Sympathetic and Immune Interactions During Dynamic Exercise
Mediation Via a $\beta_2$-Adrenergic-Dependent Mechanism

David R. Murray, MD; Michael Irwin, MD; C. Ann Rearden, MD; Michael Ziegler, MD; Harvey Motulsky, MD; and Alan S. Maisel, MD

Background. The relation between the sympathetic nervous system and the immune system has not been fully defined. Recent investigations have suggested an adrenergically driven efflux of specific $\beta_2$-receptor-rich lymphocyte subsets into the circulation with either exercise or infusion of exogenous catecholamines.

Methods and Results. To determine whether acute sympathetic stimulation mediates immunoregulatory cell traffic and function via a $\beta_2$-receptor mechanism, we exercised 20 healthy volunteers before and after 1 week of treatment with either the nonselective $\beta$-antagonist propranolol or the $\beta_1$-selective antagonist metoprolol. Before treatment, exhaustive exercise according to the Bruce protocol led to a marked lymphocytosis. $T_{\text{suppressor/cytotoxic}}$ ($T_{\text{sc}}$) and natural killer cells, subtypes with the largest density of $\beta$-receptors, showed the most pronounced increases after exercise, with less impressive elevations in $T_{\text{helper}}$ and B cells. With respect to function, exhaustive exercise led to a decrease in concanavalin A–stimulated IL-2 receptor expression and $[^3H]$thymidine incorporation while enhancing natural killer cell activity. One week of propranolol therapy blunted the exercise-induced increases in circulating $T_{sc}$ and natural killer subpopulations as well as the previously observed alterations in cellular immune function. Treatment with the $\beta_1$-selective antagonist metoprolol, however, did not impair the influence of exercise on any of the above parameters.

Conclusions. Acute sympathetic stimulation by exhaustive exercise leads to selective release of immunoregulatory cells into the circulation with subsequent alterations in cellular immune function, either secondary to subset changes or as a result of direct catecholamine effects on function. These changes are attenuated by propranolol but not metoprolol, suggesting a $\beta_2$-mediated mechanism.

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Key Words • nervous system, sympathetic • mitogens • lymphocytes • receptors, IL-2

Growing evidence suggests that immune function is regulated in part by the sympathetic nervous system. Sympathetic nerve endings densely innervate lymphoid tissues such as spleen, lymph nodes, and thymus, and lymphoid cells have $\beta$-adrenergic receptors.1,2 We and other investigators have previously shown that acute sympathetic stimulation via dynamic exercise leads to changes in circulating lymphocyte subsets, including a sharp rise in the $T_{\text{suppressor/cytotoxic}}$ ($T_{sc}$) and natural killer cells but only a modest increase in $T_{\text{helper}}$ ($T_b$) cells.3–5 Numerous in vitro studies have shown that $\beta$-adrenergic agonists can inhibit the activation and proliferation of lymphocytes and cytotoxicity of natural killer cells.6–8 To determine whether acute sympathetic stimulation mediates immunoregulatory cell traffic and function via a $\beta_2$-receptor mechanism, we exercised 20 healthy volunteers before and after 7 days of treatment with either propranolol (nonselective $\beta_1$/$\beta_2$-blocker) or metoprolol (selective $\beta_1$-blocker). Specifically, we examined the effects of exercise and differential $\beta$-blockade on the number and subset distribution of circulating lymphocytes as well as functional parameters of cellular immunity such as mitogen-stimulated lymphocyte proliferation, natural killer cell activity, and interleukin-2 (IL-2) receptor expression.

Methods

Subjects

Twenty healthy volunteers (18 men, 2 women; age range, 24–43 years) without history of hypertension, diabetes, or coronary artery disease participated in the study after having given informed consent. The study protocol was approved by the University of California committee for investigations involving human subjects.

Protocol

Fasting subjects lay supine for 20 minutes, and a venous blood sample was drawn. They then exercised to exhaustion on a treadmill according to the Bruce protocol. On completion of the treadmill test, the subjects...
immediately resumed the supine position, and another venous sample was drawn. A final sample was drawn 1 hour after completion of the treadmill. Twelve ECG leads were recorded during exercise and for 6 minutes afterward, and neither ST segment changes nor treadmill-induced angina was evidenced in any subject. A mercury sphygmomanometer and stethoscope were used at 3-minute intervals during and after exercise to measure arterial blood pressure. The double product (product of systolic blood pressure and heart rate) was calculated for each participant as a measure of cardiac output. Twelve of the volunteers were subsequently given the nonselective β-blocker propranolol (40 mg t.i.d.), and the remaining eight subjects took the β-selective antagonist metoprolol (50 mg b.i.d.). After 1 week of treatment, they were again subjected to treadmill testing until exhaustion according to the above protocol with similar venous blood draws before, upon completion, and 1 hour after exercise.

