Exercise Benefits in Chronic Graft versus Host Disease: A Murine Model Study

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ABSTRACT

FIUZA-LUCES, C., L. SOARES-MIRANDA, Á. GONZÁLEZ-MURILLO, J. M. PALACIO, I. COLMENERO, F. CASCO, G. J. MELÉN, A. DELMIRO, M. MORÁN, M. RAMÍREZ, and A. LUCIA. Exercise Benefits in Chronic Graft versus Host Disease: A Murine Model Study. *Med. Sci. Sports Exerc.*, Vol. 45, No. 9, pp. 1703–1711, 2013. **Introduction**: Chronic graft versus host disease (cGVHD) is a life-threatening complication of allogeneic hematopoietic stem cell transplantation that generates considerable morbidity and compromises the physical capacity of patients. We determined the effects of an exercise training program performed after allogeneic hematopoietic stem cell transplantation on clinical and biological variables in a minor histocompatibility antigen–driven murine model of cGVHD treated with cyclosporine A. **Methods**: Recipient BALB/C female mice (age 8 wk) received bone marrow cells and splenocytes from donor B10.D2 male mice and were randomly assigned to an exercise (n = 11) or control group (n = 12). For approximately 11 wk after transplant, the exercise group completed a moderate-intensity treadmill program. Variables assessed were clinical severity scores, survival, physical fitness, cytokine profile, immune cell reconstitution, molecular markers of muscle exercise adaptations, and histological scores in affected tissues. **Results**: Exercise training increased survival (P = 0.011), diminished total clinical severity scores (P = 0.002), improved physical fitness (P = 0.030), and reduced blood IL-4 and tumor necrosis factor α levels (P = 0.03), while increasing circulating B220 (P = 0.008) and CD4 lymphocytes (P = 0.043). **Conclusions**: A moderate-intensity exercise program that mimics widely accepted public health recommendations for physical activity in human adults was well tolerated and positive effects on survival as well as on clinical and biological indicators of cGVHD. **Key Words:** EXERCISE IS MEDICINE, HEMATOPOIETIC STEM CELL TRANSPLANTATION, INFLAMMATION, IMMUNE RECONSTITUTION

Recent advances in allogeneic hematopoietic stem cell transplantation (allo-HSCT) have led to an expanding population of long-term survivors, many of whom suffer severe side effects, particularly those related to graft versus host disease (GVHD) (13,20). GVHD encompasses several clinical and histological manifestations caused by activated donor T cells interacting with tissue antigens in the immunosuppressed host (13). In the acute disease form (aGVHD), exacerbated inflammation leads to lesions in skin, liver, lungs, and the gastrointestinal tract, whereas virtually any organ can be affected in the chronic form (cGVHD) (40). The latter characteristically presents as autoimmune and alloimmune deregulation and a preferential cytokine production pattern linked to CD4+ T helper 2 cells (Th2) (36). cGVHD occurs in 30%–65% of allo-HSCT recipients and shows a 5-yr mortality rate of 30%–50% mainly due to immune deregulation and opportunistic infections (5). In addition, this disease can be highly debilitating and lead to a poor health state, considerably impairing patients' quality of life and physical functioning (8,16,22). Once the disease has developed, immunosuppressive drug treatment is the only current therapeutic option (11), yet the likelihood of a good response decreases with the severity of disease (26). When successful, survivors have to live with treatment side effects, particularly muscle toxicity and an increased risk of infections, which further deteriorates their health and physical capacity (13,30).

There is strong evidence supporting a broad range of therapeutic benefits associated with regular, moderate-intensity aerobic exercise, including decreased systemic inflammation and improved immune function (6). Despite numerous studies addressing the effects of exercise in human allo-HSCT recipients, no study has specifically examined the effects of exercise on

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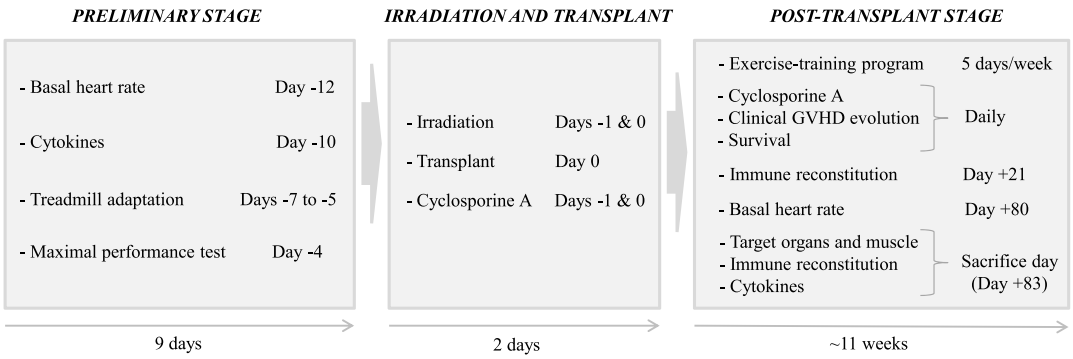

