Regular and moderate exercise before experimental sepsis reduces the risk of lung and distal organ injury

Carla C. de Araújo,1 Johnatas D. Silva,1 Cynthia S. Samary,1 Isabela H. Guimarães,1 Patrícia S. Marques,1 Gisele P. Oliveira,1 Luana G. R. R. do Carmo,1 Regina C. Goldenberg,2 Ilka Bakker-Abreu,3 Bruno L. Díaz,3 Nazareth N. Rocha,3,4 Vera L. Capelozzi,5 Paolo Pelosi,6 and Patricia R. M. Rocco1

1Laboratory of Pulmonary Investigation. 2Laboratory of Cell and Molecular Cardiology, and 3Laboratory of Inflammation, Carlos Chagas Filho Institute of Biophysics, Federal University of Rio de Janeiro; 4Department of Physiology, Fluminense Federal University, Niterói; 5Department of Pathology, Faculty of Medicine, University of São Paulo, São Paulo, Brazil; and 6Department of Surgical Sciences and Integrated Diagnostics, University of Genoa, Genoa, Italy

Submitted 23 August 2011; accepted in final form 17 January 2012

Sepsis is a complex clinical syndrome characterized by end-organ dysfunction away from the primary site of infection. Despite many research efforts, sepsis remains associated with high incidence and mortality rates (40–60%) (13, 14).

Many studies focus on a wide range of therapies for sepsis, but only a few investigators have analyzed the effect of therapies to minimize the systemic damage caused by sepsis. In this line, regular and moderate exercise has been linked to improvement in the immune response against infection due to the establishment of a balance between pro- and anti-inflammatory cytokines (11, 12, 18, 33, 35). Chen et al. (6) observed that regular exercise prior to lipopolysaccharide-induced sepsis led to beneficial effects on the cardiac system, attenuating the reduction in systemic arterial pressure and inflammatory response, as well as decreasing the release of free radicals and inflammatory mediators. To our knowledge, however, the possibility that regular and moderate exercise modulates the inflammatory and remodeling processes, not only in the heart, but also in lung, liver, kidney, and small intestine villi, thus increasing the survival rate in sepsis, has not been investigated.

The purpose of this study was to evaluate the effects of regular and moderate exercise before cecal ligation and puncture-induced sepsis on lung and distal organ injury and survival.

METHODS

This study was approved by the Health Sciences Center Ethics Committee at the Federal University of Rio de Janeiro (CEUA-CCS-019). All animals received humane care in compliance with the Principles of Laboratory Animal Care formulated by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences.

Animal preparation and experimental protocol. One hundred twenty-four male BALB/c mice (weighing 25–30 g) were kept under specific pathogen-free conditions in the animal care facility at the Laboratory of Pulmonary Investigation, Federal University of Rio de Janeiro. Fifty-two mice (n = 13/each group) were used to evaluate lung mechanics and histology, as well as echocardiography. Thirty-two animals (n = 8/each) were submitted to the same protocol described above to obtain aliquots of bronchoalveolar lavage (BALF) and peritoneal (PLF) fluids and plasma. These parameters were obtained in another group of animals because the collection of BALF may influence on lung and systemic data, sedentary animals were handled by 10.220.33.3 on October 14, 2017 http://jap.physiology.org/ Downloaded from

This study was approved by the Health Sciences Center Ethics Committee at the Federal University of Rio de Janeiro (CEUA-CCS-019). All animals received humane care in compliance with the Principles of Laboratory Animal Care formulated by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences.

Animal preparation and experimental protocol. One hundred twenty-four male BALB/c mice (weighing 25–30 g) were kept under specific pathogen-free conditions in the animal care facility at the Laboratory of Pulmonary Investigation, Federal University of Rio de Janeiro. Fifty-two mice (n = 13/each group) were used to evaluate lung mechanics and histology, as well as echocardiography. Thirty-two animals (n = 8/each) were submitted to the same protocol described above to obtain aliquots of bronchoalveolar lavage (BALF) and peritoneal (PLF) fluids and plasma. These parameters were obtained in another group of animals because the collection of BALF may influence on lung and systemic data, sedentary animals were handled by 10.220.33.3 on October 14, 2017 http://jap.physiology.org/ Downloaded from
identically to the T group, including the same number of times removed from cages each day, except for treadmill running.

