Endurance Training Modulates Lymphocyte Function in Rats with Post-MI CHF

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ABSTRACT

BATISTA, M. L., JR., R. V. T. SANTOS, R. D. LOPES, A. C. LOPES, L. F. B. P. COSTA ROSA, and M. C. L. SEELAENDER. Endurance Training Modulates Lymphocyte Function in Rats with Post-MI CHF. *Med. Sci. Sports Exerc.*, Vol. 40, No. 3, pp. 549–556, 2008. **Purpose:** Exercise training restores innate immune system cell function in post–myocardial infarction (post-MI) rats. However, studies of the involvement of lymphocyte (Ly) in the setting of the congestive heart failure (CHF) are few. To address this issue, we investigated the function of Ly obtained from cervical lymph nodes from post-MI CHF rats submitted to treadmill running training. **Methods:** Twenty-five male Wistar rats were randomly assigned to the following groups: rats submitted to ligation of the left coronary artery, which were sedentary (MI-*S*, $N = 7$, only limited activity) or trained (MI-*T*, $N = 6$, on a treadmill (0% grade at 13–20 $m \cdot min^{-1}$) for 60 $min \cdot d^{-1}$, 5 $d \cdot wk^{-1}$, for 8–10 wk); or sham-operated rats, which were sedentary (sham-*S*, $N = 6$) or trained (sham-*T*, $N = 6$). The incorporation of [2-^{14}C]-thymidine by Ly cultivated in the presence of concanavalin A (Con A) and lipopolysaccharide (LPS), cytokine production by Ly cultivated in the presence of phytohemagglutinin (PHA), and plasma concentration of glutamine were assessed in all groups, 48 h after the last exercise session. **Results:** Proliferative capacity was increased, following incubation with Con-A in the MI groups, when compared with the sham counterparts. When incubated in the presence of PHA, MI-*S* produced more IL-4 (96%) than sham-*S* ($P < 0.001$). The training protocol induced a 2.2-fold increase in the production of interleukin-2 ($P < 0.001$) of the cells obtained from the cervical lymph nodes of MI-*T*, compared with MI-*S*. **Conclusion:** The moderate endurance training protocol caused an increase in IL-2 production, and a trend toward the reversion of the Th_1/Th_2 imbalance associated with IL-4 production increased in the post-MI CHF animal model. **Key Words:** TREADMILL RUNNING, INTERLEUKIN-2, INTERLEUKIN-4, GLUTAMINE, MYOCARDIAL INFARCTION

Congestive heart failure (CHF) is associated with changes in the immune system, especially regarding lymphocyte function (3,29,37). To contribute to adaptive immunity, the rare antigen-specific lymphocyte (Ly) must proliferate extensively before differentiating into functional effector cells of a particular immunogenic specificity (24). Cytokines are soluble glycoproteins that are produced by Ly and other cells, and they mediate the communication between immune cells and of these with nonimmune cells, organs, and organ systems throughout the body, playing an important role in controlling homeostasis. Ly-derived cytokines may be divided into proinflammatory (IL-6, IL-8, and TNF-α) and T Ly helper type 1(Th_1) cytokines, such as IL-2 and IFN-γ, or T Ly helper type 2 (Th_2) cytokines, IL-4 and IL-10 (31). The dominating cytokine profile may vary according to the etiologies of heart failure, although the balance of Th_1/Th_2 cytokines is disrupted under these circumstances, and, in this way, CHF might also be considered a Th_1/Th_2 imbalance–associated disease (36).

Moderate regular activity modulates both the innate and the adaptive branches of the immune system, contributing to improved immune response (5,24). In fact, regular exercise offers protection against all-cause mortality, and there is evidence from randomized intervention studies that exercise training is effective as a treatment in patients with chronic heart disease, type 2 diabetes, and in those presenting symptoms related to the metabolic syndrome (20,24,37).

During exercise, there is recruitment of natural killer cells (NK) and B and T Ly to the blood, leading to an increased total Ly count (20). After a prolonged and intense bout of exercise, however, the number of NK and Ly in the peripheral blood is reduced, as well as the function of NK

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and B Ly (20,21). The response of Ly to mitogens after exercise is still subject to controversy, because both decreases and increases in this parameter are reported in the literature (33). Despite different findings across studies, it is clear that exercise modifies the cellular and humoral branches of the immune system, and regular physical activity at moderate levels can increase the host's resistance to disease, whereas heavy exertion will enhance the risk of illness (21,32).

Besides the well-established beneficial effects of endurance exercise training in patients with chronic CHF, in which an enhancement of the maximal oxygen uptake ($\dot{V}O_{2max}$), maximal stroke volume, and perfusion of skeletal muscles during exercise (8) is reported, its role in the regulation of immune response has led the medical community to adopt it as a complementary therapeutic strategy for the management of heart disease (1,4,7).