FIGURE 1—Diagram of the study protocol.

cGVHD. To the best of our knowledge, only one previous study has assessed the effects of exercise in a murine GVHD model with no immunosuppressive treatment (14). We hypothesized that adding an exercise intervention to the standard immunosuppressive therapy for GVHD could improve the clinical course of the disease and survival while minimizing impaired functional capacity with no harmful effects on immune function. Our study was designed 1) to assess the effect of an exercise program on the clinical course of the disease (severity), survival, and physical capacity (primary study outcomes) in mice with cGVHD receiving standard immunosuppressive treatment (cyclosporine A) compared with a control, nonexercise cGVHD group receiving the same drug; and 2) to evaluate the effects of this intervention on inflammatory markers, immune reconstitution, skeletal muscle molecular adaptations to exercise, and histological/clinical state of target organs of the disease (secondary outcomes).

METHODS

The study was in adherence with the animal care standards of the American College of Sports Medicine. All procedures were carried out according to European and Spanish legislative and regulatory guidelines (European convention ETS 1 2 3, on the use and protection of vertebrate mammals in experimentation and for other scientific purposes and Spanish Law 32/2007, and R.D. 1201/2005 on the protection and use of animals in scientific research). The study protocol received animal experimentation institutional review board approval (Centre of Energy, Environment and Technical Research [CIEMAT], Madrid, Spain).

Animals

The experimental animals were BALB/C (recipient) and B10.D2 (donor) mice bred at the animal house (registration no. ES280790000183) of the CIEMAT (Madrid, Spain) from breeding pairs originally obtained from Jackson Laboratory (Bar Harbor, ME). All mice were housed in Eurostandard type IIL microisolator cages (five mice maximum in each) under controlled conditions of temperature and humidity (20°C ± 2°C and 55% ± 10%, respectively). The cages were lit (fluorescent lighting) from 7:00 a.m. to 7:00 p.m., and food (Harlan Teklad Global Diets 2914) and water (50 μm filtered and UV irradiated) were provided *ad libitum*. Mice were routinely screened for pathogens in accordance with recommendations of the Federation of European Laboratory Animal Science Associations.

Twenty-three female mice (age, 8 wk; weight, 18–20 g) underwent allo-HSCT to induce cGVHD. Transplanted mice were randomly assigned to the groups: exercise (n = 11, subjected to ~11 wk of treadmill exercise training) or control (n = 12, no exercise training and movement confined to their cages).

Study Design

All mice were treated with cyclosporine A (15 mg·kg^{-1}; Sigma Chemical Co., EEUU) each day of the week (Monday–Sunday) from the first day of irradiation to day +82. Cyclosporine A was prepared in sterilized conditions and diluted in a final volume of 100 μL of 1× phosphate-buffered saline (PBS). Mice were weighted every day (Monday–Sunday) before drug administration for adjusting dosage and handled by the same researcher to minimize subjectivity. The study protocol (which is summarized in Fig. 1) included three main stages: (i) preliminary, (ii) irradiation and transplant, and (iii) posttransplant, when the exercise training intervention was performed.

Preliminary stage. On day −12 (i.e., healthy conditions), basal heart rate was measured during the animal's dark cycle using a throat sensor for anesthetized mice (MouseOx®; STARR Life Sciences Corp., Oakmont, PA). This procedure was conducted for 20–30 min, and when heart rate stabilized, we calculated the average heart rate for the stabilization period. As anesthetic, we used isoflurane gas (IsoFlo; Abbot Laboratories, UK) in an MSS.3 continuous system (Medical Supplies and Services International Ltd., Keighley, UK) with oxygen as the carrier gas. Doses were 4% for anesthesia induction and 1.5%–2% for maintenance.

On day −10, blood was drawn from each mouse (100 μL, tail vein) to determine serum cytokine levels (see following sections). Serum was separated by two 10-min centrifugations at 1600 rpm, and the samples were stored at −80°C until analysis.

All mice from the two study groups were allowed to adapt to the treadmill (Harvard Apparatus; Panlab, Barcelona, Spain) in three sessions (days −7, −6, and −5). This adaptation period involved a gradual increase in running time and intensity, starting with placement on the treadmill the first day (0% inclination and 0 $cm \cdot s^{-1}$ speed for 1 min, with no electrical stimulation) and ending with a 15-min period on the treadmill at a low running intensity on the third day (25% and 15 $cm \cdot s^{-1}$, electrical stimulation 0.2 mA, 1 Hz, 200 ms) (23).