**Echocardiography.** To evaluate cardiac adaptation to exercise, echocardiography (with a 30 MHz mechanical transducer, Visual Sonics, Toronto, Canada) was performed before the training protocol and at 8 wk. Mice were anesthetized with 1.5–2.0% isoflurane by mask, the chest was shaved, and the animal was placed in the supine position. Images were obtained from the subcostal and parasternal views. Short-axis two-dimensional views of the left ventricle were acquired at the level of the papillary muscles to obtain the M-mode image. Systolic and diastolic volumes and ejection fraction were obtained from the B-mode long axis tracings according to Simpson’s method. The following M mode parameters were analyzed: left atrium and aorta diameters, left ventricular septum and posterior wall thickness in diastole, and left ventricular mass. The measurements were obtained following American Society of Echocardiography Guidelines (5).

**Experimental model of sepsis.** In CLP groups, mice were fasted for 16 h before surgery, anesthetized with sevoflurane, and submitted to a midline laparotomy (2-cm incision). The cecum was carefully

---

**Fig. 1.** Schematic flow chart and timeline of the study design. S, sedentary; T, trained. In CLP groups, animals were submitted to cecal ligation and puncture. A sham-operated group was used as control (C) for animals undergoing CLP.

**Fig. 2.** Short-axis B-dimensional views of left ventricle (LV) (top) in S and T animals. Echocardiographic data (left ventricular diameter, left ventricular mass, systolic volume, and diastolic volume) were measured before training protocol and at 8 wk. Values are means ± SD of 26 mice in each group. *Significantly different from T0 (*P < 0.05).* #Significantly different from S (*P < 0.05).*
isolated to prevent damage to blood vessels. A 3.0 cotton ligature was placed below the ileocecal valve to prevent bowel obstruction. Finally, the cecum was punctured once with an 18-gauge needle and the animals recovered from anesthesia (4). In C animals, the abdominal cavity was opened and the cecum was isolated without ligation and puncture. Anesthesia procedures were similar in C and CLP groups. All animals received subcutaneous injections of 1 ml of warm (37°C) saline with tramadol hydrochloride (20 mg/g body wt) during the postoperative period.

Mechanical parameters. At 24 h, C and CLP animals were sedated (diazepam 1 mg ip), anesthetized (thiopental sodium 20 mg/kg ip), tracheotomized, paralyzed (vecuronium bromide, 0.005 mg/kg iv), and ventilated with a constant flow ventilator (Samay VR15; Universidade de la Republica, Montevideo, Uruguay) with the following parameters: frequency of 100 breaths/min, tidal volume (VT) of 0.2 ml and fraction of inspired oxygen of 0.21. The anterior chest wall was surgically removed, and a positive end-expiratory pressure (PEEP) of 2 cmH$_2$O was applied. After a 10-min ventilation period, lung mechanics were computed. Airflow and tracheal pressure (Ptr) were measured (3). In an open chest preparation, Ptr reflects transpulmonary pressure (PL). Lung resistive ($\Delta$P1) and viscoelastic/inhomogeneous ($\Delta$P2) pressures, as well as static elastance (Est), were computed by the end-inflation occlusion method (4). Lung mechanics measurements were performed 10 times in each animal. All data were analyzed using the ANADAT data analysis software (RHT-InfoData, Montreal, Quebec, Canada).

Lung histology. A laparotomy was done immediately after determination of lung mechanics, and heparin (1,000 IU) was intravenously injected in the vena cava. The trachea was clamped at end-expiration (PEEP = 2 cmH$_2$O), and the abdominal aorta and vena cava were sectioned, yielding a massive hemorrhage that quickly killed the animals. The right lung was then removed, fixed in 3% buffered formaldehyde and paraffin embedded. Four-micrometer-thick slices were cut and stained with hematoxylin-eosin. Lung morphometry analysis was performed with an integrating eyepiece with a coherent system consisting of a grid with 100 points and 50 lines of known length coupled to a conventional light microscope (Olympus BX51, Olympus Latin America, Brazil). Fraction areas of collapsed and normal lung areas were determined by the point-counting technique (10, 32) across 10 random, noncoincident microscopic fields (27). Neutrophils and mononuclear (MN) cells and lung tissue were evaluated at $\times$1,000 magnification. Points falling on neutrophils and MN cells were counted and divided by the total number of points falling on lung tissue in each microscopic field.