CHF is a syndrome characterized by fatigue, shortness of breath, congestion, and cachexia, symptoms related to the inadequate perfusion of tissue and often fluid retention (14). Several models have evolved to explain this syndrome, and 20–25 yr ago the cardiorenal and cardiocirculatory and neurohormonal pathophysiologic theories were introduced, leading to important changes in the pharmacological treatment of CHF. However, despite state-of-the-art treatment, the morbidity and mortality rates of CHF are still high, indicating that important pathogenic mechanisms are not fully encompassed in those pathophysiologic models and remain unchallenged by current treatment strategies (38). Recently, several studies have emphasized the importance of biologically active molecules, the cytokines, in the development and progression of the syndrome of CHF, suggesting the important role of the immune system in the setting of CHF (6,29,37).

Therefore, with a growing body of evidence to support a role of immune mechanisms in the pathogenesis of CHF, several theories have been suggested to explain immune activation and the origin of cytokines in CHF patients, including the myocardium itself, attributable to myocardium tissue injury. Also, autoimmunity, infection, mechanical overload, bacterial–endotoxin translocation, and peripheral tissue ischemia–hypoxia owing to hypoperfusion may activate different types of mononuclear cells to release proinflammatory mediators (3,6,23). Several recent studies have shown that peripheral blood mononuclear cells (i.e., T cell, B cells, NK cells, and monocytes) from CHF patients have enhanced gene expression and release of inflammatory cytokines, including various chemokines and ligands of the TNF-α superfamily (38). However, only 1–2% of Ly are found in the blood, yet this is usually the only site available for clinical sampling (39).

Among leukocyte subsets, Satoh et al. (29) report that the subpopulation of cytokine producing T Ly ($CD4^+$) was larger in patients with symptomatic heart failure than in their asymptomatic subjects, suggesting that T Ly could be even more important as a cellular source for inflammatory cytokines in CHF (37). Few studies, however, have addressed this aspect of Ly function in this disease.

Considering that light- to moderate-intensity exercise is capable of immune modulation, we have sought to examine the effect of endurance training on Ly function, specially the cytokine secretion profile and the proliferative capacity in the cells obtained from rats with post-MI CHF. The mitogenic response and the Th_1 or Th_2 of cell-related cytokine production, such as IL-2 and IL-4, respectively, were examined for assessment of Ly function.

MATERIAL AND METHODS

Animals. A total of 31 male Wistar rats ranging in age from 6 to 8 wk (weighing about 250 g), obtained from the animal breeding unit, Institute of Biomedical Sciences, University of São Paulo, were used. They were housed, five per cage, with food and water *ad libitum*, in an animal room under 12/12-h light–dark cycle, with lights on at 7:00 p.m. (inverted light cycle), at a temperature of 22 ± 1°C and humidity of 60 ± 5%. The experiment was carried out after acclimation for a week. The investigation conforms to the guide for the care and use of laboratory animals published by U.S. National Institute of Health (NIH publication no. 85–23, revised 1996), and all protocols were approved by the animal use and care committee of the Institute of Biomedical Sciences, University of São Paulo (protocol no. 067/2002).

Surgical preparation. Rats were initially anesthetized with 3% halothane, intubated via tracheotomy, placed under a rodent respirator apparatus (Harvard model 680), and maintained under a 2% halothane oxygen mixture. The heart was exposed through left thoracotomy, between the fifth and sixth ribs (1.5 cm in diameter), and the pericardium was opened. In animals in which a myocardial infarction (MI) was produced, a 9-0 Ethilon suture was placed under the left main coronary artery at a point 1–2 mm distal to the edge of the left atrium, and the artery was ligated (26). Sham-operated animals underwent the same procedure, except that the suture under the coronary artery was left untied. Muscle and skin incisions were closed with separate pursestring silk sutures (size 0), and the lungs were fully expanded. The heart was then returned to its normal position, and the thorax was immediately closed. Each animal was allowed a minimum of 4 wk of recovery.

Experimental designs. After 4 wk (this being the necessary time to achieve the development of the heart failure state) (25), they were randomly assigned to either a sedentary or a trained group. Four groups were used in the study: two sedentary groups (sham-operated sedentary, $N = 6$, and MI sedentary, $N = 7$) and two trained groups (sham-operated trained, $N = 6$, and MI trained, $N = 6$).