Mononuclear Cell Isolation

Venous blood was placed in tubes containing heparin, and an equal volume of RPMI medium was added.

| Table 1. Antibodies Used for Identification of Lymphocyte Subsets |
|---------------------|-------------------|------------------|
| Cluster | Antibody | Isotype | Distribution |
| CD3 | Anti-leu 4 | IgGk | Mature T lymphocytes |
| CD4 | Anti-leu 3a | IgGk | Helper, inducer T lymphocytes |
| CD8 | Anti-leu 2a | IgGk | Suppressor/cytotoxic lymphocytes |
| CD19 | Anti-leu 12 | IgGk | B lymphocytes |
| CD16 | Anti-leu 11 | IgGk | IgG Fc receptors on natural killer cells and neutrophils |
| CD57 | Anti-leu 7 | IgMk | T lymphocytes and NK subsets |
| CD56 | Anti-leu 19 | IgGk | NK cells, cytotoxic T cell subsets |

Figure 1. Graphs showing circulating epinephrine (epi) (panel A) and norepinephrine (N-epi) (panel B) both before and after 1 week of treatment with either propranolol (panel A) or metoprolol (panel B). Exhaustive exercise led to marked lymphocytosis (p<0.0001, paired t test, n=20). Propranolol blunted exercise-induced lymphocytosis (p<0.01, paired t test), whereas metoprolol had no effect. Values are given as mean±SEM of 12 individuals in the propranolol group and eight individuals in the metoprolol group.

Figure 2. Graphs showing circulating lymphocytes (cells/mm³) both before and after 1 week of treatment with either propranolol (panel A) or metoprolol (panel B). Exhaustive exercise led to marked lymphocytosis (p<0.0001, paired t test, n=20). Propranolol blunted exercise-induced lymphocytosis (p<0.01, paired t test), whereas metoprolol had no effect. Values are given as mean±SEM of 12 individuals in the propranolol group and eight individuals in the metoprolol group.
Mononuclear leukocytes (MNLs) were isolated over a Ficoll-Hypaque gradient and washed according to previous methods. More than 90% of these cells were viable, as assessed by trypan blue exclusion. A separate tube of blood with EDTA was used for automated white blood cell count and differential.

**Flow Cytometric Analysis of MNLs**

Lymphocyte subsets were detected by mouse monoclonal antibodies (Becton Dickinson, Mountain View, Calif.) conjugated directly with either fluorescein or phycoerythrin. Antibodies used for identification of lymphocyte subsets are shown in Table 1.

Twenty microliters of monoclonal antibody reagent was reacted with 50 μl of MNLs (10⁶ cells) in 12×75-mm tubes for 30 minutes in an ice-cold water bath. Cells were washed twice with phosphate-buffered saline supplemented with 0.1% sodium azide and finally resuspended in 1 ml of phosphate-buffered saline supplemented with 0.05% paraformaldehyde. Cells were stored at −20°C until analyzed.

Immunofluorescence was measured with a flow cytometer (FAC-Scan, Becton Dickinson) equipped with a 15-mW argon-ion laser and interfaced with a model 310 computer (Hewlett-Packard Co., Palo Alto, Calif.). Data analysis was performed with the Consort 30 Data Management program supplied by the manufacturer.

Five thousand cells were analyzed per sample. Electronic “gating” of the lymphocyte population was based on forward and side-scatter parameters. Percent positive fluorescence of the gated population was determined relative to the fluorescence of the negative control cells. The relative proportion of each subset was obtained as a percentage of the total lymphocytes counted, and the absolute number in each subset was calculated by multiplying the percentage of each subset by the absolute lymphocyte count derived from the white blood cells and differential count.

