Exercise-Induced Enhancement of Immune Function in the Rat

Janice C. Kaufman, MD; Tamara J. Harris, BA; Joseph Higgins, BA; Alan S. Maisel, MD

Background There have been many anecdotal reports that regular, moderate exercise confers some protective immunity against infection. There has been little scientific evidence to support this. It is also unclear whether training alters lymphocyte trafficking from the spleen to the periphery after a bout of exhaustive exercise. 

Methods and Results To determine the effect of moderate training on in vivo antibody production, using rats as an animal model, we gradually trained 18 rats using a swimming protocol for a 4-week period after injection and booster with Keyhole limpet hemocyanin antigen. There were 9 age-matched controls. At the conclusion of training, both groups underwent a short-term exhaustive swim. The trained group showed marked enhancement of IgM and IgG production. After short-term exercise, both groups had acute lymphocytosis, mainly T\textsubscript{helper} (trained), B cells, and the T\textsubscript{Th} to T\textsubscript{cyte} ratio. The changes in the splenocyte subsets were the opposite of the changes in the peripheral blood. With respect to function, after exhaustive exercise, there was a slight increase in mitogenesis and interleukin-2 receptor expression to concanavalin A (untrained more than trained) compared with controls.

Conclusions Regular, moderate training enhances antibody production to specific de novo antigen both early and late. In addition, short-term exercise leads to selective release of immune cells from the spleen and results in slightly enhanced function of splenocytes. Direct stimulation by the sympathetic nervous system and catecholamines is the proposed mechanism for the changes seen after short-term exercise and possibly antibody production during training. (Circulation. 1994;90:525-532.)

Key Words • antibodies • lymphocytes • splenocytes • exercise • immunity

The dampening effect of exhaustive physical exercise on the immune system has been well documented. 1-4 This is in agreement with numerous studies, in both humans and animals, that demonstrate that catecholamines generally suppress immune function. 5-7 Despite this seemingly “bad news” for people living during the fitness craze of the 1980s and 1990s, there have been many anecdotal reports extolling the virtues of regular exercise. Among the claims recreational athletes make is that exercise confers some protective immunity against infections, although there has been little scientific evidence to document this. 5 Most studies have used elite athletes, and the results are not generalizable to those who perform regular but only moderate degrees of exercise. 

Although animal models have been used effectively to investigate alterations in immunoregulatory cells and blastogenic responses in trained and untrained subjects, until recently there has been no way to study in vivo antibody production during the period of moderate training. We have developed an ELISA to measure IgG and IgM responses to a novel antigen in rats undergoing a moderate training protocol. This enabled us to study whether an animal undergoing training might have enhancement of antibody production in response to booster injections of this antigen. In addition, we sought to investigate whether training would alter lymphocyte trafficking from the spleen to the periphery after a final short-term, exhaustive bout of exercise, thereby affecting mitogenesis.

Methods

Rats

Thirty-six Sprague-Dawley, age-matched male rats were kept two to a cage in a climate-controlled area with 12-hour light-dark schedules and unlimited water and Purina rat chow. The rats were taken out only for swimming and bleeding.

Selection of Rats

Thirty-six rats were injected intraperitoneally with Keyhole limpet hemocyanin (KLH) (Calbiochem) antigen (10 µg/kg) on day 0. Subsequently, blood (about 300 µL) was withdrawn 2 days a week via tail veins; on day 13, the blood was analyzed by ELISA (see below) for antibody response. Nonresponders (a total of 9) were eliminated from the remainder of the study. The rats who responded to the antigen were randomly divided into two groups. Eighteen animals were trained; nine were left untrained.

Training

Our training protocol is well established and is considered equivalent to moderate training. 9,10 Training consisted of having the rats swim in a 22 × 31-in cylindrical container with a water depth of 24 in and temperature of 34°C. The rats were initially acclimated to the water by 15-minute swim intervals. Training times then were slowly increased to 2 hours over a 2.5-week period. The rats continued swimming for 2 hours a day for 5 days a week for an additional 1.5-week period. At the end of the training period, both the trained and the untrained rats were subjected to an exhaustive swim. The rats were killed immediately after exercise. After sedation with 100 mg/kg ketamine and 6.7 mg/kg xylazine, peripheral blood was removed by cardiac puncture, and the spleen was removed and retained for further study.

