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ORIGINAL ARTICLE

Temporal development of muscle atrophy in murine model of arthritis is related to disease severity

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Abstract
Background Rheumatoid arthritis (RA) is an inflammatory autoimmune disease of unknown etiology, affecting mainly the joint but also other tissues. RA patients usually present weakness and muscle atrophy, nonarticular manifestations of the disease. Although causing great impact, the understanding of muscle atrophy, its development, and the mechanisms involved is still very limited. The objective of this study is to evaluate the development of muscle atrophy in skeletal muscle of a murine model of arthritis.
Methods The experimental murine model of collagen-induced arthritis (CIA) was used. DBA/1J mice were randomly divided into three groups: control (CO, n=25), sham arthritis (SA, n=25), and arthritis (CIA, n=28), analyzed in different time points: 25, 35, and 45 days after the induction of arthritis. The arthritis development was followed by clinical scores and hind paw edema three times a week. The spontaneous exploratory locomotion and weight were evaluated weekly. In all time points, serum was collected before the death of the animals for cytokine analysis, and myofiber cross-sectional areas (CSA) of gastrocnemius (GA) and tibialis anterior (TA) skeletal muscles were evaluated.
Results The clinical parameters of arthritis progressively increased in CIA in all experimental times, demonstrating the greatest difference from other groups at 45 days after induction (clinical score: CO, 00±00; SA, 1.00±0.14; CIA, 3.28±0.41 p>0.05). The CIA animals had lower weights during all the experimentation periods with a difference of 6 % from CO at 45 days (p>0.05). CIA animals also demonstrated progressive decrease in distance walked, with a reduction of 54 % in 35 and 74 % at 45 days. Cytokine analysis identified significant increase in IL-6 serum levels in CIA than CO and SA in all experimental times. CSA of the myofiber of GA and TA was decreased 26 and 31 % (p> 0.05) in CIA in 45 days after the induction of disease, respectively. There was significant and inverse correlation between the disease clinical score and myofiber CSA in 45 days (GA: r=−0.71; p=0.021).
Conclusion Our results point to a progressive development of muscle wasting, with premature onset arthritis. These observations are relevant to understand the development of muscle loss, as well as for the design of future studies trying to understand the mechanisms involved in muscle wasting. As far as we are concerned, this is the first study to evaluate the relation between disease score and muscle atrophy in a model of arthritis.

Keywords Muscle wasting · Arthritis animal model · Chronic inflammation

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1 Introduction

Rheumatoid arthritis (RA) is a systemic inflammatory disease of unknown etiology, characterized by symmetrical and erosive chronic synovitis, preferentially affecting peripheral

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joints. RA is the most common autoimmune disease, affecting about 1 % of the world population [1, 2]. In addition to articular manifestations, RA has several systemic manifestations that significantly influence its morbidity and mortality. Rheumatoid cachexia [3] is one of these systemic manifestations that has been receiving more attention due to its impact in function and quality of life, occurring in approximately 10 to 66 % of RA patients. It is characterized by disruption of muscle protein synthesis and degradation of balance, increased basal metabolic rate, total energy expenditure, insulin resistance, and inflammation, as well as decreased muscle mass, strength, and function [3–7].

The mechanisms involved in muscle wasting of RA patients have yet to be fully elucidated, but probably include cytokine-driven hypermetabolism, particularly by tumor necrosis factors-alpha (TNF-α), limitation of physical activity, insulin resistance, and inadequate protein ingestion [8–10]. Better understanding of the relative participation of these and other factors involved in muscle atrophy would be important for the development of preventive and therapeutic strategies for this complication, particularly now that a number of innovative therapeutic approaches are available for this disease, such as direct cytokine blockade using monoclonal antibodies and fusion-receptor proteins [11]. However, investigations of muscle atrophy mechanisms in humans are difficult, and to our knowledge, there are none on RA patients. On the other hand, studies with animal models of arthritis have been of critical importance for our current understanding of the diverse pathophysiological processes involved in chronic immune-mediated arthritis, and this knowledge greatly contributed to the development of modern target-based therapies. There are some reports evaluating muscle atrophy in murine models of arthritis. Ozawa et al. [12] demonstrated reduction about 20 % in skeletal muscle and in muscle weight in a model adjuvant-induced arthritis (complete Freund's adjuvant, CFA). Hartog et al. [13] have described reduced weight and spontaneous locomotion in collagen-induced arthritis (CIA), demonstrating generalized muscle loss. In the same way, we have recently observed a reduction of about 31 % in the relative weight of the gastrocnemius muscle in the rat CIA model at 21 days after the induction of arthritis [14].