When the mice had adapted to the treadmill (day −4), they were subjected to a maximal treadmill test to determine training intensity based on the maximal velocity reached by the mice (V_{max}) (23). The test was performed after a warm-up period of 10 min at 10 $cm \cdot s^{-1}$. The initial velocity was 5 $cm \cdot s^{-1}$, and this was followed by increases of 3 $cm \cdot s^{-1}$ every 2 min until exhaustion. The treadmill inclination was kept constant at 25% because this gradient elicits maximal cardiovascular intensity while preventing injuries (23). Mice were defined as exhausted when they spent more than five continuous seconds on the electric grid and were unable to continue running at the next speed (3).

Irradiation and transplant. On days −1 and 0, the mice were given a myeloablative regimen consisting of two sessions of lethal total body irradiation (300 kV, 10 mA; Philips MG-324, Hamburg, Germany) with a 24-h interval between sessions to minimize gastrointestinal toxicity (dose per session, 4.75 Gy). For the transplant, we used B10.D2 male mice (12–18 wk of age). Donor bone marrow cells were prepared by flushing femurs and tibias. Splenocytes were harvested by passing spleens through steel mesh with 1× PBS, 2% fetal bovine serum, and 0.5 M ethylenediaminetetraacetic acid (EDTA). Recipient mice were transplanted intravenously through the tail vein with a single inoculum of 10×10^6 splenocytes and 10×10^6 bone marrow cells. The allo-HSCT model used in this study was a major histocompatibility complex–matched, minor histocompatibility antigen–mismatched model: B10.D2 (H-2^d) ⟶ BALB/C (H-2^d) (37). This is a CD4 T-cell-dependent chronic sclerodermatous model of GVHD (38).

Posttransplant stage. Two days after allo-HSCT (day +2), mice were started on the ~11-wk exercise training program, which consisted of treadmill running 5 $d \cdot wk^{-1}$ (Monday–Friday). Exercise duration, treadmill speed, and inclination were gradually increased during the program, that is, beginning at very low workloads in the first session (25 min at 35% V_{max} and 0% gradient in the first day) and ending with 60 min at 70% V_{max} and 25% gradient). During the training sessions between 7:15 a.m. and 12:00 p.m., no electrical stimulation was applied.

On day 80 posttransplant, basal heart rate measurements were made as described above to assess the functional state of the mice. Survival and clinical variables indicative of GVHD were assessed daily. On day +21, 100 μL of blood was drawn from the tail vein to assess cellular immune reconstitution. Finally, on day +83, mice were killed by intraperitoneal injection with a lethal dose of Avertin (0.2%, 0.15 $mL \cdot g^{-1}$), and whole blood was extracted (axillary vein) for cytokine analysis. Skeletal muscle tissue from the posterior limbs was then dissected and immediately frozen in liquid nitrogen and stored at −80°C until the molecular analysis of muscle adaptations. Target tissues of the disease (liver, intestine, and skin) were fixed in formalin for histological analysis, and spleens were stored in 1× PBS and 0.5 M EDTA to assess immune reconstitution.

Study Outcomes

Primary outcomes. Each day after allo-HSCT, animals were individually scored by the same researcher (who was blinded to animals' group assignment) for disease severity parameters on a scale from 0 (absence) to 2 (maximal severity grade) (14): percentage of weight loss, score of 0, 1, or 2 for weight loss <10%, >10%, and <25% or >25%, respectively; posture, 0, 1, or 2 for normal, hunching noted only, and rest or severe hunching impairing movement; fur texture, 0, 1, or 2 for normal, mild to moderate ruffling, or severe ruffling/poor grooming; skin integrity, 0, 1, or 2 for normal, scaling of paws/tail, or obvious areas of denuded skin; and activity, 0, 1, or 2 for normal, mild to moderately decreased, or stationary unless stimulated. A total GVHD score (0–10) was generated daily by adding individual scores. Survival data were provided as Kaplan–Meier plots. Changes in physical capacity were determined as the difference between pre- and posttransplant heart rate (corresponding to pre- versus posttraining, respectively, for the exercise group) (12).

Secondary outcomes. The following cytokine levels were quantified in serum samples (days −10 and +83) using BioPlex ProTM, Mouse Cytokine Standard Group I 23-Plex (Laboratories Inc., Hercules, CA) according to the manufacturer's instructions: interleukin (IL)-2, IL-4, IL-6, IL-10, IL-17A, interferon γ (IFN-γ), and tumor necrosis factor α (TNF-α). Absolute numbers of peripheral blood leukocytes were obtained using an AC.T Cell Counter (Beckman Coulter Spain S.A., Madrid, Spain). Blood (day +21) and spleen (day +83) immune reconstitution analyses were performed by flow cytometry using the following antibodies purchased from BD Bioscience (San Agustin de Guadalix, Madrid, Spain): antimouse B220 (clone RA3-6B2), antimouse CD3 (clone 145–2C11), antimouse CD4 (clone RM4–5), antimouse CD8 (clone 53–6.7), antimouse CD11b/Mac1 (clone ICRF44), and their respective isotype controls. Cells were acquired and analyzed with a FACS Canto II flow cytometer (BD Bioscience; San Agustin de Guadalix).