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age-matched controls that did not undergo the exhaustive swim were also killed as above.

**Antigen Exposure**

Both trained and sedentary rats were injected intraperitoneally with KLH antigen, a novel T-cell antigen (10 μg/kg; 0.3 mL) on day 0 and then received boosters on days 14 and 35. The dose and days for boosting were determined in preliminary studies in our laboratory. For the antibody analysis, approximately 0.3 mL of blood was removed via the tail veins 2 days a week, always before exercise. Samples were centrifuged, and the plasma was retained and diluted 1:10 with phosphate-buffered saline and Tween 20 with 0.05% sodium azide and then frozen for later ELISA analysis.

**ELISA**

Anti-KLH IgG and IgM antibody levels were quantified by ELISA. In this assay, a plasma sample was incubated for 4 hours in a 96-well plate coated with KLH. The serum was then washed from the plate, and an alkaline phosphatase–conjugated F(ab')₂ fragment goat anti-rat antibody against IgG and IgM (Fc fragment specific) was added to each well and incubated overnight. The plate was again washed, and p-nitrophenyl phosphate was added; the spectrophotometric reaction was measured 60 minutes later with an automatic plate reader at 405 to 410 nm. Samples from all three groups were assayed at the same time.

**Lymphocyte Preparation**

Immediately after collection, blood was placed into tubes containing 0.5 mol/L EDTA and diluted 1:4 with 1× PBS. Ficoll-Paque was subfused, and the resulting suspension was centrifuged at 1400 rpm for 60 minutes. Lymphocytes were collected at the Ficoll-PBS interface and washed three times at 1200 rpm for 15 minutes. Cells were resuspended in 1× PBS with 0.1% sodium azide to an approximate concentration of 15×10⁶ cells/mL. A separate tube of blood with EDTA was used for automated white blood cell count and differential count.

**Sterile Splenocyte Preparation**

Spleens were teased apart in 1× PBS using sterile technique. Ficoll-Paque was subfused, and the resulting suspension was centrifuged at 1600 rpm for 30 minutes. Splenocytes were collected at the Ficoll-PBS interface and washed twice at 1400 rpm for 15 minutes. Cells were resuspended in supplemented RPMI 1640 (20% fetal bovine serum, 2 mmol/L glutamine, and 25 μg/mL gentamicin). Cells were counted on a hemacytometer in Trypan blue to ensure viability.

**Mitogen-Stimulated Cell Proliferation and [³H]Thymidine Uptake**

Isolated under sterile conditions, splenocytes were resuspended in supplemented RPMI 1640 and placed in 96-well flat-bottom plates at a final concentration of 2×10⁴ cells/mL. Additional media with or without Concanavalin A (Con A) at 0.5, 1, 2, 4, or 8 μg/mL were added to give a final volume of 210 μL per well. Cells were incubated for 4 days in a 37°C humidified incubator with 95% O₂-5% CO₂. During the final 16 hours of incubation, 2 μCi of [³H]thymidine was added to each well. At the end of the incubation, cells were harvested over GF/C filter paper using a Cell Harvester (Brandel). The incorporated radioactivity was quantified in a liquid scintillation counter using 4.5 mL of Ecoscint (Natural Diagnostics). Each data point was assessed in quadruplicate in each experiment.

**Immunofluorescent Lymphocyte Subtyping**

Both direct and indirect immunofluorescence was required to label the different subpopulations. Splenocytes and PBL mononuclear leukocytes were diluted to a concentration of 15×10⁶ cells/mL with 1× PBS plus 0.1% sodium azide. Then, 50 μL of cells was added to 20 μL of FITC-conjugated mouse anti-CD monoclonal antibodies to either T total (W3/13), T<sub>suppressor/cytotoxic</sub> (MRC OX-8), T<sub>helper</sub> (W3/25), or natural killer cells (CD16), with mouse IgG1-FITC conjugate as a control. Indirect staining was required for the detection of the B-cell population. The primary antibody was mouse anti-rat L-CA present on B lymphocytes (MRC OX-33). Mouse IgG1 purified served as a control. The secondary antibody was F(ab')₂ rabbit anti-mouse IgG-FITC conjugate. Cells were incubated in the dark on ice for 30 minutes and washed twice at 1600 rpm for 10 minutes. The cells were fixed with 1% formaldehyde and refrigerated until analyzed. Immunofluorescence of the tagged cells was measured using a flow cytometer (model 50-F; Ortho Cytofluorograf).