One important issue that to our knowledge has not been explored yet is the time course of occurrence of muscle atrophy during the development of chronic arthritis. This information, besides being very informative for the design of future experimental studies, could also shed some light on the relative importance of the several factors that have been implicated in the generation of muscle atrophy, such as the coupling of muscle atrophy intensity and the severity of arthritis or its occurrence even before clinical arthritis has developed, as well as being informative for the design of preventive and therapeutic strategies for patients with chronic arthritis. Therefore, we decided to undertake the current series of experiments trying to describe the time course of the development of muscle atrophy in the CIA mouse model, a widely used animal model for rheumatoid arthritis, with particular attention to its correlation with the development and severity of the arthritis.

2 Materials and methods

2.1 Mice and experimental groups

Male DBA/1J mice from 8 to 12 weeks of age were used. The mice were reared at 20 °C, with 12-h light–dark cycle, controlled access to food, and free access to water. All experiments were performed according to the Guiding Principles for Research Involving Animals (NAS) and to the Ethics Committee of Research and Postgraduate Group of the Hospital de Clínicas de Porto Alegre.

The animals were randomly allocated into three experimental groups: control without intervention (CO=25); sham arthritis injected with only adjuvant, without collagen (SA=25), and collagen-induced arthritis injected with type II collagen emulsified in adjuvant (CIA=28). Mice were killed at 25, 35, or 45 days after induction of arthritis for histological analysis of muscles and for measurements of serum cytokines. Arthritis was induced, day 0, with bovine type II collagen (CII, Chondrex, Inc.; 2 mg/ml) dissolved in 0.1 M acetic acid at 4 °C for 12 h and CFA (Sigma, St Louis, MO, USA; 4 mg/ml) containing inactivated *Mycobacterium tuberculosis*. On the 18th day, the animals received a booster of CII emulsified with incomplete Freund's adjuvant (IFA—without *M. tuberculosis*) in the tail [15].

2.2 Experimental procedures

During the procedures, mice were anesthetized with isoflurane (1 ml/ml, Abbott). At day 0, 50 μL of emulsion (CII + CFA) was injected intradermally at the base of the tail to induce arthritis. After 18 days, mice received a reinforcement of the emulsion (CII + IFA) in another site of the tail. Animals were killed in different times: 25, 35, and 45 days after the first immunization. The tibiotarsal joint was removed to confirm the development of arthritis by histological analysis. These times of analyses were chosen based on a pilot experiment, when muscle atrophy was checked at 25, 30, 35, 40, 45, 50, 60, 70, and 80 days, and no additional muscle wasting was observed after the 45th day.

2.3 Clinical severity and measurement of edema

Arthritis severity was quantified clinically according to a graded scale of 0–4 as follows: 0, no evidence of erythema

and swelling; 1, erythema and mild swelling confined to the tarsals or ankle joint; 2, erythema and mild swelling extending from the ankle to the tarsal; 3, erythema and moderate swelling extending from the ankle to metatarsal joints; and 4, erythema and severe swelling encompassing the ankle, foot, and digits, or ankylosis of the limb [15]. Joint edema was assessed by size of latero-lateral extent of the height of the metatarsus of the mice hind paws with digital calipers (Myoto), as previously described [16]. Paw size measurements were obtained at the time of the booster (18 days) and every 3 days.