Treadmill testing ($\dot{V}O_{2max}$). $\dot{V}O_{2max}$ was assessed by having each rat perform a maximal exercise test adapted from Musch et al. (18). The parameters were measured using the Oxymax gas-analyzing system for small animals

(Columbus Instruments). The test was always carried out after a 24-h recovery period. The volume of the supplied air was 4.5 $L \cdot min^{-1}$. The gas analyzer was calibrated with a reference gas mixture before each test. The $\dot{V}O_{2max}$ test protocol involved stepwise increases in the treadmill speed as follows: 15-min period of acclimation, the treadmill was then started at 10 $m \cdot min^{-1}$, and the speed was incrementally increased 5 $m \cdot min^{-1}$ every 3 min until the rat reached exhaustion. Exhaustion was defined as enduring the electrical stimulus without attempting to reengage the treadmill within 15 s. The highest $\dot{V}O_{2max}$ measured at each workload was taken as a measure of each rat's running economy ($\dot{V}O_{2submax}$) for that workload and, at the last step, as $\dot{V}O_{2max}$. The training sessions were performed at the same time each day, in order to avoid circadian interference.

Training protocol. Rats in the training groups ran for 5 $d \cdot wk^{-1}$ for 8 wk at a work rate that ranged between 55 and 65% $\dot{V}O_{2max}$. $\dot{V}O_{2max}$ was determined for each rat at the end of 2 wk of training, and workloads were adjusted upward according to the increase found in $\dot{V}O_{2max}$. On the first day of training, all the rats ran for 30 min. On the subsequent days of training, the running time was extended 10 min each day, until all the rats were running 60 $min \cdot d^{-1}$. Familiarization with the treadmill was maintained also in the sedentary groups by having each rat run on the treadmill (0% grade) for 10 $min \cdot d^{-1}$, 2 $d \cdot wk^{-1}$, at a speed of 15 $m \cdot min^{-1}$. After a resting period of 48 h after the last workout session, the animals were killed by decapitation without anesthesia.

Tissue weight determination. To obtain the wet/dry weight ratio of the lungs and liver, these organs were removed and separated from adhering tissues. In each case, the tissue was weighed, dissected into smaller pieces, and placed in the oven at 65°, until a constant weight was obtained, which was usually after about 24 h. The heart was then removed, the right ventricle was surgically separated from the left ventricle (LV) and septum, and LV tissues were weighed. In addition, rats were considered to have CHF when LV weight–body weight and lung weight–body weight ratios were increased, compared with their sham-operated counterparts.

Determination of left ventricular infarct size. After fixation in formalin for a minimum of 24 h, the left ventricle was cut into four transverse sections from base to apex in parallel with the atrioventricular groove. The four sections of the left ventricle were then dehydrated in alcohol, cleaned in xylene, and embedded in paraffin. Transverse sections (10 μm thick) were cut, mounted, and stained with Masson's trichrome stain, from which hematoxylin was omitted to provide maximum discrimination between the fibrous area of infarct and muscle (structural index). These sections were then measured with a planimeter digital image analyzer.

Determination of citrate synthase activity. Citrate synthase (CS) activity, an index of oxidative capacity, was determined for the soleus muscle of each rat. Tissue samples were homogenized at 0°C in a volume of 100 mM KPO_4 buffer, so that a 1:20 (weight/volume) homogenate was obtained. CS activity was measured with the spectrophotometric method, as previously described (7). The homogenates were frozen under liquid nitrogen and thawed four times to disrupt the mitochondria. The assay system contained, in a total volume of 200 μL, 100 mM Tris buffer (pH 8.35), 5 mM 5,5- dithiobis(2-nitrobenzoate) (DTNB), 22.5 mM acetyl-CoA, 25 mM oxaloacetate (OAA), and 4 μL of muscle homogenate. The principle of the assay was to initiate the reaction of acetyl-CoA with OAA and link the release of free CoA-SH to a colorimetric reagent, DTNB (acetyl-CoA + OAA + $H_2O \leftrightarrow$ citrate + CoA-SH, then CoA-SH + DTNB $\rightarrow$ mercaptide ion). The rate of color change was monitored at a wavelength of 405 nm in 15-s intervals for a period of 3 min, by using a Dynex MRX plate reader controlled through personal computer software (Revelation, Dynatech Laboratories). All assays were linear in respect to time and dilution, and each sample was analyzed in duplicate, in the same setting, at 37°C. The solubilized protein extracts of the homogenates were quantified in duplicate by using bicinchoninic acid reagents (Pierce, Rockford, IL) and bovine serum albumin standards. CS activity was then normalized to the total protein content and expressed as nanomoles per milligram of protein per minute.