**Concanavalin A–Stimulated Proliferation**

Sterile isolated MNLs were resuspended in RPMI medium supplemented with 25 μg/ml gentamicin, 2 mM glutamine, and 20% fetal calf serum at a final density of 10⁶ cells/ml. Cell suspension (200 μl) was transferred to each well of 96-well flat-bottom plates. In some wells, concanavalin A (Con A) (Sigma, St. Louis, Mo.), 4.5, 9, 18, or 36 μg/ml (final concentration), was present. Cells were incubated for 4 days in a humidified incubator with 95% air/5% CO₂. During the last 16 hours of the incubation, 2 μCi of [³H]thymidine dissolved in 20 μl of RPMI was added to each well. At the end of the incubation, the cells were harvested over GFC filters. The filters were washed with 10 ml of water and placed into vials, and 4.5 ml of Liquiscint (National Diagnost-
FIGURE 4. Graphs showing numbers of circulating B cells before, immediately after, and 1 hour after exercise both before and after 1 week of treatment with either propranolol (prop) (panel A) or metoprolol (metop) (panel B). Graphs in panels C and D portray the percentages of circulating B cells per total lymphocytes under similar conditions. Exercise led to a significant increase in circulating B cells (p<0.0001, paired t test, n=18). Although the absolute numbers of circulating B cells are not affected by either propranolol or metoprolol, the decrease in percent B cells per total lymphocytes after exercise is significantly blunted in the propranolol group (p<0.05, paired t test). Values are given as mean±SEM of 10 individuals in the propranolol group and eight individuals in the metoprolol group.

IL-2 Receptor Expression
Sterile isolated MNLs were suspended in 10 ml supplemented RPMI medium (see above) in the absence and presence of Con A (18 μg/ml). After 48, 72, and 96 hours, the cells were centrifuged, and the expression of IL-2 receptors was determined fluorometrically with anti-CD-25 antibodies.

Natural Killer Cell Activity
Cells were prepared as described above and were suspended in RPMI with 10% fetal calf serum at a concentration of 2×10^6 cells per milliliter and incubated with ^51Cr-labeled K-562 target cells in triplicate at effector-to-target (E:T) cell ratios of 40:1, 20:1, 10:1, and 5:1. After 3 hours of incubation in a 37°C incubator with 5% CO₂, the plate was removed and spun at 200g. An aliquot (100 μl) was removed, and ^51Cr release was measured in a gamma counter. Assay results for natural killer cell activity were expressed as lytic units.

Miscellaneous
Plasma catecholamines were measured radioenzymatically by the method of Durrett and Ziegler.

Data Analysis
Data shown are mean±SEM. The significance of differences between groups was assessed by unpaired or paired t tests without correction for multiple comparisons. We report two-tailed probability values unless otherwise noted.

Results

Exercise Data
All subjects were exercised to exhaustion before and 1 week after administration of propranolol (40 mg t.i.d.) or metoprolol (50 mg b.i.d.). As can be seen in Table 2, baseline exercise studies were similar in both groups. After 1 week of treatment with either medication, subjects did not suffer any impairment in their exercise capacity. As was expected, heart rate and blood pressure response were blunted similarly in both groups after treatment with medication.
toward baseline 1 hour after exercise (Figure 1). Treatment with metoprolol or propranolol did not lead to alteration in either basal or exercise-stimulated catecholamine levels.

Changes in Lymphocyte Subsets

Dynamic exhaustive exercise led to marked leukocytosis and lymphocytosis, manifested predominantly by a striking increase in circulating $T_{\text{cv}}$ and natural killer cells, with only a modest increase in $T_{\text{s}}$ and B cells (Figures 2–6). The total number and distribution of lymphocytes returned to baseline 1 hour after exercise. As a consequence of this altered distribution of lymphocyte subsets immediately after exercise, the $T_{\text{helper}}/T_{\text{suppressor}}$ ratio dropped dramatically (Figure 6). These changes occurred in each subject.