2.4 Histological score system

The following histological score system was used to evaluate individual joints and measure arthritis severity [16]: *synovial inflammation*: five high-power magnification fields (HMF) were scored for the percentage of infiltrating mononuclear cells as follows: 0, absent; 1, mild (1–10 %); 2, moderate (11–50 %); 3, severe (51–100 %); *synovial hyperplasia*: 0, absent; 1, mild (5–10 layers); 2, moderate (11–50 layers); 3, severe (>20 layers); *extension of pannus formation based on the reader's impression*: 0, absent; 1, mild; 2, moderate; 3, severe; *synovial fibrosis*: 0, absent; 1, mild (1–10 %); 2, moderate (11–50 %); 3, severe (51–100 %); *cartilage erosion*: percentage of the cartilage surface that was eroded: 0, absent; 1, mild (1–10 %); 2, moderate (11–50 %); 3, severe (51–100 %); *bone erosion*: 0, none; 1, minor erosion(s) observed only at HMF; 2, moderate erosion(s) observed at low magnification; 3, severe transcortical erosion(s).

2.5 Cytometric bead array analysis for cytokines

The cytometric bead array (CBA) kit employed here allows for the discrimination of the following cytokines: IL-2, IL-4, IL-6, IL-10, IL-17, IFN-γ, and TNF-α. Sample processing and data analysis were performed according to the manufacturer's instructions. Briefly, serum samples were incubated with the six cytokine capture beads and PE-conjugated detection antibodies for 3 h, at room temperature and protected from light. Afterward, samples were washed, and sample data were acquired using a FACSCalibur flow cytometer (BD Biosciences, San Diego, CA). Sample results were generated in graphical and tabular format using the BD CBA Analysis Software (BD Biosciences, San Diego, CA) [17].

2.6 Assessment of locomotion

Assessment of locomotion was performed at booster (day 18) and at different times of death: 25, 35, and 45 days. The mice were placed individually inside an acrylic box (Monitor de Atividade IR) of 60 cm×40 cm. Spontaneous exploratory locomotion of animals was detected by sensor bars located in the sides of the movement box during 5 min, after 30 s of adaptation time (adapted [13]). The movement's detection was evaluated by the computer software Insight Equipamentos Ltda® using the following parameters: route design, walked distance, mean velocity, resting time, and number of times standing.

2.7 Evaluation of body weight and fiber cross-sectional area

The body weight measurements were obtained before induction (day0), booster (day18), and 25, 35, and 45 days after induction of arthritis. One transverse section of each muscle was stained with hematoxylin–eosin and analyzed with and optic microscope (×400). Two straight lines crossing at a right angle at the fiber center were drawn in each myofiber. The mean of these diameters (in micrometer) was used to calculate the transverse section area, based on circle area formula (in square micrometer). For the measurement of myofiber cross-sectional area (CSA) from the whole muscle, ten pictures were taken of each section, and 20 fibers were measured from each picture using the software Image-Pro Express (version 5.1.0.12, Media Cybernetics).

2.8 Statistical analysis

Results are expressed as mean ± SE. Variables observed systematically were analyzed using two-way ANOVA: animal weight, edema, arthritis score, and locomotion—distance and velocity (Bonferroni as post hoc test), and variables observed once were analyzed using one-way ANOVA: gastrocnemius and tibialis muscle cross-sectional area, joint histological analysis, and cytokine levels (Turkey as post hoc test). In addition, Pearson correlation was used to test the relation among two continuous variables. Significance was accepted at $p<0.05$ (Graph Pad Prism 5).

3 Results

3.1 Assessment of disease activity and progression

Arthritis score and hind paw edema were evaluated using a score system for arthritis severity and digital calipers, respectively. Onset of disease, characterized by the development of erythema and/or paw swelling, was observed at day 25 (mean [CI 95 %] arthritis score CIA, 0.72 [0.53–0.91], vs. CO, 0.04 [0.02–0.06], $p>0.05$), increasing at 35 days (CIA, 1.90 [1.67–2.13], vs. CO, 0.11 [0.04–0.16], $p>0.05$) and at 45 days (CIA, 3.28 [3.03–3.53], vs. CO, 0.00 [0.0–0.0], $I>0.05$) (Fig. 1a and b). The histopathological study of tibiotarsal joints confirmed these observations, with the