Collagen (picrosirius-polarization method) and elastic fibers (Weigert’s resorcin fuchsin method with oxidation) were quantified in the alveolar septa (4, 25). The alveolar septa quantification was carried out with the aid of a digital analysis system and specific software (Image-Pro Plus 5.1 for Windows; Media Cybernetics, Silver Spring, MD) under $\times$200 magnification. The images were generated by a microscope (Axioplan, Zeiss) connected to a camera (Sony Trinitron CCD, Sony, Tokyo, Japan), fed into a computer through a frame grabber (Oculus TCX, Coreco, St Laurent, PQ, Canada) for off-line processing. The thresholds for collagen and elastic fibers were established after enhancement of contrast up to the point where the fiber was easily identified as either birefringent (collagen) or black (elastic) bands. Bronchi and blood vessels were carefully avoided during the measurements. To avoid any bias due to septal edema or alveolar collapse, the areas occupied by the elastic and collagen fibers were measured by digital densitometric recognition, divided by the length of each studied septum, and expressed as the amount of elastic and collagen fibers per unit of septum length ($\mu$m$^2$/\mu$m).

Transmission electron microscopy. Three $2 \times 2 \times 2$ mm slices were cut from three different segments of the left lung and fixed [2.5% buffered formaldehyde and paraformaldehyde (10:2)]. The specimens were dehydrated in ethanol, embedded in 1% Epon 812, and polymerized for 3 days at room temperature. Thin sections were cut and stained with toluidine blue. Electron microscopy was performed with a transmission electron microscope (Philips CM120, Philips, Eindhoven, The Netherlands).

Fig. 3. Kaplan-Meier survival curves during the training protocol and 7 days after surgery. Data represent percentage survival of S (S-C, S-CLP, n = 10/group) and T mice (T-C and T-CLP, n = 10/group). *Significantly different from S-C group ($P < 0.05$). **Significantly different from T-C group ($P < 0.05$).

Fig. 4. Lung static elastance (Est) (A); resistive ($\Delta$P1, gray bar), viscoelastic ($\Delta$P2, white bar), and total pressures ($\Delta$Ptot = $\Delta$P1 + $\Delta$P2) (B). Values are means $\pm$ SD of 13 animals in each group (10 determinations per animal). *Significantly different from S-C group ($P < 0.05$). **Significantly different from T-C group ($P < 0.05$). 

#Significantly different from S-CLP group ($P < 0.05$).
Fig. 5. Representative photomicrographs of lung stained with hematoxylin-eosin. Photographs were taken at an original magnification of ×200.

Fig. 6. Electron micrographs of lung parenchyma. Type II cell (PII), Type I cell (PI), alveolar space (AS), neutrophils (N), Type III collagen fibers (CIII), erythrocyte (E), interstitial cell (IC), endothelial cell (EC), macrophages (M), casement membrane (BM), lamellar bodies (LB). *Degeneration of lamellar bodies. Note the endothelium lesion in CLP groups (arrows). Electron photomicrographs are representative of data obtained from lung sections derived from 5 animals.
RESULTS

Regular and moderate exercise training for 8 wk induced cardiac hypertrophic adaptations, as evidenced by significant increase in left ventricular mass and diameter, as well as systolic and diastolic volume (Fig. 2).

Effects of training in control groups. No animals in the control groups died during the investigation period (Fig. 3). Although lung mechanics (Fig. 4), histology (Figs. 5 and 6, Tables 1 and 2), and the degree of apoptosis in lung, heart, liver, kidney, and small intestine villi (Table 3) were similar in S and T groups, the fraction area of total cells was smaller in lung tissue associated with a reduction in mononuclear cells and an increase in neutrophils in T group (Table 1). Conversely, the total cell count in BALF and plasma were higher in T than S groups. Neutrophils were also more elevated in T than S groups in PLF and plasma (Fig. 7). No significant differences between T and S groups were observed concerning pro- and anti-inflammatory mediators in BALF, PLF, and plasma (Fig. 8).