Treatment with the nonselective $\beta$-blocker propranolol attenuated exercise-induced increases in total lymphocyte number, circulating $T_{\text{cv}}$, and natural killer cells but did not alter levels at baseline or 1 hour into recovery (Figures 2, 3, and 5, left panel). Unlike its effect on $T_{\text{cv}}$ and natural killer cells, propranolol treatment increased baseline circulating levels of the CD4 $T_{\text{s}}$ subtype slightly, though significantly ($p=0.026$), consistent with previous results13 (Figure 3, left panel). Although the absolute number of circulating CD4 cells immediately after exercise was not affected by treatment with propranolol, the increase in circulating $T_{\text{s}}$ cells above baseline after exercise was significantly different (271 cells/mm$^3$ increase after exercise with propranolol versus 445 cells/mm$^3$ without propranolol) (Figure 3, left panel; Figure 7). Because propranolol limited migration of $T_{\text{cv}}$ to a greater extent than that of $T_{\text{s}}$ cells, exercise-induced decreases in the $T_{\text{helper}}/T_{\text{suppressor}}$ ratio were not nearly as dramatic (Figure 6, left panel). Propranolol treatment did not alter the increase in B cell number with exercise (Figure 4). However, because propranolol reduced the exercise-induced increase in $T_{\text{cv}}$ and natural killer cells, the exercise-induced decrease in the percentage of circulating B cells was significantly blunted (Figure 4).

Contrary to the results with propranolol, treatment with the selective $\beta_1$-adrenergic blocking agent metoprolol did not consistently attenuate exercise-induced alterations in circulating lymphocytes. In fact, pretreatment with metoprolol appeared to have the opposite effect to that of propranolol; that is, it enhanced exercise-induced increases in circulating lymphocytes (Figures 2–6, right panels). Figure 7 summarizes the effects of $\beta$-blockers on exercise-induced increases in

**Catecholamines**

Epinephrine and norepinephrine increased more than fivefold immediately after exercise and returned

### Table 2. Exercise Data

<table>
<thead>
<tr>
<th>Exercise time</th>
<th>Metabolic equivalents (O$_2$ ml/kg)</th>
<th>Heart rate maximum (bpm)</th>
<th>Heart rate (% target)</th>
<th>Blood pressure maximum systolic (mm Hg)</th>
<th>Blood pressure maximum diastolic (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before propranolol</td>
<td>16 Minutes, 57 Seconds</td>
<td>17.2±0.6</td>
<td>187±4</td>
<td>98±2</td>
<td>184±5</td>
</tr>
<tr>
<td>After propranolol</td>
<td>15 Minutes, 55 seconds</td>
<td>17±0.7</td>
<td>156±6</td>
<td>79±2</td>
<td>178±8</td>
</tr>
<tr>
<td>Before metoprolol</td>
<td>17 Minutes, 33 seconds</td>
<td>18.1±0.7</td>
<td>187±2.4</td>
<td>98±1.3</td>
<td>194±7</td>
</tr>
<tr>
<td>After metoprolol</td>
<td>17 Minutes, 3 seconds</td>
<td>18.1±0.7</td>
<td>157±3</td>
<td>82±2</td>
<td>174±8</td>
</tr>
</tbody>
</table>

bpm, Beats per minute. Values are mean±SEM.
Figure 6. Graphs showing T_{helper}/T_{suppressor/cytotoxic} (CD4/CD8) ratio in subjects before, immediately after, and 1 hour after exercise both before and after 1 week of treatment with either propranolol (panel A) or metoprolol (panel B). Exhaustive exercise led to a dramatic drop in the CD4/CD8 ratio with normalization into recovery (p<0.001, n=20, paired t test). Propranolol treatment significantly blunted (p<0.05) this exercise-induced decrease in the helper/Suppressor ratio. Values are given as mean±SEM of 12 individuals in the propranolol group and eight individuals in the metoprolol group.

Natural Killer Cell Activity

Natural killer cell activity was determined before, immediately after, and 1 hour after exercise using four E:T ratios. Figure 8 demonstrates lytic activity at an E:T ratio of 20:1. Results were similar for other target cell ratios. The increase in lytic activity seen immediately after exercise was significantly blunted when patients were pretreated with propranolol (p<0.01) but not with metoprolol.