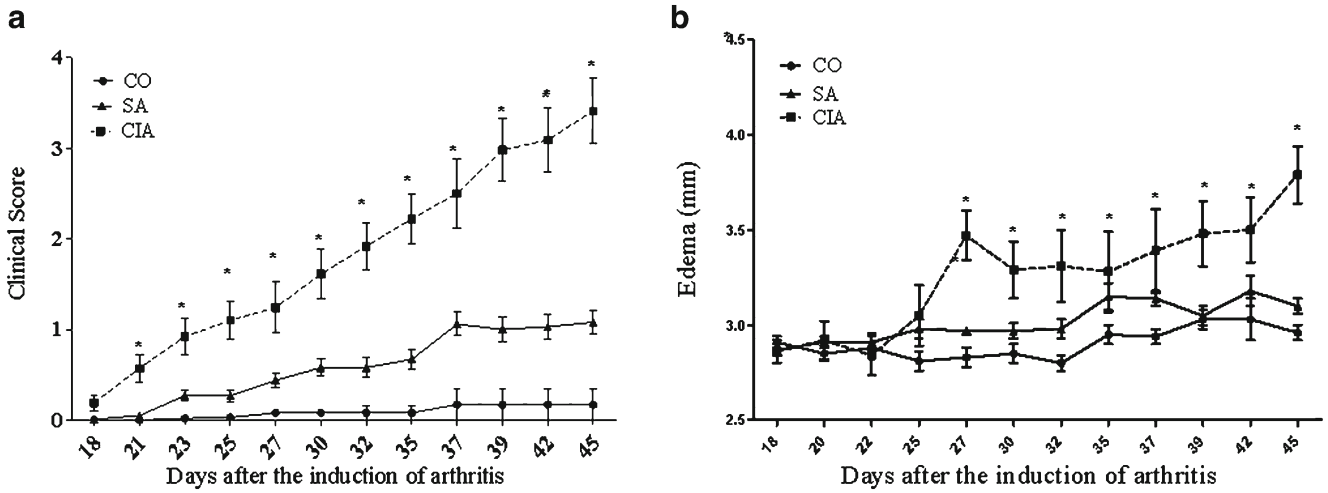

Fig. 1 Arthritis clinical score progression (**a**) and hind paw edema (**b**) were evaluated three times a week initiating from booster injection. In **a** can be observed the severity progression of arthritis score through the experimental period. *$p>0.05$ vs. CO and SA groups, by one-way ANOVA followed by Bonferroni post hoc test. Mean ± SEM results in each group are shown

presence of all the parameters associated with destructive inflammatory joint disease. At 25 days, light infiltration by inflammatory cells and synovial hyperplasia with pannus formation in the joint space could be observed, as well as mild cartilage and bone erosions, indicating the presence of an early arthritis. At 35 days, there was an important pannus formation and increased bone erosion, with significant progression of the disease. Finally, at 45 days, established destructive disease was observed, with marked infiltration of inflammatory cells and synovial hyperplasia with pannus formation in the joint space, as well as severe cartilage and bone erosions, confirming the presence of a severe arthritis (Supplementary Table 1). Joints of sham arthritis animals had the same pattern as the CO.

3.2 Cytokine profile

The serum cytokine profile (Table 1) demonstrated that IL-2, IL-6, IL-10, and IFN-γ were significantly increased in CIA animals compared to the control at 25 days. At 35 days, IL-2 and IL-6 were enhanced in CIA animals compared to CO. On the other hand, at 45 days, IL-2 remained similar to the control, while IL-6 and IFN-γ were significantly increased in CIA.

3.3 Animal locomotion

Since animal paws were clearly inflamed and swollen, we explored to what extent physical performance was affected. Spontaneous locomotion was evaluated in different experimental times: 18, 25, 35, and 45 days. At 25 days, CIA animals showed about 34 % decrease in the walking distance and velocity in comparison with CO and sham arthritis animals (Fig. 2a and b, respectively). This difference was increased at 35 days (reduction in distance, 54 %, and velocity, 52 %) and 45 days (reduction in distance, 74 %, and velocity, 70 %) ($p<0.05$ for all comparisons).

3.4 Animal weight and muscle fiber cross-sectional area

CIA animals had significantly less weight during the experimentation period compared to the control group ($p<0.05$) (Fig. 3). At day25 there was no difference in muscle fiber CSA among the experimental groups. The CSA of gastrocnemius (GA) and tibialis anterior (TA) muscles were significantly smaller at 35 and 45 days in CIA than CO (Fig. 4a and b). During the experimentation period, the CO group increased myofiber CSA by 6 %, while the CIA group had a reduction of 15 %. Comparing CIA to CO animals, there is a reduction of 26 % in myofiber CSA at 45 days. There was a significant and inverse correlation between fiber CSA and disease score just at 45 days (TA, −0.68, $p=0.029$; and GA, −0.71, $p=0.021$).