Citrate synthase activity, a classic marker of muscle oxidative capacity (35), was spectrophotometrically determined in skeletal muscle homogenates at 30°C in the presence of 0.1% Triton X-100 following the formation of 5-thio-2-nitrobenzoic acid at 412 nm, as previously described (39). The activity of mitochondrial respiratory chain complexes I, II, III, IV, I + III, and II + III was also determined as an indicator of oxidative capacity in skeletal muscle homogenates (in 225 mM mannitol, 75 mM sucrose, 0.1 mM EDTA,

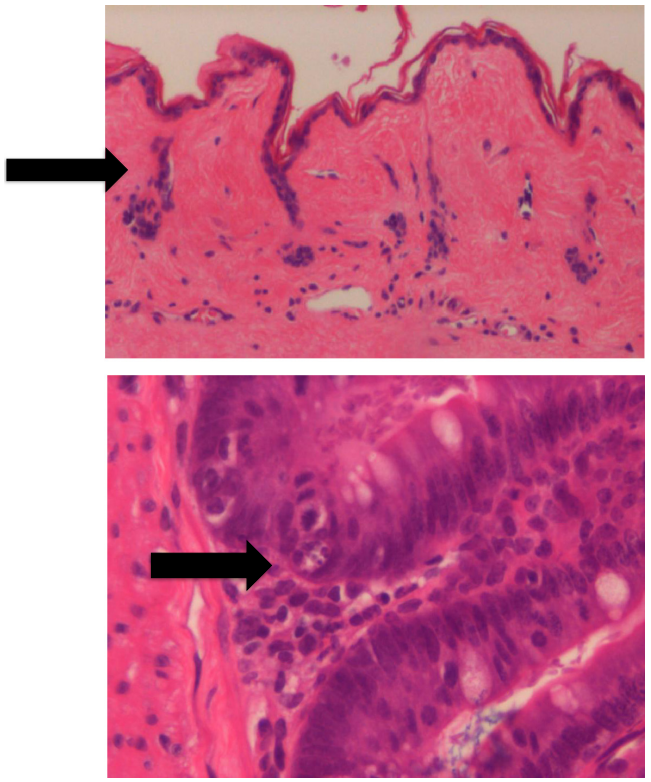

FIGURE 2—Skin histology, showing thickening of the dermis (scleroderma) (upper panel). Gut histology, showing cell apoptosis in crypts (lower panel).

and 10 mM Tris–HCl pH 7.4) according to the standardized protocols for spectrophotometric assays of the respiratory chain described by Medja et al. (28). In addition, samples of skeletal muscle homogenates (40 and 20 mg) were also used for the semiquantitative analysis of p70 S6 kinase and phospho-p70 S6 kinase protein levels by immunoblotting (Western blot). Sodium dodecyl sulfate–polyacrylamide gel electrophoresis was performed on a 7.5% separation gel. Resolved proteins were transferred to a PVDF membrane. Blots were blocked and incubated with the following primary antibodies: rabbit anti-p70 S6 kinase and rabbit anti-phospho-p70 S6 kinase (Thr389) (Cell Signalling Technology, Inc., Izasa, Barcelona, Spain). Primary antibodies were immunodetected with peroxidase-conjugated goat antirabbit antibodies (GE Healthcare, Madrid, Spain) using the NovexR ECL HRP Chemiluminescent Substrate Reagent Kit (Life Technologies S.A.; Alcobendas, Spain) to detect the signal. Band densities were determined by densitometric scanning (Image J software). To check that the total protein amount loaded in each lane was the same, α-tubulin was immunodetected with mouse anti-α tubulin (Sigma-Aldrich, Madrid, Spain) coupled to peroxidase-conjugated goat antimouse antibody (GE Healthcare). We calculated the ratio of phospho-p70 S6 kinase to p70 S6 kinase as a marker of muscle anabolism (33).

Samples of the disease's target organs were histologically examined by fixing in 7% formaldehyde solution and embedding in paraffin followed by staining 5 mm-thick sections with hematoxylin and eosin (see Fig. 2 for an example). Cumulative histopathology scores were calculated according to a mouse GVHD disease grading system in which lesions are assigned 0–4 points: 0 = normal, 0.5 = focal and rare, 1.0 = focal and mild, 2.0 = diffuse and mild, 3.0 = diffuse and moderate, and 4.0 = diffuse and severe (41). Scores were added to provide a total score for each specimen. Two independent pathologists analyzed the slides in a blinded fashion to assess disease intensity.