Con A-Induced Lymphocyte [3H]Thymidine Uptake

Con A–induced [3H]thymidine uptake was assayed as a measure of cellular proliferation. Basal cellular proliferation (no mitogen), generally averaging less than 3% of peak Con A–stimulated values, did not change after exercise and was unaffected by treatment with either β-blocker drug (data not shown). Figure 9 shows the effects of propranolol and metoprolol on peak thymidine uptake after exposure of lymphocytes to the T cell mitogen Con A. Exercise attenuated Con A–stimulated peak [3H]thymidine incorporation in each of 20 subjects. The mean reduction was 21.6±0.5% (p<0.0001 by paired t test). The effect of the β-blockade on exercise-induced decrements in lymphocyte proliferation, on the other hand, was not consistent with every individual. Treatment with propranolol blunted the inhibitory effects of exercise in eight of 12 subjects. Compared with baseline postexercise values, propranolol therapy led to a 24% increase in [3H]thymidine uptake. This finding was consistent at several doses of Con A, as shown in Figure 10. Conversely, metoprolol treatment did not significantly affect lymphocyte proliferation, though there was a tendency for reduced [3H]thymidine incorporation before, immediately after, and 1 hour after exercise.
FIGURE 8. Graphs showing natural killer cell activity in subjects before, immediately after, and 1 hour after exercise both before and after 1 week of treatment with either propranolol (panel A) or metoprolol (panel B). Exhaustive exercise enhanced natural killer cell (NK) activity (p<0.001, paired t test, n=10). The increase in lytic activity seen immediately after exercise was significantly blunted when patients were pretreated with propranolol (p<0.01) but not metoprolol. Data are mean±SEM of five individuals in each treatment group.

**IL-2 Receptor Expression**

Exercise reduced Con A–stimulated IL-2 receptor expression in 14 of 19 subjects by an average of 8.7±3.4% (p=0.02 by paired t test). Figure 11 shows the effect of propranolol and metoprolol treatment on IL-2 receptor expression. Propranolol attenuated this exercise-associated decrement in IL-2 receptor expression (p<0.05, paired t test). Metoprolol again had no effect. No changes were seen in the preexercise and 1 hour postexercise IL-2 receptor studies after β-blockade with either drug. These data were collected after 3 days of mitogen exposure; similar results were obtained after 2 and 4 days.

**Discussion**

Considerable evidence suggests that the immune system, like other major organ systems in the body, is regulated by hormones and neurotransmitters. Peripheral lymphoid organs such as spleen and lymph nodes are densely innervated by adrenergic fibers in direct contact with pools of lymphocytes. Lymphocytes have well-characterized homogeneous β-receptors that couple to adenylate cyclase, leading to production of cyclic AMP (cAMP). Interestingly, lymphocyte subsets have
been shown to have varying densities of β2-receptors. HLA class I restricted CD-8 T lymphocytes (suppressor and cytotoxic cells) have 2–3 times more functional β2-receptors than do class II restricted CD4 T cells.3,14–16 B cells and monocytes also have many β2-receptors.3,15,16 Natural killer cells have the greatest density of β2-receptors among the various subsets, nearly 50% more than Tc1 cells.3,16 The function of these lymphocyte β2-receptors is not clear. Several in vitro studies have suggested that β-adrenergic stimulation of isolated lymphocytes dampens immune performance. Treatment of isolated human lymphocytes with the β-adrenergic agonist isoproterenol (and other agents that elevate intracellular cAMP concentration) has been shown to reduce mitogen-induced increases in intracellular inositol-(1,4,5)-triphosphate and calcium, plant lectin–induced [3H]thymidine incorporation, pro-

production of IL-2 and interferon, expression of IL-2 receptors, IgE-mediated histamine and slow-reacting substance of anaphylaxis release, as well as IL-1 production by monocytes.5,7,17 Patients with congestive heart failure, known to have markedly elevated circulating catecholamines, have altered natural killer and suppressor cell activity.18,19

Lymphocyte traffic from peripheral lymphoid organs into the circulation may also be mediated via the adrenergic system through lymphocyte membrane β2-adrenergic receptors. Endogenous sympathetic activation via dynamic exercise3–5 or mental stress,4 as well as exogenous infusions of either epinephrine20 or isoproterenol,15 dramatically increases the number of circulat-

FIGURE 10. Bar graphs showing dose–response curves for concanavalin A (Con A)–induced thymidine incorporation (as a measure of lymphocyte proliferation) immediately after exercise, before and after propranolol (panel A) or metoprolol (panel B). Although thymidine incorporation was increased immediately after exercise after treatment with propranolol, there was a significant decrease in thymidine uptake immediately after exercise in those patients taking metoprolol. Values are given as mean±SEM of 12 individuals in the propranolol group and eight individuals in the metoprolol group.