Effects of training in CLP groups. At day 7 following CLP, the T group exhibited a lower mortality rate compared with S group (30 vs. 70%) (Fig. 3). Lung static elastance, resistive, and viscoelastic pressures were lower in T than S (Fig. 4). The fraction area of alveolar collapse, neutrophil infiltration, interstitial edema (Table 1, Fig. 5) as well as collagen and elastic fiber content in alveolar septa were more reduced in T group than S.

Furthermore, S group showed distortion of lung parenchyma structure, degeneration of lamellar bodies, denudation of basement membrane, damage in microvilli and hyaline membrane, and apoptosis of type II pneumocytes (Fig. 6). In T group, type II pneumocytes were well preserved, with the integrity of lamellar bodies and typical microvilli projecting from the cell surface. The greater integrity of type I pneumocytes, alveolar-capillary membrane, endothelial cells, basement membrane, and alveolar space could also be observed (Fig. 6, Table 2).

Table 1. Lung morphometry, tissue cellularity, collagen, and elastic fiber content

<table>
<thead>
<tr>
<th>Groups</th>
<th>Normal</th>
<th>Alveolar Collapse</th>
<th>Total Cells</th>
<th>Neutrophil</th>
<th>MN</th>
<th>Collagen Fibers</th>
<th>Elastic Fibers</th>
</tr>
</thead>
<tbody>
<tr>
<td>S C</td>
<td>89.1 ± 1.1</td>
<td>10.9 ± 1.1</td>
<td>54.3 ± 2.1</td>
<td>2.9 ± 0.4</td>
<td>35.6 ± 0.8</td>
<td>0.20 ± 0.01</td>
<td>0.026 ± 0.006</td>
</tr>
<tr>
<td>T C</td>
<td>89.8 ± 0.6</td>
<td>10.2 ± 0.6</td>
<td>49.8 ± 1.6*</td>
<td>4.4 ± 0.4*</td>
<td>32.1 ± 1.2*</td>
<td>0.23 ± 0.03</td>
<td>0.027 ± 0.005</td>
</tr>
<tr>
<td>S CLP</td>
<td>80.8 ± 0.8*</td>
<td>19.2 ± 1.5*</td>
<td>51.8 ± 0.8*</td>
<td>16.6 ± 1.9*</td>
<td>24.8 ± 1.7*</td>
<td>0.68 ± 0.06*</td>
<td>0.044 ± 0.005*</td>
</tr>
<tr>
<td>T</td>
<td>87.3 ± 0.9†‡</td>
<td>12.7 ± 0.9†‡</td>
<td>56.6 ± 1.7†‡</td>
<td>9.7 ± 1.0†‡</td>
<td>30.9 ± 1.6‡</td>
<td>0.26 ± 0.03‡</td>
<td>0.032 ± 0.007‡</td>
</tr>
</tbody>
</table>

Values are means ± SD in % of 13 animals in each group. All values were computed in 10 random, noncoincident fields per mice. Fraction area of normal and collapsed alveoli. MN, mononuclear cells; S, sedentary; T, trained. In CLP groups, animals were submitted to cecal ligation and puncture. A sham-operated group was used as control (C) for animals undergoing CLP. *Significantly different from S-C group (P < 0.05). †Significantly different from T-C group (P < 0.05). ‡Significantly different from S-CLP (P < 0.05).