Lymphocyte function analysis. After decapitation without anesthesia, the cervical lymph nodes were removed and disrupted in a stainless steel mesh, as previously described (10), in phosphate-buffer saline 0.9% (w/v), pH 7.4. Cell suspensions were centrifuged at 500*g* for 15 min at 37°C, and cell viability was determined with the Trypan blue exclusion test. Ly were cultured (Microprocessor CO_2 incubator LAB LINE) in 96-well plates (Corning, NY) at a density of 2×10^5 cells per well (total volume, 200 μL) with RPMI-1640 medium without glutamine, 10% homologous serum, 20 μg per well of concanavalin A (Con A), and 10 μg per well lipopolysaccharide (LPS) (a mitogenic stimulus), for 48 h at 37°C, under an artificially humidified atmosphere of 5% CO_2 in air. After 48 h in culture, more than 98% of the mononuclear cells were still viable. After 48 h in culture, the cells were pulsed with 20 μL (0.02 μCi) [2-^{14}C]-thymidine (specific activity: 56.0 mCi nM^{-1}), diluted in sterile phosphate-buffered saline, and maintained under these conditions for an additional 16 h, after which they were harvested automatically by a multiple cell harvester (Skatron Instruments, Norway) onto a filter paper (cat. no. 11731 Skatron Combi, Suffolk, UK). The paper discs containing the labeled cells were placed into vials with 5 mL of Ecolume (ICN, CA, scintillation cocktail), and the radioactivity was measured in a Beckman-LS 5000TD liquid scintillation counter (Beckman Instruments, Fullerton, CA). The precision within the assay of thymidine incorporation was 3.2–8.9%, and between-assay precision was 4.2–9.5%. The recovery of the Skatron cell harvester is 95%, according to the manufacturer.

For determination of cytokine production, mononuclear cells obtained from the cervical lymph nodes (±79% lymphocytes) were plated (1.0×10^6 cells per milliliter) onto plastic petri dishes with RPMI 1624 medium enriched with 2 mM glutamine and 10% homologous serum, in the presence of 10 $\mu g \cdot mL^{-1}$ phytohemagglutinin (PHA) to stimulate IL-2 and IL-4 production. After 48 h, the concentration of the cytokines was measured in the supernatant with commercially available ELISA-kits (Quantikine Elisa Kits, R&D Systems, Abingdon, UK). All kits showed less than 10% variation of precision and reproducibility for both within-assay and between-assay measurements, a recovery of 95%, and no cross-reaction with other cytokines, according to the manufacturer.

Plasma metabolite measurement. Blood was placed in test tubes containing sodium heparin and was kept on ice. The samples were centrifuged at 4°C, 690 *g* for 25 min, and an aliquot of plasma was added to 1.4 volumes of 8.6% trichloroacetic acid. After centrifugation, the clear supernatant fraction was extracted twice with six volumes of diethyl ether (ACS grade) saturated with water, and residual ether was removed under a stream of N_2. All operations during these and other tissue-extraction procedures described below were performed in capped tubes at 0–4°C, previously stored at −80°C. Plasma glutamine concentration was determined by the modified enzymatic assay, according to the method described by Windmueller and Spaeth (35). The reaction mixture, yielding 1 mL, contained the following: plasma, 50 mM KH_2PO_4, glycerol 50%, 4 mM NADH, BSA 10%, GDH (5 $U \cdot mL^{-1}$), 4 M alpha-ketoglutaric acid, and asparaginase (5 $U \cdot mL^{-1}$), pH 8.0. Absorbance changes at 340 nm from the stepwise decrease were determined using a Beckman analyzer (Beckman Instruments, Fullerton, CA). Each sample was analyzed in duplicate with a coefficient of variation of < 5%.

Statistical analysis. The statistical analysis was performed with the statistical package from SigmaStat (version 3.1, SYSTAT, Point Richmond, CA). Data are expressed as means ± SD of the values, and the number of experiments is shown in each of the tables and figures. A primary observation indicated that the results of experiments were distributed normally. Posttraining measurements were analyzed by two-way ANOVA of 2 × 2 design (sedentary/trained vs sham-operated/MI). With this type of analysis, the data were partitioned into main effects (sedentary vs trained group effects, A; and MI vs sham group effects, B). The interaction effects consisted of A × B.

When a significant F value was found by two-way ANOVA, a Holm–Sidak *post hoc* test was performed to demonstrate all pairwise multiple comparison between the means. The 0.05 probability level was considered to indicate statistical significance.