FIGURE 11. Bar graphs showing percent of lymphocytes expressing IL-2 receptors after 3 days of concanavalin A stimulation. Lymphocytes were isolated and assessed before, immediately after, and 1 hour after exercise, both before and after 1 week of treatment with either propranolol (panel A) or metoprolol (panel B). Exercise impaired IL-2 receptor expression (p=0.02, paired t test, n=19). Propranolol therapy significantly (p<0.05, paired t test) blunted the observed decrease in IL-2 receptor expression after exercise. Values are shown as mean±SEM of 11 individuals in the propranolol group and eight individuals in the metoprolol group.
ing lymphocytes, with a marked rise in T<sub>ink</sub> and natural killer cells, variable increases in peripheral B cells, but only a modest change in T<sub>S</sub> cell numbers. This generalized lymphocytosis may be further attributed to the adrenergic system, as it has been shown to be prevented by pretreatment with the β-adrenergic antagonist propranolol.<sup>21</sup>

Our present investigation sought to characterize changes in circulating lymphocyte traffic and function associated with dynamic exhaustive exercise and to determine whether these alterations occur via a β<sub>T</sub>-mediated mechanism. As with previous studies,<sup>3-5</sup> we have shown that acute dynamic exhaustive exercise led to a marked lymphocytosis, predominantly of the T<sub>ink</sub> and natural killer subtypes. With 1 hour of rest, the subset distribution of circulating lymphocytes returned to preexercise values, as did blood levels of epinephrine and norepinephrine, documenting a transitory phenomenon. Propranolol, a nonselective β<sub>1</sub>/β<sub>2</sub>-antagonist, blunted the exercise-induced changes in circulating lymphocyte pools, whereas metoprolol, a β<sub>T</sub>-selective agent, either had no effect or tended to augment release. Both groups of subjects had similar suppression of heart rate before and after exercise with either agent, so our results cannot be attributed to a dosing discrepancy. Similarly, the drugs did not show differential effects upon exercise tolerance or exercise-induced release of norepinephrine and epinephrine. Thus, it appears that the differences between propranolol and metoprolol with respect to their effects on lymphocyte traffic after exercise is accounted for largely by propranolol’s additional β<sub>T</sub>-blocking capabilities.

Other studies have also suggested that β<sub>T</sub>-adrenergic receptors influence lymphocyte migration. Acute administration of exogenous β-adrenergic agonists such as isoproterenol<sup>15</sup> and epinephrine<sup>20</sup> but not the α<sub>1</sub>/β<sub>T</sub>-selective agent norepinephrine can mimic the subset-selective increases in circulating lymphocytes seen with dynamic exercise. We have also recently exercised a group of patients with congestive heart failure, in whom redistribution of circulating lymphocytes did not occur. In those particular subjects, basal levels of norepinephrine and epinephrine were increased. However, unlike the healthy individuals used in this study, postexercise epinephrine levels did not increase, whereas norepinephrine levels did, again supporting a β<sub>T</sub>-mediated mechanism for lymphocyte release into the circulation.<sup>3</sup> How β<sub>T</sub>-receptor stimulation mediates lymphocyte traffic is still unclear. A recent observation that a 20-minute infusion of the β-adrenergic agonist isoproterenol increases the number of circulating T<sub>ink</sub> and natural killer cells in control subjects but not in splenectomized patients suggests that the spleen may be the greatest reservoir for circulating lymphocytes.<sup>15</sup> Perhaps lymphocytes are released from the spleen via stimulation of receptors on contractile elements and blood vessels of the spleen.<sup>1</sup> Then again, the subtypes of lymphocytes that entered the circulation with exercise are the subtypes known to have the greatest density of β<sub>T</sub>-receptors.<sup>3,14,15</sup> The greater density of receptors may make these subsets more accessible to the effects of agonists such as epinephrine and allow for more effective generation of secondary messages. Direct stimulation of these receptors might reduce adherence to high-endothelial venules of lymph nodes and spleen.<sup>22,23</sup> Preferential histological location and proximity of certain lymphocyte subgroups to neurally released norepinephrine may also play a role in the propensity for migration.<sup>1</sup>