Table 2. Semi-quantitative analysis of electron microscopy

<table>
<thead>
<tr>
<th>Groups</th>
<th>Type I Epithelial Lesion</th>
<th>Type II Epithelial Lesion</th>
<th>Denudation of Basement Membrane</th>
<th>Alveolar Collapse</th>
<th>Endothelial Cell Lesion</th>
<th>Endothelial Cell Apoptosis</th>
<th>Hyaline Membrane</th>
</tr>
</thead>
<tbody>
<tr>
<td>S C</td>
<td>0 (0–0)</td>
<td>0 (0–0)</td>
<td>0 (0–0)</td>
<td>0 (0–0)</td>
<td>0 (0–0)</td>
<td>0 (0–0)</td>
<td>0 (0–0)</td>
</tr>
<tr>
<td>T</td>
<td>0 (0–0)</td>
<td>0 (0–0)</td>
<td>0 (0–0)</td>
<td>0 (0–0)</td>
<td>0 (0–0)</td>
<td>0 (0–0)</td>
<td>0 (0–0)</td>
</tr>
<tr>
<td>S CLP</td>
<td>2 (1.75–2.25)*</td>
<td>3 (2.25–3.25)*</td>
<td>2 (1–2.25)*</td>
<td>3 (2.75–3.25)*</td>
<td>4 (3–4)*</td>
<td>2 (1.75–2.25)*</td>
<td>2 (1.75–2.25)*</td>
</tr>
<tr>
<td>T</td>
<td>1 (1–1)†‡</td>
<td>2 (1.75–2.25)†‡</td>
<td>1 (0.75–1.25)†‡</td>
<td>2 (1–2)†‡</td>
<td>2 (1–2)†‡</td>
<td>1 (0–1)†‡</td>
<td>1 (0–1)†‡</td>
</tr>
</tbody>
</table>

Values are median (25th–75th percentile) of 5 animals per group. Pathologic findings were graded according to a 5-point semi-quantitative severity-based scoring system: 0 = normal lung parenchyma, 1 = changes in 1 to 25%, 2 = 26 to 50%, 3 = 51 to 75%, and 4 = 76 to 100% of examined tissue.

*Significantly different from S-C group (P < 0.05). †Significantly different from T-C group (P < 0.05). ‡Significantly different from S-CLP (P < 0.05).
The number of lung, heart, liver, kidney, and small intestine villi apoptotic cells was lower in T compared with S (Table 3). The total and differential cell count in PLF and plasma were lower in T than S. In contrast, no significant changes were observed in total cell count in BALF between S and T groups, but there was a reduction in neutrophils in T group (Fig. 7). Moreover, in PLF, IL-1β, IL-6, and KC levels were lower in T compared with S group, whereas in the plasma, only KC was more reduced. In BALF, PLF, and plasma, IL-10 level was higher in T group (Fig. 8).

**DISCUSSION**

In the current study, normal mice submitted to regular and moderate exercise presented an increase in the total cell count and neutrophils in plasma, with no lung or systemic effects. The total and differential cell count in PLF and plasma were lower in T than S. In contrast, no significant changes were observed in total cell count in BALF between S and T groups, but there was a reduction in neutrophils in T group (Fig. 7). Moreover, in PLF, IL-1β, IL-6, and KC levels were lower in T compared with S group, whereas in the plasma, only KC was more reduced. In BALF, PLF, and plasma, IL-10 level was higher in T group (Fig. 8).

The number of lung, heart, liver, kidney, and small intestine villi apoptotic cells was lower in T compared with S (Table 3). The total and differential cell count in PLF and plasma were lower in T than S. In contrast, no significant changes were observed in total cell count in BALF between S and T groups, but there was a reduction in neutrophils in T group (Fig. 7). Moreover, in PLF, IL-1β, IL-6, and KC levels were lower in T compared with S group, whereas in the plasma, only KC was more reduced. In BALF, PLF, and plasma, IL-10 level was higher in T group (Fig. 8).

Table 3. *Cell apoptosis*

<table>
<thead>
<tr>
<th>Groups</th>
<th>Lung</th>
<th>Heart</th>
<th>Liver</th>
<th>Kidney</th>
<th>Villi</th>
</tr>
</thead>
<tbody>
<tr>
<td>S C</td>
<td>0 (0–1)</td>
<td>0 (0–0.25)</td>
<td>0 (0–1)</td>
<td>0 (0–1)</td>
<td>0 (0–1)</td>
</tr>
<tr>
<td>T C</td>
<td>0 (0–1)</td>
<td>1 (0–1)</td>
<td>0 (0–1)</td>
<td>0 (0–1)</td>
<td>0 (0–0.25)</td>
</tr>
<tr>
<td>S CLP</td>
<td>2 (1.75–2.25)*</td>
<td>3 (2–3.25)*</td>
<td>2 (1.75–3)*</td>
<td>3 (2–3.25)*</td>
<td>3 (2–3.25)*</td>
</tr>
<tr>
<td>T CLP</td>
<td>1 (1–1)‡</td>
<td>1 (0.75–1.25)‡</td>
<td>1 (0–1)‡</td>
<td>1 (0.75–1.25)‡</td>
<td>1 (0.75–2)†‡</td>
</tr>
</tbody>
</table>