4 Discussion

To the best of our knowledge, this is the first study to evaluate the time course of the occurrence of muscle atrophy, defined as decreased muscle fiber cross-sectional area, during the development of chronic arthritis in an animal model. This muscle wasting caused by chronic disease has been demonstrated in several studies [13, 18, 19], and additional investigations on its molecular mechanisms have been reported [11, 20–22]. There are evidences of the involvement of the ubiquitin–proteasome pathway, as indicated by increased expression of MuRF-1 and MAFbx [20, 22], and downregulation of growth factors [11]. These effects are

Table 1 Cytokine profile was measured by CBA in three experimental times: 25, 35, and 45 days after induction of arthritis. Results are depicted as means ± SEM

	CO	SA	CIA
IL-6			
25 days	0.55 (0.32–1.70)	2.96 (1.36–19.91)	27.90 (15.92–56.73)*
35 days	0.65 (0.59–0.71)	1.20 (1.13–6.93)	13.60 (4.56–29.57)
45 days	0.76 (0.53–8.65)	2.08 (1.70–10.24)	50.65 (32.94–132.90)*
IL-2			
25 days	1.50 (1.49–1.59)	1.53 (1.50–1.57)	1.66*† (1.65–1.87)
35 days	1.47 (1.42–1.52)	1.47 (1.46–1.65)	1.65 (1.59–1.77)*
45 days	1.55 (1.49–1.66)	1.58 (1.47–1.61)	1.60 (1.55–1.67)
IL-10			
25 days	16.27 (6.39–17.94)	4.72 (2.74–4.83)	5.11 (4.29–4.29)*†
35 days	4.87 (4.17–5.58)	3.97 (3.97–5.94)	4.00 (3.20–4.34)
45 days	3.87 (3.08–5.94)	4.27 (2.44–5.69)	4.79 (4.09–8.16)
INF			
25 days	0.44 (0.41–0.58)	1.09 (0.78–2.13)	3.61 (1.65–10.41)*
35 days	0.58 (0.54–0.63)	0.60 (0.39–1.20)	1.49 (0.87–4.50)
45 days	0.26 (0.21–0.51)	0.74 (0.66–1.07)	1.39 (0.99–2.76)*
TNF			
25 days	8.730 (6.16–12.51)	14.42 (9.47–18.63)	18.87 (11.07–23.46)
35 days	9.47 (9.36–9.59)	13.40 (10.51–13.40)	12.60 (11.10–13.99)
45 days	8.50 (6.22–9.53)	8.90 (8.27–13.64)	12.16 (9.70–13.62)

*$p<0.05$ vs. CO group and sham arthritis mice, by one-way ANOVA followed by Tukey post hoc test

†$p<0.05$ arthritis mice vs. CO group, by one-way ANOVA followed by Tukey post hoc test

probably mediated by proinflammatory cytokines, such as TNF-α and IL-6, and appear to be distinct from the mechanisms of muscle wasting due to immobilization, as we have recently demonstrated in a rat arthritis model (submitted).

However, in all these studies, the histological and molecular analyses of the muscles were performed in a single time point, usually 21 days after induction in mice and 15 days in rats. These time points were chosen presumably because they represent the time of greatest disease severity, when the investigators expected that muscle atrophy would also be at its peak. However, this would only be true if we consider that the two processes, arthritis and muscle atrophy, are somehow coupled, either by a direct causal relationship or by the involvement of common pathways, such as the participation of inflammatory cytokines. Although this correlation of severity of arthritis and muscle atrophy could be considered probable, in light of the current evidence on the mechanisms