RESULTS

Characterization of the State of Post-MI CHF

Rats submitted to MI procedures or sham operation showed no changes in body weight: sham-operated (sedentary (*S*): 325 ± 32 g, $N = 6$ and trained (*T*): 334 ± 37 g, $N = 6$) and MI (sedentary (*S*): 381 ± 61 g, $N = 7$ and trained (*T*): 325.7 ± 38 g, $N = 6$) (Table 1). Perioperative mortality in the MI group was 16.1% ($N = 5$, defined as death within 24 h after MI preparation) and 3.2% ($N = 1$) in the sham-operated group. The structural index (discrimination between the fibrous area of infarct, attributable to MI and muscle) indicative of left ventricle dysfunction was assessed through quantification of left ventricle infarct size for all rats in the different groups. For MI-*S* and trained groups, it was 35.7 ± 5.7% and 33.8 ± 3.8%, respectively (Table 1). No detectable infarcts were found in the sedentary and trained sham-operated rats. Heart weight showed significant MI versus sham group effects with no difference in sedentary versus trained condition. This difference pointed to a greater heart weight in MI-*S* when compared with sham-*S* (1.19 ± 0.2 and 0.94 ± 0.1 g., respectively: $P < 0.05$). Left ventricle weight normalized to body weight was elevated in MI-*S* and MI-*T* (20% and 23%, respectively, $P < 0.05$) when compared with their sham-operated counterparts. Moreover, these increases in left ventricle weight coincided with increases in lung wet/dry weight–body weight ratio (12% and 17%, respectively, $P < 0.05$), demonstrating that these animals presented significant pulmonary congestion. Because increases (8%, MI-*S* and 6%, MI-*T*, $P < 0.05$) in liver wet/dry weight normalized to body weight were also found in these animals, they appeared to be in a chronic state of compensated congestive heart failure.

TABLE 1. Left ventricular infarct sizes, heart and left ventricular weights (LV wt), lung and liver wet/dry weight ratios, and total body weights in sedentary and trained rats with MI and in rats in which sham operations were performed.

	Infarct (%)	Body Weight (g)	Heart Weight (g)	LV Weight/Body Weight ($mg \cdot g^{-1}$)	Lung Wet/Dry Weight (Ratio)	Liver Wet/Dry Weight (Ratio)
Sham-operated rats						
Sedentary ($N = 6$)		335 ± 32	0.94 ± 0.2	2.51 ± 0.11	4.15 ± 0.12	3.01 ± 0.05
Trained ($N = 6$)		334 ± 47	0.96 ± 0.1	2.48 ± 0.16	4.19 ± 0.11	3.03 ± 0.07
MI rats						
Sedentary ($N = 7$)	35.7 ± 5.7	381 ± 65	1.19 ± 0.2†	3.01 ± 0.08†	4.63 ± 0.19†	3.24 ± 0.10†
Trained ($N = 6$)	33.8 ± 3.8*	325 ± 38	1.22 ± 0.2†	3.06 ± 0.78†	4.91 ± 0.14†	3.22 ± 0.13†

Values are means ± SD in grams and/or milligrams per gram. *N*, number of rats; IM, myocardial infarction; sham, sham-operated rats; LV, left ventricular. The percentage of *infarct size* and ratios of LV, as well as lung weight to body weight ratio, are shown. * $P < 0.05$, training effect for sedentary vs trained (two-way ANOVA); † $P < 0.05$, MI effect for MI vs sham (two-way ANOVA).

TABLE 2. Maximal oxygen uptake ($\dot{V}O_{2max}$) and citrate synthase (CS) maximal activities measures in sedentary and trained rats with MI and in rats in which sham operations were performed.

	$\dot{V}O_{2max}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	CS Activity ($nmol \cdot mg^{-1} \cdot min^{-1}$)
Sham-operated rats		
Sedentary ($N = 6$)	55.9 ± 3	99 ± 8
Trained ($N = 6$)	63.1 ± 4*	115 ± 7*
MI rats		
Sedentary ($N = 7$)	46.4 ± 2†	90 ± 4†
Trained ($N = 6$)	52.9 ± 3*	107 ± 5*

Values are means ± SD. $\dot{V}O_{2max}$ and CS activities were determined after 8 wk of endurance training or sedentary control conditions. * $P < 0.05$, training effect for sedentary vs trained (two-way ANOVA); † $P < 0.05$, MI effect for MI vs sham (two-way ANOVA).

Effects of Exercise Training

Moderate endurance protocol. Decrements in the maximal consumption of oxygen ($\dot{V}O_{2max}$) were found in MI-*S* compared with the sham-*S* (21%, MI vs sham group effect) after the exercise protocol (Table 2). However, this decrement was attenuated in MI-*T* after 8 wk of training (sedentary vs trained effect). $\dot{V}O_{2max}$ for the different groups showed significant sedentary versus trained group effects (Table 2). $\dot{V}O_{2max}$ was 13% and 14% higher, respectively, in sham-operated and MI-*T* animals than in their sedentary counterparts after moderate endurance training ($P < 0.05$).

CS activity measured in the soleus muscle showed a significant sedentary versus trained group effect (Table 2). CS activity in the trained sham-*T* and MI-*T* groups was 16% and 20% greater than in the sedentary counterparts, respectively ($P < 0.05$).

Lymphocyte function. The influence of MI on the immune system was addressed by evaluating the proliferative capacity of Ly obtained from the cervical lymph node cells, as shown in Table 3, as well as by the ability of these cells to produce interleukins 2 and 4 after 48 h in culture (Fig. 1).