Statistical Analysis

Statistical significance was set at 0.05, and data were shown as mean ± SD. We used a two-factor (group × time) ANOVA with repeated measures on time to assess the effect of an exercise intervention on clinical GVHD total and individual scores as well as on blood inflammatory (cytokine) profiles. Survival data in Kaplan–Meier plots were compared between groups using the log-rank test. The Mann–Whitney *U* test was used to compare changes in physical capacity (heart rate pretransplant minus posttransplant) between the two groups as well as the variables collected only once: immune cell reconstitution in blood (day +21) and spleen immune reconstitution, muscle molecular markers of exercise adaptation (citrate synthase and respiratory complexes activities, and phospho-p70 S6 kinase–p70 S6 kinase ratio), and histological findings in organs (all on day +83).

RESULTS

Primary outcomes. The exercise intervention successfully reduced the clinical severity of cGVHD, as indicated by a significant group–time interaction effect on the total severity score ($P = 0.002$) and individual scores of weight ($P < 0.001$), posture ($P = 0.002$), and activity ($P < 0.001$) (Fig. 3). In parallel with a better clinical outcome, the survival rate was significantly higher ($P = 0.011$) in the exercise group (54.5%) than that in the control group (16.7%) (Fig. 4).

Significant between-group differences were also detected in changes in physical capacity, as determined by the difference between pre- and posttransplant basal heart rate ($P = 0.030$), that is, basal heart rate remained virtually stable in the control group (356 ± 4 and 346 ± 29 beats·min^{-1} at pre- and posttransplant, respectively, mean difference pre- minus posttransplant = 10 ± 23 beats·min^{-1}) but decreased considerably in the exercise group after the training program (395 ± 45 and 316 ± 70 beats·min^{-1} at pre- and posttransplant, respectively; mean difference = 79 ± 11 beats·min^{-1}), reflecting an improved fitness status in the latter.

Secondary outcomes. We quantified the levels of IL-2, IL-4, IL-6, IL-17A, IFN-γ, and TNF-α cytokines

