mice only. Values are means ± SE.

Fig. 6. Number of CD44+ (memory) lymphocytes in young (A) and old (B) Ex or Con, Nad or saline-treated male BALB/cNJIA mice. No significant effects of exercise or nadolol treatment were observed in young mice. In old mice, a significant group × drug interaction was found (P = 0.042). Saline-treated Ex mice had fewer CD44+ cells than saline control mice (++), whereas Nad Ex mice had a greater number of CD44+ cells (+ +) than Nad Con mice. Values are means ± SE.
The finding that catecholamines appear to mediate some but not all of the immunomodulatory effects of exercise training may be related to the number of β-adrenergic receptors expressed on cells of the immune system or the relative intensity of exercise training in young compared with older mice. It has been shown that resting and activated Th1 cells express a detectable level of β2-adrenergic receptors, whereas Th2 cells do not (33). The repeated increase in circulating catecholamines that occurs with each bout of exercise may have a greater impact on the Th1 cells that produce IL-2 and IFN-γ, considering that β-adrenergic receptors have been detected on Th1 cells but not on Th2 cells that produce IL-10. B cells also express adrenergic receptors, and it is therefore possible that the effects of exercise on improved IgM production can be mediated by catecholamines. One study elegantly demonstrated that norepinephrine stimulation of the β2-adrenergic receptor is necessary for optimal IgM and IgG production in vivo (18). Others have demonstrated that adrenergic receptor blockade can blunt the immunomodulatory changes associated with acute exercise such as NK cell number (27), soluble intercellular adhesion molecule (34), or macrophage antiviral response (21). Chronic exercise training has been associated with a downregulation in the actual number of β-adrenergic receptors present on peripheral blood lymphocytes (6) and an enhancement of activity per receptor, as assessed by isoproterenol cAMP production (35). Taken together, there is evidence that immunomodulatory changes associated with exercise are mediated by catecholamines, yet the specific cellular pathways involving β-adrenergic receptors and how these interact with intracellular responses to antigen remain to be established.

Another interesting finding observed in these experiments is that the role of catecholamines as mediators of exercise immunomodulation occurs in the aged mice but not in the young mice. It is possible that catecholamines do modulate responses in young mice, but to a smaller degree than in older mice, or that the aged mice may have exercised at a greater relative percentage of maximal exercise than the young mice, resulting in greater circulating concentrations of catecholamines. The young mice reached the peak speed of 18 m/min by week 5 of the study, whereas the old mice did not reach this speed until week 8. Although the duration remained the same between groups, the young mice ran at a higher speed for the majority...
of the study in an attempt to control for relative percentage of maximal exercise in old compared with young mice. However, we cannot completely rule out the possibility that running at a different intensity contributed to the β-adrenergic effect seen in old, but not young, mice. It is also possible that the aged animal is more sensitive to neuroendocrine influences than the younger animal. For example, 2 mo of chronic β-adrenoceptor blockade in 18-mo-old mice altered the percentage of thymocyte subpopulations but had no effect in young mice (25). Aging also influences the ability of immune cells to respond to catecholamines. Macrophage chemotaxis is altered by norepinephrine in young mice, but this effect was not observed in older mice (30). In light of the evidence suggested by these other studies, it remains possible that the β-adrenergic adaptations to exercise training may vary by age. Finally, it is also possible that other neuroendocrine factors, such as endogenous opioids or central opioids (13, 15), may also have a role in modulating immune response during exercise training.

In summary, our results demonstrate that β-adrenergic receptor activation is involved in mediating the exercise training-associated increase in antibody production and IL-2 and IFN-γ kinetics in aged mice. These findings are important in terms of elucidating the mechanisms of neuroendocrine-immune adaptations that occur during exercise training. Although further research is required to identify specific intracellular mechanisms involved, we have determined that it may be fruitful to examine postadrenoceptor cellular signaling pathways in modulating immune responsiveness. In addition, our findings highlight the importance of age and exercise intensity as factors to be considered when neuroendocrine mechanisms of immunomodulation induced by exercise are evaluated.

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