The control proliferative index, measured in the absence of mitogen stimulation, was higher in MI-*S* (24%, $P < 0.05$) and MI-*T* (21%, $P < 0.05$) as compared with their respective controls. The mitogenic response of Ly to concanavalin A was 35% higher in MI-*S* than in the sham-operated counterparts ($P < 0.05$), as well as 55% higher in MI-*T* than in sham-*T* ($P < 0.05$); however, no significant sedentary versus trained group effects (MI-*S* × MI-*T*, $P = 0.150$) were found. When stimulated with LPS, a mitogen specific to B Ly, no changes regarding training or MI effects were observed.

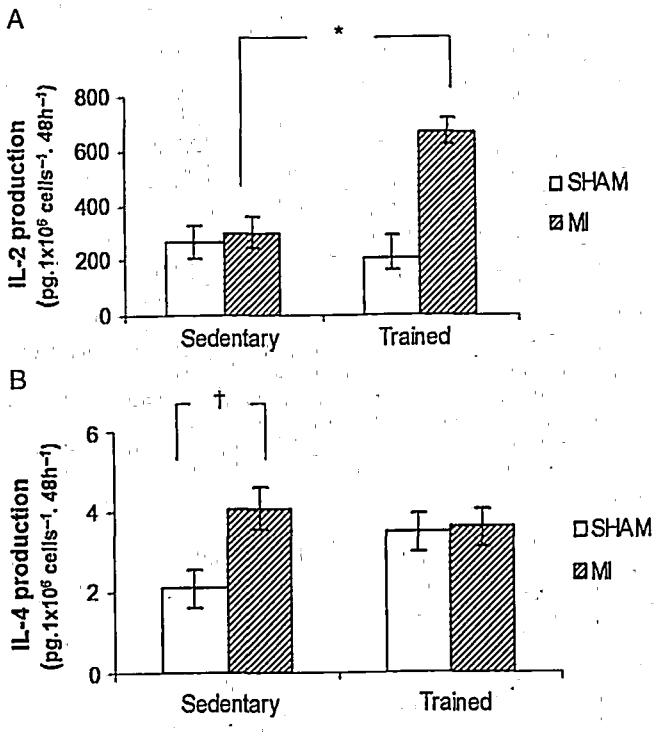

FIGURE 1—Effect of endurance training (8 wk) on IL-2 (*A*) and IL-4 (*B*) cytokine production in cervical lymphocytes stimulated by phytohemagglutinin (PHA) of sham-operated (sedentary, $N = 6$ and trained, $N = 6$) and MI (sedentary, $N = 7$ and trained, $N = 6$) rats. Cervical lymphocytes were cultured in the presence of autologous serum and PHA (10 $\mu g \cdot mL^{-1}$) for 48 h. Cytokine production in culture supernatants was measured by ELISA system. * $P < 0.05$: training effect, sedentary vs trained (two-way ANOVA); † $P < 0.05$: MI effect, MI vs sham (two-way ANOVA).

Figure 1 shows IL-2 (A) and IL-4 (B) production by cervical Lys cultured for 48 h and stimulated with phytohemagglutinin (PHA). IL-4 production by MI-*S* cells was significantly higher (96%) than that of the sham-operated sedentary group ($P < 0.001$). IL-2 production showed significant sedentary versus trained group effects ($P < 0.001$), increasing 2.2-fold in MI-*T* compared with MI-*S*.

TABLE 3. Proliferative response of lymphocytes obtained from the cervical lymph nodes in sedentary and trained rats with MI and in rats in which sham operations were performed.

	Controls	Con A		LPS	
	Mean ± SD	Mean ± SD	Δ	Mean ± SD	Δ
Sham-operated rats					
Sedentary ($N = 6$)	617 ± 63	1208 ± 78	95%	1645 ± 206	167%
Trained ($N = 6$)	599 ± 69	1190 ± 88	98%	1974 ± 225	229%
MI rats					
Sedentary ($N = 7$)	764 ± 89	1630 ± 109†	113%	1708 ± 291	124%
Trained ($N = 6$)	722 ± 69	1848 ± 83†	156%	1686 ± 225	133%

Values are presented as means ± SD and expressed as disintegrations per minute. Δ, Percentage of proliferation compared with control (absence of mitogen stimulation). Cervical lymphocytes were cultured in the presence of autologous serum and concanavalin A (Con A, 20 $\mu g \cdot mL^{-1}$) and lipopolysaccharide (LPS, 10 $\mu g \cdot mL^{-1}$). [^{3}H] Thymidine incorporation was measured during the final 16 h of a 64-h culture period and is expressed as stimulation index. * $P < 0.05$, training effect for sedentary vs trained (two-way ANOVA); † $P < 0.05$, MI effect for MI vs sham (two-way ANOVA).