**Interleukin-2 Receptor Expression**

Splenocytes isolated using sterile technique were suspended in 10 mL supplemented RPMI medium at a final concentration of 1.4×10⁶ cells/mL in the absence and presence of Con A. After 72 hours, the cells were centrifuged, and the expression of interleukin-2 (IL-2) receptors was determined fluorometrically with anti-CD-25 antibodies. For catecholamine determination, catecholamines were determined from plasma immediately after the final exhaustive swim. They were measured by the radioenzymatic method of Durrett and Ziegler.

**Data Analysis**

Values are given as mean±SEM. The significance of differences among the groups was assessed using ANOVA with Bonferroni corrections.

**Results**

**Exercise Data**

At the end of the training period, both untrained and trained groups of rats underwent an exhaustive swim before they were killed. The average time of the untrained group in the pool was 30 to 90 minutes, and the average time for the trained group was 120 to 180 minutes. The animals were killed immediately after exercise as described above. Catecholamines are listed in Table 1. Trained animals had lower epinephrine levels after acute exhaustive exercise compared with control and untrained animals.

<table>
<thead>
<tr>
<th>WBC</th>
<th>MNL</th>
<th>%MNL</th>
<th>T&lt;sub&gt;1&lt;/sub&gt;</th>
<th>T&lt;sub&gt;av&lt;/sub&gt;</th>
<th>T&lt;sub&gt;b&lt;/sub&gt;</th>
<th>NK</th>
<th>B</th>
<th>T&lt;sub&gt;b&lt;/sub&gt;-to-T&lt;sub&gt;av&lt;/sub&gt; Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6050±848</td>
<td>4395±363</td>
<td>76±5</td>
<td>2954±295</td>
<td>1314±192</td>
<td>2199±181</td>
<td>239±28</td>
<td>478±116</td>
</tr>
<tr>
<td>Sedentary</td>
<td>6844±480</td>
<td>5643±441</td>
<td>82±2</td>
<td>4258±311</td>
<td>1991±218</td>
<td>3030±212</td>
<td>512±66</td>
<td>286±43</td>
</tr>
<tr>
<td>Trained</td>
<td>5962±418</td>
<td>5017±362</td>
<td>84±2</td>
<td>3498±320</td>
<td>1887±192</td>
<td>2332±179</td>
<td>623±58</td>
<td>299±23</td>
</tr>
</tbody>
</table>

WBC indicates white blood cell count; MNL, mononuclear leukocytes; T<sub>1</sub>, T cells; T<sub>av</sub>, T<sub>suppressor/cytotoxic</sub> Cells; T<sub>b</sub>, T<sub>helper</sub> Cells; NK, natural killer cells; and B, B cells. Values are given as mean±SEM, pg/mL.
**Peripheral Lymphocyte Subsets**

Absolute numbers of total white blood cells and mononuclear leukocytes are given in Table 2. Dynamic exercise led to minimal leukocytosis in the untrained group only; this trend was mitigated in the trained animals. There was an increase in the absolute number and percentage of mononuclear leukocytes in both groups (trained and untrained). Subsets are presented as a percentage of the total mononuclear leukocytes in Figs 1 through 5. There was a substantial release of $T_{\text{suppressor/cytotoxic}}$ cells into the circulation after short-term exercise in both untrained and trained groups compared with controls (Fig 1, $P=.04$). The $T_{\text{helper}}$ cells showed a small, insignificant increase in the untrained rats but a moderate decrease in the trained rats compared with controls (Fig 2, $P=.01$). Thus, there was a 33% decrease in the $T_{\text{helper}}$-$T_{\text{suppressor/cytotoxic}}$ ratio ($T_{h}/T_{vo}$) in the trained group compared with controls (Fig 3, $P<.037$ two-tailed $t$ test). The most dramatic changes occurred in the natural killer cell population. Although both untrained and trained groups showed an increase in circulating natural killer cells after exhaustive exercise, the trained group had 2.5 times more cells than controls (Fig 4, $P<.001$ by Bonferroni). The percentage of B cells uniformly decreased in both trained and untrained