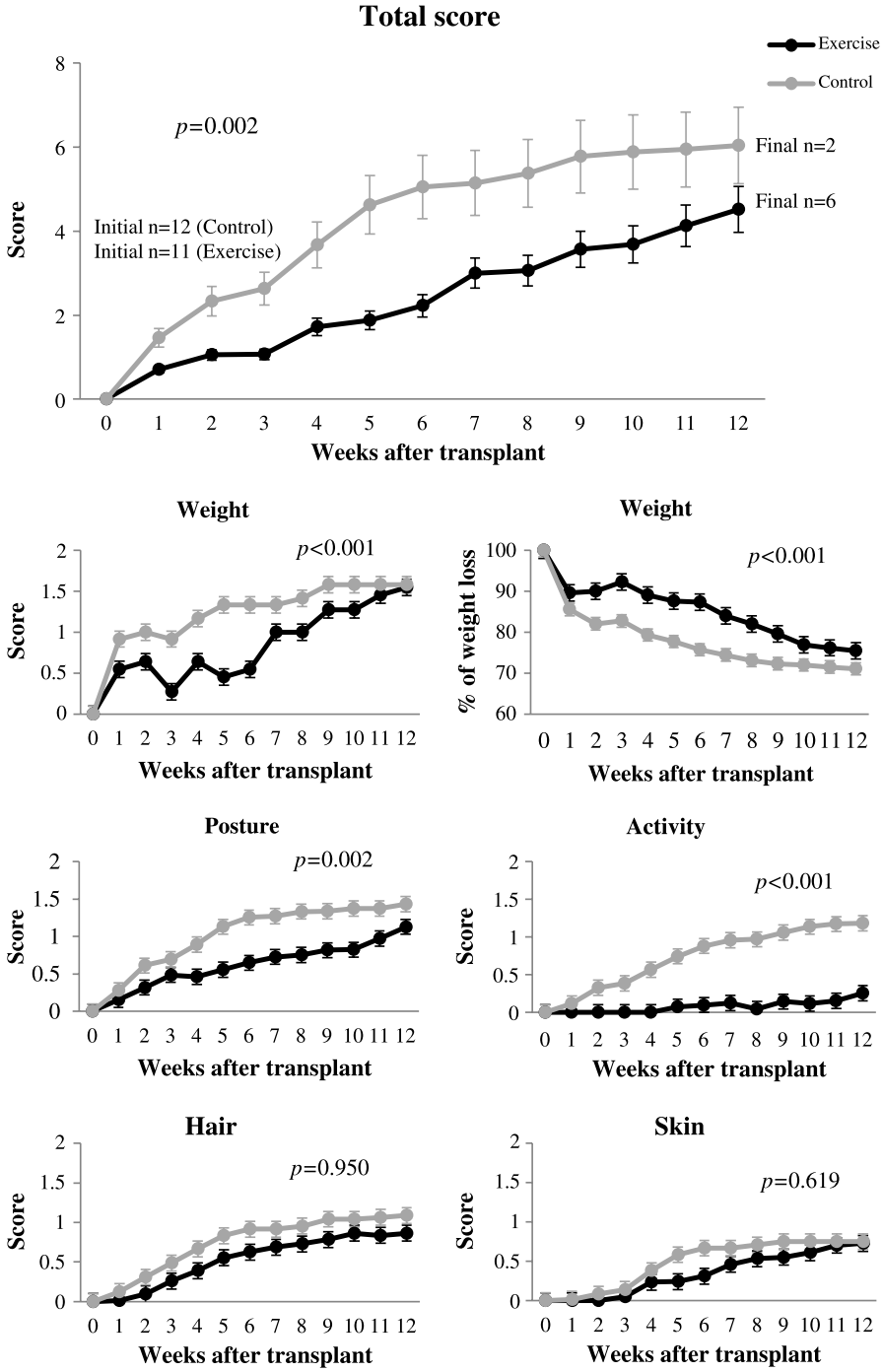

FIGURE 3—Daily clinical disease progression (total and individual cGVHD severity scores) by group. The *P* value for each score corresponds to the interaction (group–time) effect. Effect size (η^2) and the statistical power of significant interaction (group–time) effects were as follows: 0.115 and 0.982 (total clinical score), 0.133 and 0.995 (weight), 0.114 and 0.981 (posture), and 0.284 and 1.000 (activity), respectively.

in the peripheral blood of transplanted mice at baseline (day −10, before transplant) and at 12 wk posttransplant. The exercise intervention resulted in a significant decrease in IL-4 and TNF-α levels (both $P = 0.03$ for the interaction effect), and the mean levels of these two cytokines showing severalfold increases after transplantation in the control group but minimal changes in the exercised mice (Table 1).

Immune reconstitution was assessed in peripheral blood (day +21) and in spleen (day +83). On day +21, the exercise group showed significantly higher numbers of circulating B220 ($P = 0.008$) and CD4 lymphocytes ($P = 0.043$) and a trend toward higher CD3 lymphocyte counts ($P = 0.069$) (Fig. 5). No between-group differences emerged for spleen immune reconstitution upon animal sacrifice (day +83), except

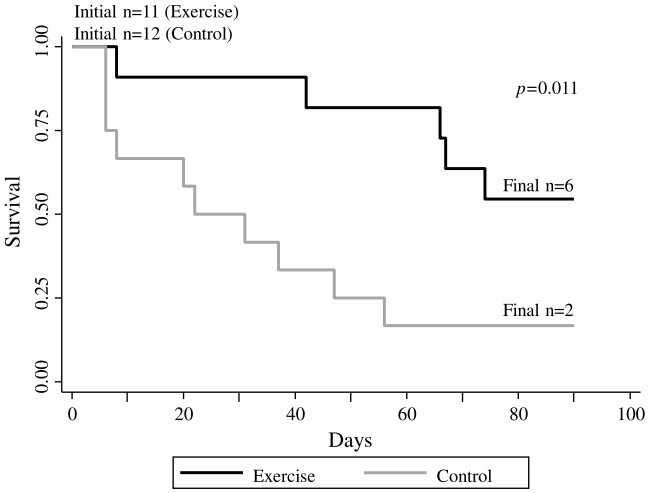

FIGURE 4—Survival estimates by group.

for a trend toward higher B220 and CD8 lymphocyte levels in the exercise group ($P = 0.061$).

Our analysis of muscle molecular markers of exercise adaptations revealed no between-group difference in citrate synthase activity (750 ± 162 versus 699 ± 135 $nmol \cdot min^{-1} \cdot mg^{-1}$ for the control and exercise groups, respectively, $P = 0.322$) or in respiratory chain complexes' activities (see Figure, Supplemental Digital Content, http://links.lww.com/MSS/A265, Activities of mitochondrial respiratory chain complexes in mice muscle homogenates by group. Abbreviations: C I to IV, complex I to IV) and a trend toward higher muscle anabolism (as determined by the phospho-p70 S6 kinase–p70 S6 kinase ratio) in the exercise group compared with the control group (98.3% ± 39.6% versus 67.4% ± 44.3% respectively, $P = 0.083$). Mice surviving the whole study period showed no between-group differences in histological scores recorded for GVHD target organs (liver: 0 ± 0 in both groups, $P = 1.000$; gut: 1.4 ± 1.1 [exercise] and 1.0 ± 0.0 [control], $P = 0.335$), except a trend ($P = 0.079$) toward less skin damage detected in the exercise (0.2 ± 0.4) compared with the control group (1.0 ± 1.4).