Our current investigation has shown an impairment in lymphocyte function, as manifested by a decrement in IL-2 receptor expression and Con A-induced [<sup>3</sup>H]thymidine incorporation immediately after exercise, with a normalization of these parameters after 1 hour of rest. The proliferation of T lymphocytes is known to be, at least in part, dependent on the presence of IL-2 produced by a population of activated lymphocytes, presumably acting through the IL-2 receptor. The IL-2 receptor, normally not detected on resting lymphocytes, is expressed in a population of activated T cells and upregulated by IL-2.<sup>24</sup> Exercise-induced impairment of IL-2 receptor expression and subsequent proliferation may be directly related to large increases in circulating epinephrine. β-agonists in vitro (isoproterenol> epinephrine> norepinephrine) have been shown to inhibit de novo expression of IL-2 receptors, downregulate previously expressed IL-2 receptor populations on mature T cells, and impair IL-2 production.<sup>17</sup> Exogeneously administered isoproterenol<sup>15</sup> and epinephrine<sup>25</sup> have the ability to decrease lymphocyte mitogen responsiveness ex vivo. However, the in vitro data are conflicting,<sup>6,7,17,25</sup> calling into question whether the increase in catecholamines immediately after exercise is directly responsible for the observed decrements in lymphocyte function. The impairment in lymphocyte IL-2 receptor expression and proliferation immediately after exercise, like that seen with exogenously administered epinephrine and isoproterenol, was dramatic and might simply be a consequence of alterations in the composition of the circulating lymphocyte pool. The relative increase in peripheral T<sub>ink</sub> cells after a surge of catecholamines might lead to an increased production of a putative suppressive factor. Then again, the T<sub>S</sub> cells have a much greater capacity than T<sub>ink</sub> or natural killer cells to generate IL-2 in response to a mitogenic stimulus<sup>26</sup>; perhaps the relative decrease in peripheral T<sub>S</sub> cells after exercise is associated with a proportional decrement in IL-2–stimulated proliferation. Finally, Con A in and of itself might have variable effects among the different lymphocyte subgroups in its ability to induce activation and proliferation. Regardless of mechanism, the fact that propranolol, but not metoprolol, blunted exercise-induced changes in mitogen-stimulated activation and IL-2 receptor expression suggests β<sub>T</sub>-mediated alterations in function.

Although exercise reduced lymphocyte function, as measured by Con A–stimulated expression of IL-2 receptors and [<sup>3</sup>H]thymidine incorporation, it dramatically enhanced natural killer cell activity, consistent with previous observations.<sup>27-29</sup> This finding appears to conflict with in vitro data that micromolar concentrations of epinephrine and norepinephrine inhibit natural killer cell activity.<sup>8</sup> Perhaps the concentration of circulating catecholamines after exercise is not sufficient to
alter peripheral blood natural killer cell cytotoxicity. Alternatively, suppression of natural killer cell activity after activation of the sympathetic nervous system may occur only in the spleen, in which direct noradrenergic innervation is present. The most plausible explanation for the discrepancy between ex vivo data and published in vitro data is that the enhanced cytotoxic response after exercise is related to a dramatic increase in circulating natural killer cells. Further studies using single-cell cytotoxicity assays need to be performed to accurately determine exercise-induced changes in the binding and kinetics of natural killer cell–directed cytosis. Propranolol, but not metoprolol, attenuated exercise-induced enhancement of natural killer cell cytotoxicity, probably through its ability to suppress efflux of natural killer cells into the circulation.

In summary, we have demonstrated that acute dynamic exhaustive exercise in healthy volunteers leads to a marked lymphocytosis, predominantly of the T<sub>ac</sub> and natural killer cell subtypes. These alterations in subset distribution are associated with decrements in lymphocyte function, as manifested by decreased Con A–stimulated lymphocyte activation and IL-2 receptor expression, and enhanced natural killer cell–directed cytotoxicity. These functional changes are most likely secondary to the changes in the relative numbers of the various lymphocyte subsets or changes in the function of certain subsets. Transient in nature, these parameters return to baseline after 1 hour of rest. Nonelective β-blockade with propranolol, but not β<sub>1</sub>-selective blockade with metoprolol, significantly blunts all of the above changes. Our results support the hypothesis that the sympathetic nervous system modulates immune function, at least in part, by β-adrenergic–mediated control of immunoregulatory cell traffic. Further work in this area might elucidate the clinical implications of linking “stress” with immune alterations and subsequent clinical illnesses.

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