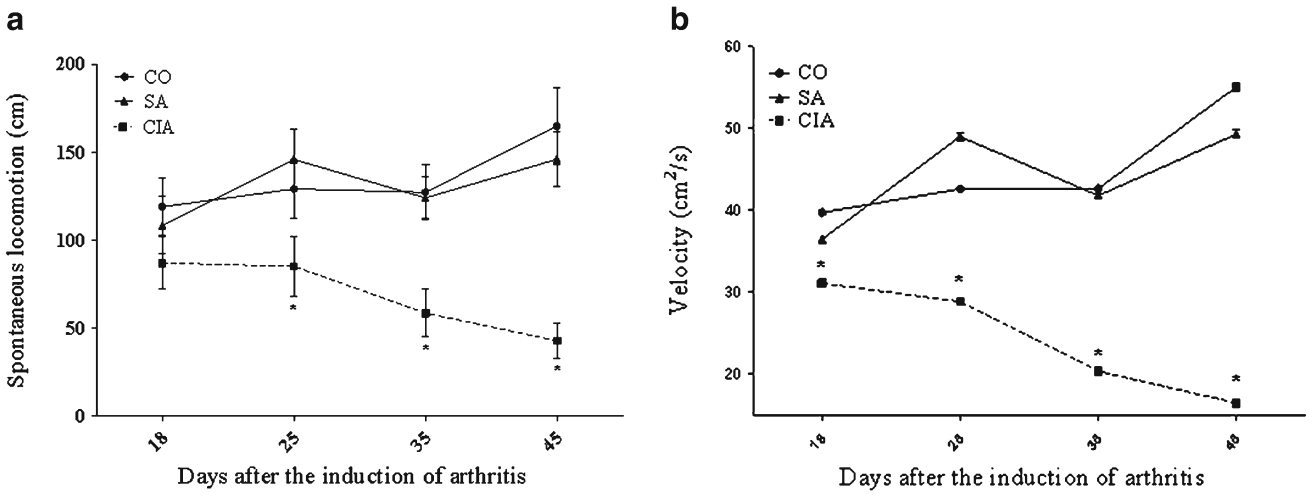

Fig. 2 **a** and **b** Spontaneous exploratory locomotion was evaluated before booster injection and in three experimental times: 25, 35, and 45 days after induction arthritis. The CIA group demonstrated decreased locomotion (distance and velocity) at 25, 35, and 45 days after induction of arthritis. Results are depicted as means ± SEM. *$p<0.05$ vs. CO group and SA mice, by one-way ANOVA followed by Bonferroni post hoc test

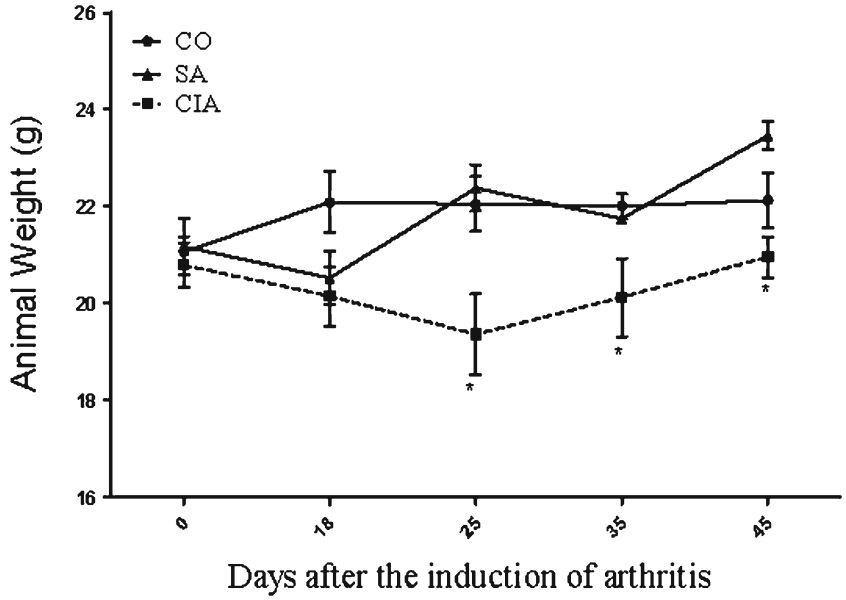

Fig. 3 Animal weight was measured before the induction and booster injections and in three experimental times: 25, 35, and 45 days after induction of arthritis. The CIA group was lighter during all experimentation periods. Results are depicted as means ± SEM. *$p<0.05$ vs. CO group and sham arthritis mice, by one-way ANOVA followed by Bonferroni post hoc test

of muscle protein degradation during inflammatory processes, it remains essentially untested.