DISCUSSION

This study identifies the effects of a regular moderate-intensity exercise program mimicking the widely accepted public health recommendations for physical activity in humans (≥30 $min \cdot d^{-1}$ in most days of the week [21]) added to standard immunosuppressive therapy for cGVHD on both clinical and biological markers of disease progression and exercise adaptations. The exercise training intervention proposed was well tolerated by the transplanted mice receiving immunosuppressive therapy and had beneficial effects on survival and the clinical course of disease besides improving physical fitness. In addition, rather than compromising immune cell reconstitution, the exercise intervention had a beneficial effect on immune cell kinetics and led to a less aggressive inflammatory profile. Nevertheless, our findings must be interpreted with caution, owing to the low sample size of both groups at the end of the study, which is a consequence of the aggressive nature of cGVHD.

The present findings extend previous results emerging from our laboratory (14). We recently evaluated the effect of the same type of treadmill exercise program in a murine model of allo-HSCT-induced GVHD in the absence of immunosuppressive treatment. In this aGVHD model, exercise improved the physical capacity of the mice several weeks after transplant, returning it to its pretransplant healthy state, and animals in the exercise group showed a clinical course that was improved over that observed in control mice not undertaking exercise. In the subsequent cGVHD model, however, exercise only benefited physical capacity, suggesting this intervention alone is not sufficient to ameliorate the course of the disease in its more aggressive form.

In the present study, we found that exercise led to the significantly lower production of two cytokines strongly linked to GVHD: TNF-α and IL-4. TNF-α plays an important role in disease pathogenesis: it has both indirect effects (through activation/proliferation of T-cell pathways, the main cell effector of GVHD) and direct effects leading to apoptosis in GVHD target tissues (24). As such, TNF-α inhibition has been used as a therapeutic strategy in experimental therapies against GVHD. IL-4 has also been related to the pathophysiology of cGVHD (1), the disease form reproduced in our murine model. IL-4 production by CD8 has been linked to cGVHD severity in human patients and is an immunological marker of this disease (31) because of its inducing effects on B-lymphocytes (9). The mechanisms whereby regular exercise could diminish the production of these two GVHD mediators remain to be elucidated. However,

TABLE 1. Inflammatory profile by group.

	Exercise		Control		Interaction (Group–Time) Effect
	Pretransplant ($n = 11$)	12 wk Posttransplant ($n = 6$)	Pretransplant ($n = 12$)	12 wk Posttransplant ($n = 2$)	*P* Value, Effect Size (η^2), and Statistical Power
IL-2	26.9 ± 1.5	34.6 ± 9.7	26.4 ± 1.1	53.8 ± 15.9	$P = 0.308$, $\eta^2 = 0.204$, power = 0.153
IL-4	3.8 ± 0.2	4.5 ± 0.6	3.1 ± 0.2	14.2 ± 6.2	**$P = 0.025$**, $\eta^2 = 0.665$, power = 0.713
IL-6	24.6 ± 2.1	45.5 ± 15.2	21.6 ± 3.2	54.2 ± 2.9	$P = 0.673$, $\eta^2 = 0.039$, power = 0.066
IL-10	265.4 ± 20.1	255.6 ± 61.3	245.4 ± 10.3	457.3 ± 64.0	$P = 0.102$, $\eta^2 = 0.444$, power = 0.368
IL-17A	441.8 ± 36.4	620.4 ± 171.1	335.2 ± 61.3	948.1 ± 282.9	$P = 0.191$, $\eta^2 = 0.314$, power = 0.235
IFN-γ	49.2 ± 2.7	45.1 ± 12.4	44.3 ± 2.4	79.0 ± 4.5	$P = 0.093$, $\eta^2 = 0.461$, power = 0.389
TNF-α	1163.5 ± 99.6	1185.4 ± 400.2	1131.1 ± 90.6	2880.3 ± 109.6	**$P = 0.031$**, $\eta^2 = 0.638$, power = 0.662

Data are presented as mean ± SD. Significant *P* values are in bold.