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**TABLE 2.** White Blood Cell Count (mm$^3$), Lymphocyte Count (MNL) (mm$^3$), Percent of Total Lymphocytes, and Absolute Numbers of $T$ Cells, $T_{\text{suppressor/cytotoxic}}$ Cells, $T_{\text{helper}}$ Cells, Natural Killer Cells, and B Cells in Control Rats and in Untrained and Trained Rats After a Bout of Short-term, Exhaustive Exercise

<table>
<thead>
<tr>
<th>Condition</th>
<th>Dopamine, pg/mL</th>
<th>Epinephrine, pg/mL</th>
<th>Norepinephrine, pg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untrained</td>
<td>201±48</td>
<td>738±413</td>
<td>698±132</td>
</tr>
<tr>
<td>Trained</td>
<td>286±35</td>
<td>287±92</td>
<td>633±60</td>
</tr>
</tbody>
</table>

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**Fig 2.** Bar graphs showing percent $T_{\text{helper}}$ cells in the blood (a) and the spleen (b) after short-term exercise for trained (n=13) and untrained (n=9) rats as well as nonexercised controls (n=6) (a: $*P<.05$ Bonferroni, control vs trained; b: $P<.05$ ANOVA). Values are given as percent of total mononuclear cells (mean±SEM).

**Fig 3.** Bar graphs showing ratio of $T_{\text{helper}}$ cells to $T_{\text{suppressor}}$ in the blood (a) and spleen (b) after short-term exercise for trained (n=13) and untrained (n=9) rats as well as nonexercised controls (n=6) (a: $*P<.05$ uncorrected, control vs trained; b: $*P<.05$ uncorrected, control vs control. Values are given as the mean±SEM of the ratio.
groups, with the most dramatic results found in the untrained group (P<.005, uncorrected).

**Splenocyte Subsets**

As illustrated in Figs 1 through 5, changes in the subsets of the splenocytes are the opposite of the changes in the peripheral blood, suggesting cellular trafficking between the spleen and the circulation. Again, the most dramatic change was in the decrease in splenic natural killer cells in the trained group (Fig 4, P<.005 Bonferroni). Likewise, there was a decrease in the percentage of splenic Tsuppressor/cytotoxic cells (Fig 1, P<.005), whereas the percentage of splenic Thelper and B-cell populations increased in the trained group (Figs 2 and 5, P=.05 and .07, respectively). The untrained group showed less of a decrease in Tsuppressor/cytotoxic cells and natural killer cells and an increase in T-helper cells. The Tc-to-Te ratio increased in the spleen in both trained and sedentary groups (Fig 3, P=.096, control versus trained).

**Con A Mitogenesis and IL-2 Receptor Expression**

Tritiated thymidine uptake after exposure to the T-cell mitogen Con A was used as a measure of splenic lymphocyte proliferation. Basal response, with no mitogen added, was 4% to 8% of maximal response (data not shown). There was a slight increase in Con A-induced [3H]thymidine uptake over the entire dose range of Con A in both the untrained and trained groups after exhaustive exercise compared with controls (Fig 6).

**Discussion**

**Short-Term Exhaustive Exercise—Effects of Training**

Many studies have documented that short-term exhaustive exercise results in alterations in lymphocyte populations in the peripheral blood. These shifts include an acute lymphocytosis with increases in Tsuppressor/cytotoxic cells and natural killer cells and a relative decrease in Thelper cells, resulting in a decreased Tc-to-Te ratio. We and
others have postulated that these effects are tantamount to a dampening of the immune system and correspond to the results of short-term and long-term catecholamine infusions.34,20-24

Data concerning the effects of moderate training on immune function are more scarce. Long-term endurance training followed by a short-term, exhaustive bout of exercise has been shown to cause a transient immunosuppression of lymphocyte mitogenesis.2,15 Light-to-moderate training is believed to confer some resistance to the immunosuppressive effects of a stressful event such as short-term, exhaustive exercise.