Therefore, we attempted to better define the correlation between joint inflammation and muscle atrophy, using three different experimental times: 25, 35, and 45 days after the induction of collagen-induced arthritis. These time points were chosen based on a prior pilot study when we tested nine different times (25, 30, 35, 40, 45, 50, 60, 70, and 80 days after induction of arthritis) and observed no further progression of arthritis and muscle wasting severity after 50 days. In each time point, clinical and histological severity of the arthritis, animal weight, locomotion activity, inflammatory-related cytokine serum levels, and muscle CSA were evaluated.

At 25 days, the onset of arthritis was confirmed through clinical score, hind paw edema, and histopathology, with mild synovitis and cartilage and bone erosions. In this experimental time, the animals also demonstrated weight loss, locomotion reduction, reduction of cytokine IL-10, and markedly increased IL-6. Despite these evidences of the presence of significant early arthritis, no major impact in fiber diameter was observed, although the slight weight loss could indicate the presence of early muscle wasting.

At 35 days, the clinical manifestations and histopathological findings of arthritis were well established. The animals continued to lose weight and locomotion, and there was clear muscle atrophy as demonstrated myofiber CSA reduction (area GA, 12 %; area TA, 9 %). Finally, at 45 days, CIA animals showed severe joint swelling and advanced histopathological arthritic changes, including extensive bone erosions and cartilage thinning, and great limitation in locomotion. These findings correlated with marked

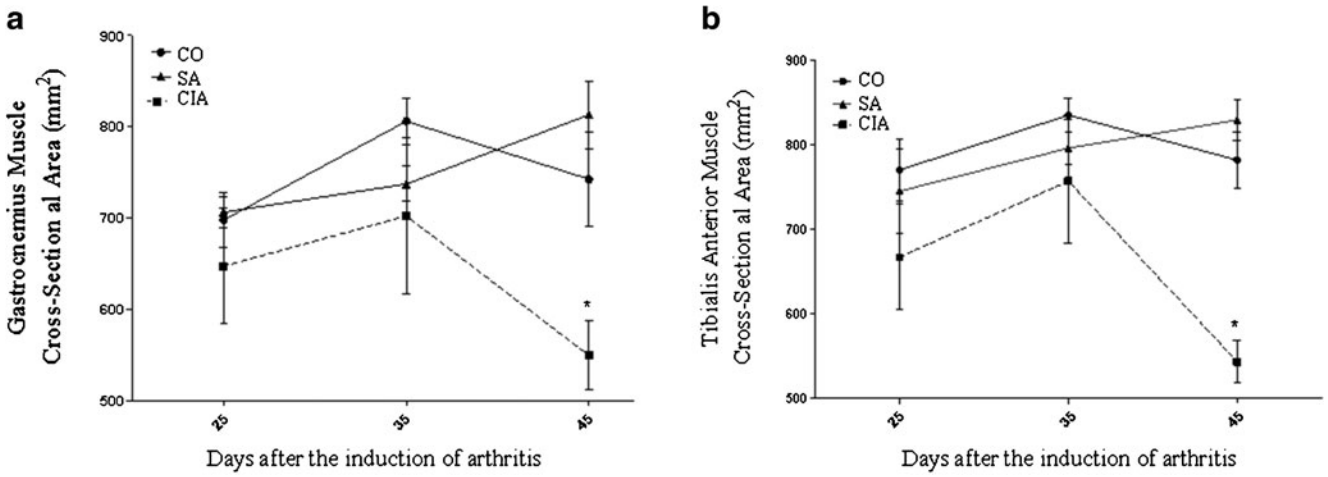

Fig. 4 CSA of GA (**a**) and TA (**b**) skeletal muscle were measured in three experimental times: 25, 35, and 45 days after induction of arthritis. CSA of GA and TA muscles were significantly smaller at 35 and 45 days in the CIA group than CO group. Results are depicted as means ± SEM. *$p<0.05$ vs. CO group and sham arthritis mice, by one-way ANOVA followed by Tukey post hoc test

decrease in CSA, with a reduction of 26 % of area GA and 31 % of area TA compared to controls. Moreover, at 45 days, there was a significant and inverse correlation between fiber CSA and disease score. Of all the analyzed cytokines in our experiment, IL-6 was the only one increased in the CIA group in all the experimental times.