Values are median (25th–75th percentile) of 5 animals per group. Apoptotic findings were graded as negative = 0, slight = 1, moderate = 2, high = 3, and severe = 4 in 10 noncoincident microscopic fields (x400 magnification). *Significantly different from S-C group (P < 0.05). †Significantly different from T-C group (P < 0.05). ‡Significantly different from S-CLP (P < 0.05).

Exercise also acted on the balance between pro- and anti-inflammatory cytokines, decreasing IL-1β, IL-6, and KC levels in PLF and increasing IL-10 in plasma, BALF, and PLF. To our knowledge, this study is the first to report the efficacy of regular and moderate exercise in attenuating the inflammatory and remodeling processes in a CLP-induced sepsis model.

The modulation of immune responsiveness as a result of exercise has been extensively studied (8, 9). Regular exercise training prior to lipopolysaccharide-induced sepsis was reported to cause important physiological responses, such as reduced organ injury from septic shock (6, 12). Nevertheless, this particular sepsis model does not comprehensively mimic the changes observed in human sepsis. In the present study, the CLP model was used because 1) it is reproducible and more comparable to human surgical sepsis, 2) apoptosis of selected cell types and host immune responses seem to mimic those of human sepsis, and 3) it is considered to be a relevant model for abdominal sepsis therapy research (2, 4, 19, 20, 24).

The trained group used a specific motorized treadmill for 30 min, during 8 wk, at a speed of 8–12 m/min and 5% grade,
corresponding to ~55–65% of maximal oxygen uptake (VO₂max), as determined for the animals' age group in previous experiments (15, 16). This mode of exercise was chosen because intensity and duration could be manipulated experimentally, unlike swimming or voluntary running (21). Additionally, the benefits of the treadmill include the repeatability of training volume and many consistent physiological adaptations, such as changes in skeletal and cardiac muscle after 8 wk of training (17). In this respect, the T group demonstrated an increase in left ventricular mass and diameter and in systolic and diastolic volumes, suggesting natural cardiac adaptation to exercise.

The lung is one of the first organs to be affected by sepsis; cellular infiltration and the release of pro-inflammatory mediators resulted in the development of acute lung injury. In this context, CLP animals showed increased lung static elastance and lung viscoelastic/inhomogeneous pressure, which was likely the result of increased amount of alveolar collapse and neutrophil infiltration (Table 1), interstitial edema, hyaline membrane, and changes in collagen fiber content. Additionally, there was an increase in ΔP1L, likely imposed by a reduction in bronchial caliber caused by airway inflammation (4). Electron microscopy revealed damaged type II pneumocytes and endothelial cells, swelling of lamellar bodies, and denudation.

![Graph showing interleukin (IL)-6, IL-1β, KC, and IL-10 levels in BALF, PLF, and plasma.](http://jap.physiology.org/)

Fig. 8. Interleukin (IL)-6, IL-1β, KC (murine IL-8 homolog), and IL-10 levels in BALF, PLF, and plasma. Each symbol represents an individual animal. Horizontal line: mean value for each group. *Significantly different from S-C group (P < 0.05). **Significantly different from T-C group (P < 0.05). #Significantly different from S-CLP group (P < 0.05).
of basal membrane (Fig. 5 and Table 2). Lung static elastance, resistive, and viscoelastic pressures were lower in T than S, and, in agreement with previous data (18), exercise reduced lung injury minimizing edema formation.