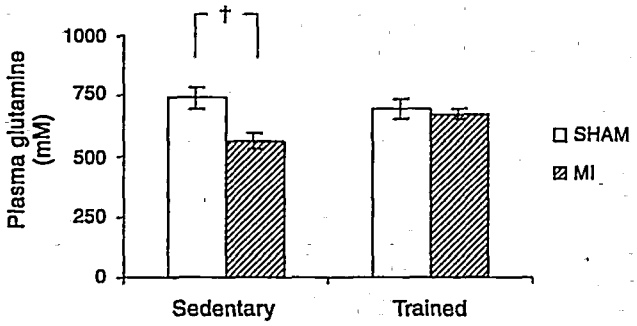

Figure 2—Effect of endurance training (8 wk) on plasma glutamine concentration of sham-operated (sedentary, $N = 6$ and trained, $N = 6$) and MI (sedentary, $N = 7$ and trained, $N = 6$) rats. Plasma glutamine concentration was determined by enzymatic assay. * $P < 0.05$: training effect, sedentary vs trained (two-way ANOVA): † $P < 0.05$: MI effect, MI vs sham (two-way ANOVA).

The animals submitted to MI showed reduced plasma glutamine concentrations (32%) (Fig. 2), with no significant sedentary versus trained group effects.

DISCUSSION

While most of the previous studies have focused on the determination of leukocyte subsets to assess the role of T Ly in the development and progression of CHF, the present study sought to investigate the mitogenic response and the production of IL-2 and IL-4 of cervical Ly *in vitro* as indicators of Ly function in post-MI CHF rats. In this way, we chose Ly from the cervical lymph nodes because of the influence of the localization of the chronic inflammation stimulus (MI) on the most closely anatomically related lymph nodes. The effect of 8 wk of treadmill running exercise on the same parameters was also examined. The moderate endurance training protocol herein adopted produced an increase in IL-2 production, a trend toward reversion of the Th_1/Th_2 imbalance associated with the increase of IL-4 production found in post-MI CHF. Also, the increase of T-Ly proliferative index with a greater responsiveness to mitogens (Con A), while no changes in this function were found for B-Ly, suggests that this response is specific in T cells obtained from animals with post-MI CHF.

The rat model of coronary artery occlusion produces moderate to severe congestive heart failure within 21–42 d after infarction (18,25). Congestive heart failure was confirmed in our MI rats by the histological examination of the infarction size (structural index) produced, which neared or exceeded 30% of the left ventricle. This method provided animals with histologically well-healed infarctions for determining the relationship between infarct size and ventricular performance (26). In addition, rats showed higher heart and left ventricle mass normalized to body weight than a progressive left ventricular dilatation might have suggested. Lung weight normalized to body weight provided evidence for pulmonary congestion, and liver wet/dry weight normalized to body weight indicated that the rats were in a chronic congestive state.

Decrements in $\dot{V}O_{2max}$ in MI rats confirmed exercise intolerance. However, the training protocol was effective in increasing $\dot{V}O_{2max}$ after the training period, yielding values similar to those observed for the sham-*S* group. Additionally, CS maximal activity in the soleus was also increased after training. These adaptations are in agreement with other studies (7,18) that have evaluated the effect of moderate treadmill running in the post-MI CHF animal model.

Immune response may be classified into either T Ly helper type 1 (Th_1) or type 2 (Th_2), according to the predominant cytokines involved (28). An imbalance between type 1 and type 2 cytokine profiles has been implicated in many human diseases (11,27). Recently, several studies have shown that leucocytes may play important functions in the course of CHF, and that the T Ly ($CD4^+$) helper population is increased in CHF patients (29,37).

In our model of post-MI CHF, an increase in cervical T Ly responsiveness to polyclonal mitogens (PHA) inducing IL-4 production was found to be specific to the post-MI CHF animal condition. Therefore, Th_2 cells may function as physiological regulators of the immune response by inhibiting potentially injurious Th_1 responses (27). Nevertheless, an appropriate immune switch to Th_2-type cells producing IL-4 at the onset of significant clinical changes in a post-MI CHF animal model could also lead to Th_2/antiinflammatory cytokine production, suggesting an adaptive response to maintain homeostasis (9). However, it is important to point out that our post-MI CHF animal model presented an infarction area ranging from 31 to 41%, which suggests a model of moderate, compensated, congestive heart failure; additional studies should to be made to evaluate the presence of these changes in infarction greater than 45% (decompensate congestive heart failure).

Moderate exercise seems to gear the immune system toward a more Th1-type cytokine response (IL-2 and IL-12) and to decrease and/or not change Th2 cytokines (IL-4 and IL-10) (17,34). Notably, the moderate endurance training protocol adopted increased IL-2 production (2.1-fold), whereas that of IL-4 was not altered, indicating that exercise training modified this specific aspect of T Ly biology. This change induced by post-MI CHF in the profile of cytokine production led to a diversion towards a Th_2-like response, whereas moderate endurance training led towards a Th_1-like response. The effect of the training protocol through modulation of the systemic or local pro/antiinflammatory and Th_1/Th_2 cytokine balance may apparently attenuate or suppress the progression of this condition. Besides, the effect of training was only evident in MI, with no effects in sham-operated animals, indicating that exercise training was only able to affect T Ly function modified by the post-MI CHF condition.