Movement of immunocompetent cells from one compartment to another may have important implications with subsequent roles in immune modulation after exercise. T cells are responsible for cell-mediated immunity to viruses and parasites as well as for delayed hypersensitivity responses.25 CD8+ cells (T

activities of lymphocytes against viruses and graft-versus-host reactions. The CD4+ cells (T

Fig 6. a, Line graph showing concanavalin A (Con A)-induced uptake of [3H]thymidine at concentrations ranging from 0.5 to 8 μg/mL as a measure of splenocyte proliferation immediately after short-term exercise in trained (n=13) and untrained (n=9) rats as well as nonexercised controls (n=6). Values are given as mean±SEM. b, Bar graph showing maximum minus basal response of splenocytes to Con A representing peak response of splenocyte proliferation. Values are given as mean±SEM. c, Bar graph showing the stimulation index: maximum/basal response of splenocytes to Con A. Values are given as mean±SEM.

thel), and delayed hypersensitivity reactions (Th2).26 We and others have shown that short-term exercise in humans produces shifts in lymphocyte populations, predominantly T

Fig 7. Bar graph showing percent interleukin-2 (II2) receptor expression in splenocytes after exposure to Concanavalin A after short-term exercise in trained (n=13) and untrained (n=9) rats as well as nonexercised controls (n=6) as a measure of splenocyte activation (P<.05 Bonferroni, control vs untrained and trained vs untrained). Values are given as mean±SEM.

Fig 8. Plot of IgG response to Keyhole limpet hemocyanin (KLH) antigen measured using the ELISA technique. KLH was initially injected on day 0 (*), with subsequent booster injections on day 14 (**) and day 35 (**). Training was started on day 14 (**) and continued through day 42. Values are given as mean absorbance 405 to 410 nm times 1000±SEM.
leases lymphocytes via a β1-adrenergic mechanism and may be mitigated by treatment with nonselective β antagonists.3

Our findings that short-term exercise enhances mitogenesis and II-2 receptor expression in both the untrained and trained groups is in agreement with some studies15,21,31 but not others.1-4 The two major factors that account for the discrepancy are the intensity and duration of the exercise14,32 and which cells are used to test blast transformation. In peripheral blood, when short-term exercise led to a large increase in T suppressor/cytotoxic cells, we found a dampening of blast transformation.3 In the present study, splenic lymphocytes, partially depleted of these suppressor cells, were incubated with mitogen, and blastogenesis increased. Therefore, the milieu in which the cells are exposed to the mitogen appears to be very important in evaluating T-cell function.35

Effects of Exercise Training on In Vivo Antibody Production

The effect of moderate training on antibody formation to a specific antigen has not been well studied. Eskola et al1 showed at the booster antibody response in marathon runners 2 weeks after a marathon during normal training regimens. Although their results showed that the antibody levels in the individual runners were greater than those in controls, their study was limited by a small number of subjects (four) as well as by poor baseline characteristics.1 Liu and Wang34 studied the in vivo response of mice to Salmonella typhi during training and found that the trained mice had elevated levels of general, nonspecific immunoglobulins compared with the untrained mice. Douglass35 studied swimming mice and their in vivo response to diptheria tetanus toxoid and found no increase in the primary immune response but a substantial increase in antibody production to subsequent booster immunizations in the trained group.

Our study is the first to examine in depth the effect of training on in vivo antibody production to a de novo antigen. We were able to examine these results in the setting of both cell-mediated and humoral immunity at the same time. We boosted the animals twice, just as they were beginning their training and at the completion of a stabilized period of training. Our results demonstrated an increase in anti-KLH antibodies in the group undergoing exercise training, both early and late.