Regular and moderate exercise promotes a specialized low-grade systemic inflammatory response that is dominated by a transient increase in IL-6 and offers protection against diseases associated with systemic inflammation. The anti-inflammatory effect of exercise-induced IL-6 reduction seems to be associated with inhibition of TNF-α and IL-1β production while increasing the production of IL-1ra and IL-10, important anti-inflammatory cytokines (23), and increased adrenal response (30). IL-6 also promotes enhanced neutrophil killing (27) and survival to sepsis and infection with E. coli, K. pneumoniae, and S. pneumoniae (7, 29, 30). Therefore, exercise may promote important changes in the balance between pro- and anti-inflammatory cytokines that are important for an appropriate immune response during sepsis. In the present study, exercise reduced the levels of IL-1β, IL-6, and KC in PLF, indicating a reduction in local inflammatory response resulting from more effective infection clearance. Additionally, increased IL-10 in BALF, PLF, and plasma was associated with a reduction in apoptosis of lung, liver, kidney, villi, and heart in T-CLP compared with S-CLP, suggesting a protective effect of exercise in systemic inflammatory response (12, 18, 23).

Systemic neutrophil activation and migration into peripheral organs are major contributors to multiple organ failure in sepsis. Of note, migration of neutrophils into the lung tissue plays a key role in the cascade of events leading to respiratory failure (12) and contributes to the high mortality rate in critically ill patients (13). Infiltrated neutrophils may release a vast array of pro-inflammatory cytokines, particularly TNF-α and IL-1β, which contribute to the propagation of tissue damage. In S-CLP group, neutrophil numbers were elevated in lung tissue, BALF, PLF, and plasma (Fig. 6), characterizing a systemic inflammatory response. Exercise markedly inhibited the neutrophilia in all compartments analyzed.

Changes in neutrophil numbers did not correlate with systemic changes in IL-1β or IL-6. Chen and coworkers (6) observed that IL-1β was elevated in the plasma of animals treated with LPS. The observed differences between our results and those of Chen and colleagues may be attributed to the particular model used to induce sepsis, and it is likely that these cytokines increase in plasma only at a later phase in CLP model (31). In S-CLP, the increase in KC in BALF may be related to endothelial damage, because KC is produced by endothelial cells that play a crucial role in monocyte/macrophage and neutrophil activation and recruitment to the inflammatory site (6, 23). Exercise was not able to modify the level of KC in the BALF, which may indicate that exercise does not modify the initial lung damage-induced sepsis, but may inhibit the perpetuation and/or amplification of the lung tissue damage by neutrophils, possibly due to increased systemic IL-10 levels.

CLP induced increased accumulation of collagen fibers and elastin in the lung, as previously reported (4). The exercise-induced modulation of lung fibrogenesis and elastogenesis may be determined by the balance between inflammatory and anti-inflammatory mediator responses, as well as differences in cellularity and apoptosis in the lung (22, 26).

The current study has some limitations that need to be addressed. First, although CLP has been considered a good model of abdominal sepsis, we do not know if these results can be directly extended to other experimental models of sepsis. Second, bacteria recovered from peritoneal and blood samples were not measured, limiting the information regarding the effect of exercise on bacteria proliferation. Third, electron microscopy and cell apoptosis data are somewhat subjective and qualitative, but these analyses were qualitatively assessed by two pathologists in a blinded fashion.

In conclusion, regular and moderate exercise prior to sepsis induced by CLP modulated the inflammatory and fibrogenic processes, prevented acute lung injury, and attenuated cell apoptosis in lung and distal organs, thus improving survival rate. These findings suggest that regular and moderate exercise induces favorable changes in immune system and may reduce the severity of sepsis in the clinical setting.

ACKNOWLEDGMENTS

The authors express their gratitude to Andre Benedicto da Silva for animal care, Thaiana Borges de Sousa for skillful technical assistance during the experiments, Ana Lucia Neves da Silva for help with microscopy, and Claudia Buchweitz and Moira Elizabeth Schöttler for assistance in editing the manuscript.

GRANTS

This work was supported by the Centers of Excellence Program (PRONEX-FAPERJ), the Brazilian Council for Scientific and Technological Development (MCT/CNPq), the Carlos Chagas Filho Rio de Janeiro State Research Supporting Foundation (FAPERJ), the São Paulo State Research Support Foundation (FAPESP), the National Institute of Science and Technology of Drugs and Medicine (INCT-INOFAR), and Coordination for the Improvement of Higher Level Personnel (CAPES).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES


EXERCISE REDUCES THE SYSTEMIC INJURY IN SEPSIS