It is interesting to note that in aging, but not young, mice, moderate exercise is associated with increased antigen-specific IL-2 and IFN-γ production in response to viral

challenges (15). In conjunction with the previous findings of Nieman et al. (21), a possible mechanistic link to the decreased infection rate among older women who exercise may be put forward; when the immune system is altered towards a Th_2-like response with aging, as occurs in post-MI CHF rats, exercise can gear it back towards the Th1-like function prevalence of younger individuals and/or sham-operated animals, respectively.

However, knowledge on cytokine production by T Ly with chronic exercise training is limited (20,32). Sugiura et al. (33) have demonstrated that voluntary exercise and chronic endurance training (12) enhanced the production of IL-2 by splenocytes under Con A stimulation. On the other hand, other studies (16,22) have shown a decrease of IL-2 production by spleen Ly stimulated with Con A, after endurance training. The results of these studies are contradictory, and these differences could be explained, at least partly, by the differences in exercise training variables (type of exercise, intensity, volume, and frequency) and origin of the lymph nodes used.

The changes in cytokine production by cultured mononuclear cells from the cervical lymph nodes were accompanied by an increase in the proliferative response to Con A, a mitogen for T cells, in the post-MI CHF groups. This result in post-MI CHF rats corroborates previous reports that have shown that peripheral lymphocyte subsets are altered in heart failure and associated with helper T Ly dominancy over cytotoxic T cells, irrespective of etiology (29). At this point, it is interesting to note that even in the absence of mitogenic stimulation, there were increases in proliferative response, suggesting a favorable environment for cell proliferation that was only evident in the cells obtained from the cervical lymph nodes of MI groups.

Contrary to the increased proliferation found in post-MI cells, Bacuau et al. (5) have shown a decrease in this function in Ly incubated in the absence of mitogen in Walker tumor-bearing rats. These changes were accompanied by alterations in the profile of glucose metabolism and increases in glutamine consumption by the cells. Besides, elevation of the plasma concentration of several proinflammatory mediators has been suggested as a determinant for the development of CHF (1,3). A trend toward TNF-α increases has been reported for post-MI CHF rats (30). Moreover, soluble CD14, a marker of endotoxin–cell interaction, and shedding from the cell membrane and LPS concentrations, are higher in CHF patients with peripheral edema (3). It might eventually be found that all of the above mechanisms take part in what is now known as immune activation in CHF, notably T Ly proliferation. However, we have not tested this hypothesis.

In addition to that, Ly migration is not always random, and lymphocytes may be directed selectively to particular body regions, suggesting that lymphocyte traffic may in some cases play a more specific role in controlling the nature of local immune responses (39). In our study, we demonstrated that Ly from the cervical lymph nodes are more responsive to mitogen stimulation, and this may represent an important source of circulating T Ly in the setting of CHF. On the other hand, changes in Ly from other peripheral lymph nodes (mesenteric), and spleen and medulla in animal models of CHF, have not been previously reported.

Anker and Rauchhaus (2) have proposed that CHF is a progressive disorder affecting different physiological parameters and protein metabolism pathways. Indeed, several studies have demonstrated the role of plasma glutamine in the maintenance of normal Ly function, as well as Th_1/Th_2 cytokine balance, proliferative responsiveness to mitogens, and metabolism on catabolic states as sepsis (19), energy restriction (13), and cachexia (5). In this way, we can suggest that the decrease in plasma glutamine in post-MI CHF may have an impact on normal Th_1/Th_2 cytokine balance, leading to a diversion toward a Th_2-like response, as well as in the increased proliferative response to Con-A. However, whether those changes were possibly correlated with decreased glutamine concentration in the blood or changes in Ly function is a matter requiring further investigation.

Although previous work by our group and by others has shown alteration of the innate immune function in the post-MI CHF animal model, this is the first study, to our knowledge, to address and characterize the effect of post-MI CHF on cervical lymph node Ly, an important component of the adaptive immune system. Eight weeks of chronic endurance exercise were able to modulate immune cell function in post-MI CHF rats, an effect that could be, at least partially, associated with the observed increase in IL-2 production induced by training. That may suggest a trend toward normalization of cervical Ly function (i.e., sedentary sham-operated values)—notably, inducing a diversion of the immune response back toward a Th_1-type response. The data presented herein point to the relevance of the immunomodulatory effect of moderate exercise training in the setting of post-MI CHF.

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