The discrepancy between the IgM and IgG responses in our study can be explained in part by a difference in the catabolic kinetics inherent to these two types of immunoglobulins. IgM is the acute, rapid responder to the initial exposure to an antigen in the immune response. It has a relatively short half-life, which means its responses are more dramatic and evident during shorter time periods. The catabolic rate of IgM is completely independent of its concentration in the serum. The catabolic rate for IgG, however, is dependent on its concentration in serum. The higher the concentration, the faster IgG is catabolized. Its half-life can decrease from the usual 23 days to as little as 10 days. The concentration-dependent kinetics of IgG make it more difficult to accurately measure the absolute magnitude of the response. It also has a long half-life, so differences become evident only in longer time periods.36 Other investigators have found enhancement of serum immunoglobulins with exercise that have correlated this change to fewer respiratory tract infection days.8,37 Eskola et al1 showed that a tetanus booster given to marathon runners induced a clear-cut increase in the specific antitoxin concentration of the runners while causing only a transient effect of lymphocyte transformation. This suggests that it is possible to effect a specific clone of B cells if presented with a specific antigen without influencing polyclonal activation to any great degree. However, other studies have reported normal levels of IgG, IgA, and IgM in marathon runners.38,39 Alternative hypotheses include changes in IL-2–activated T cells and in vitro IL-1 production,40 alterations in natural killer cell function,41 and finally exercise-induced hydrocortisone release that enhances epinephrine-induced stimulation of immunoglobulin synthesis in vitro.42

The mechanism behind the increases in secondary antibody response in the trained population is not clear. One possible explanation for this is that the shifts in lymphocyte subtypes after exercise provide a better environment for antibody response. Specifically, the increase in B cells and the higher Tα-Tβ ratio in the spleen after acute exercise is believed to be favorable for antibody production.37 Antibody production requires the coordination of B cells, macrophages, and T-helper cells. T-helper cells secrete lymphokines that stimulate B cells to synthesize immunoglobulins to particular antigens that have been presented to them and the T-helper cells by the macrophages or other antigen-presenting cells.26 In vivo studies have proposed that epinephrine can accelerate the early phase of antibody synthesis and may be mediated through the β2-adrenergic receptor activation of adenylate cyclase and cyclic AMP.5-7,37,39 If epinephrine is given in a later phase of the immune response, there is an inhibition of antibody synthesis.20 It is documented that epinephrine
and norepinephrine levels rise during and after short-term exercise and that they are the mediators behind the shifts in lymphocyte subsets.\textsuperscript{7,18,38,40-43} It is not clear how a training regimen would affect antibody production.

What are the clinical implications of the present study? Anecdotal tales of the positive effect of exercise on the body and immune function are common. People who train regularly insist that their training confers some resistance to infection as well as an enhanced feeling of physical well-being.\textsuperscript{38,43} Scientific evidence has shown that regular exercise enhances cardiovascular parameters. It is well documented that training lowers resting heart rate, increases stroke volume, and thus decreases the efficiency of the heart as a pump.\textsuperscript{34} However, there has been little direct scientific evidence that exercise enhances immune function. In fact, studies have shown that overtraining can actually suppress immune function.\textsuperscript{11,15,21,34} Our study is unique in that it considers the effect of regular, moderate training on antibody response to novel antigen KLH, as well as on lymphocyte trafficking and T-cell function. Our results showed that regular exercise enhances immune function, as evidenced by the increases in IgM and IgG responses in the trained group and by the increase in mitogenesis and II-2 receptor expression in both the untrained and trained group immediately after exercise. In addition to the cardiovascular benefits of a regular exercise program, the possibility that routine exercise may augment immune function, perhaps protecting humans from infection or other immune-associated diseases, is an exciting prospect. Further investigation should be geared to a number of areas. One could look at changes in antibody production over longer periods of different levels of training while giving different numbers of repeated boosts of the foreign antigen. This would also help to determine whether one reaches a point where too much exercise training is harmful to the immune system. In addition, because it is clear that the sympathetic nervous system plays an important role in both the movement of immune cells from the spleen to the periphery as well as in antibody production, it would be of interest to repeat a similar set of experiments using either β-blockade or splenectomy as a way of blocking cell movement.

References


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