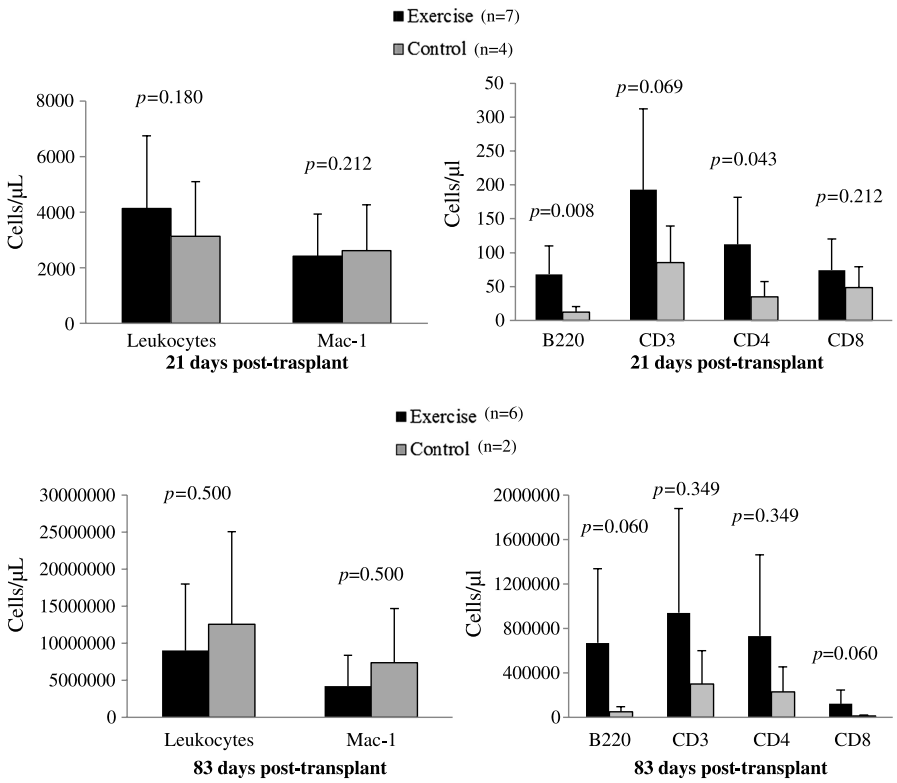

FIGURE 5—Blood and spleen immune reconstitution profiles by group.

it is known that, particularly in chronic diseases, exercise exerts its beneficial effects through the cumulative anti-inflammatory milieu created by frequent exercise bouts. During exercise, working skeletal muscles or adipose tissues transiently become active endocrine organs able to release anti-inflammatory cytokines such as the "myokine" IL-6 and the "adipokine" IL-10. Muscle-derived IL-6 exerts inhibitory effects on TNF-α production (34), and the IL-10/TNF-α ratio increases with aerobic training (25). Because we assessed levels of serum cytokines, we cannot rule out the possibility that the two study groups differed in terms of the production of inflammatory mediators in the disease's target organs.

All allo-HSCT recipients experience posttransplant deficiencies in B- and T-cell replenishment, and cGVHD has been correlated with reduced numbers of B cells and diminished availability of CD4-mediated T-cell help (10). Further, this delay in immune recovery may persist for more than a year (19). The pathophysiology of GVHD, with alloreactivity altering lymphopoiesis and T-cell selection, together with the immune suppression required to treat the disease results in long-term immune deficiency (17). Thus, the fact that exercise training did not negatively affect the kinetics of immune reconstitution in our mice (rather it led to higher circulating levels of B220 and CD4 lymphocytes) is an important finding. The presence of high T lymphocyte levels and lower TNF-α and IL-4 levels suggests that this immune reconstitution was not skewed toward an alloreactive profile. Despite higher numbers of circulating B-lymphocytes among the exercised mice, cGVHD was less severe, most likely because of the lower levels of IL-4 in those mice (9).

A traditional method for the prevention and treatment of GVHD is the administration of high doses of immunosuppressants after allo-HSCT (32). In particular, treatment with cyclosporine A, a drug that interferes with T-cell activation has proved remarkably successfully and is widely used to prevent transplant rejection and GVHD (27). However, cyclosporine A treatment induces severe side effects, including damage to those systems involved in exercise, that is, nervous, respiratory, or circulatory systems (7). Cyclosporine A can also reduce muscle capillary density (4) and mitochondrial electron chain capacity (e.g., through a toxic, inhibitory effect on the expression of COX-I and COX-IV proteins [18]) and can thus deteriorate muscle oxidative capacity and muscle performance (29). This could explain why muscle citrate synthase activity, a classic marker of muscle oxidative capacity (35), and respiratory chain complex activities were not significantly higher in our group of mice after training compared with control mice. Nonetheless, the exercise intervention resulted in a trend toward a higher phospho-p70 S6 kinase–p70 S6 kinase ratio after training. Although we cannot know if such training-induced trend toward "hypertrophic signaling" actually resulted in attenuation of muscle catabolism, we believe this preliminary finding is promising in the context of this debilitating disease (which is also known to cause skeletal muscle necrosis [2,15]). This finding is of

particular interest when considering that muscle tests were performed 83 days after transplant, when the disease was truly advanced, as indicated by clinical and histological scores.

In conclusion, our results reveal the potential benefits of regular moderate-intensity aerobic exercise combined with standard immunotherapy on the clinical course of cGVHD. Exercise may be a nonpharmacological way of improving the disease course and the quality of life of patients, attenuating in some measure the debilitating nature of this disease and its treatment without harming an already fragile immune function. Today, cGVHD is increasingly being recognized worldwide as a systemic disease complicating allo-HSCT with major implications for health care systems and workers as well as health care costs. This prompts a need for new interdisciplinary approaches designed to improve the well-being of patients with cGVHD and mitigate disease complications such as impaired physical capacity. One such approach could be supervised exercise.

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