The demonstration of close correspondence of arthritis severity, decreased locomotion, and muscle wasting is important for understanding the underlying mechanisms of this complication. Although most likely it is the result of several factors, including insulin resistance, decreased growth hormone, decreased protein ingestion, catabolism induced by proinflammatory cytokines, and impaired physical activity [8–10], the relative contribution of each of these factors needs further elucidation. Granado et al. [20] analyzed the effect of adjuvant-induced arthritis on the MuRF1 and MAFbx as well as on IGF-I and IGF-binding protein-5 gene expression in the skeletal muscle. This study demonstrates that arthritis induces a marked decrease in skeletal muscle weight along with an activation of the ubiquitin–proteasome proteolytic pathway. Besides, the parallel changes in muscular IGFBP-5 and TNF-α gene expression with the ubiquitin ligases suggest that they can participate in skeletal muscle alterations during chronic arthritis. Balasubramaniam et al. [23] also investigated the contribution the ubiquitin–proteasome proteolytic pathway (MuRF1 and MAFbx) in skeletal muscle protein breakdown in rats with thermal injury. The results suggest that the GH-releasing compound could be a powerful anticatabolic compound that reduces skeletal muscle protein breakdown by attenuating multiple burn-induced abnormalities. Castillero et al. [24] evaluated the ability of the administration of omega-3 polyunsaturated fatty acid (EPA) prevent an arthritis-induced decrease in body weight and muscle wasting in rats. The results suggest that in experimental arthritis, in addition to its anti-inflammatory effect, EPA treatment attenuates muscle wasting by decreasing MAFbx and MuRF1 gene expression and increasing the transcription factors that regulate myogenesis.

Systemic administration of cytokines results in muscle catabolism in experimental animals [25]. Furthermore, genetic [26] or pharmacologic blockade [27, 28] of cytokine signaling attenuates experimental cachexia. Although direct inflammatory cytokine action on muscle promotes atrophy, nonmuscle sites of action for inflammatory mediators are less well described. Braun et al. [29] demonstrated that central nervous system (CNS)-delimited interleukin 1β (IL-1β) signaling alone can evoke a catabolic program in muscle, rapidly inducing atrophy. This effect is dependent on the hypothalamic–pituitary–adrenal axis activation, as CNS IL-1β-induced atrophy is abrogated by adrenalectomy.

Even though experimental arthritis also induces anorexia, muscle wasting does not seem to be due to the decrease in food intake. Roubenoff et al. [30] evaluated whether adjuvant arthritis (AA) leads to changes in body composition and cytokine production similar to those seen in patients with RA. The authors suggested that AA animals lost 20 % of their body weight at the end of experimentation, and pair-fed animals lost only 5 % of the weight, indicating that anorexia alone does not explain inflammatory cachexia. Weight loss was correlated with TNF-α production by spleen mononuclear cells. In our study, CIA animals did not gain weight even before the onset of significant arthritis (edema and clinical score), and in a pilot experiment, daily food intake did not differ between controls and CIA animals (unpublished).

In systemic inflammation, the following are believed to play a role in the development of muscle wasting: the action of proinflammatory cytokines; activation of the ubiquitin–proteasome system, mainly of unregulated genes of E3 ubiquitin ligases MuRF1 and MAFbx; the action of the mammalian target of rapamycin kinase; and the satellite cell dysfunction [1, 31]. However, the current knowledge about these mechanisms and their involvement in rheumatoid-related muscle wasting is limited.

Understandably, muscle wasting could be mainly related to the reduction of spontaneous locomotion. The period of maximal decrease in fiber CSA coincides with the progression to severe disease and dramatic reduction of locomotion (−74 % at 45 days). However, there are several observations indicating that simple disuse atrophy might not be the sole mechanism responsible for muscle wasting in chronic arthritis. Immobilization-related muscle wasting and cachexia related to inflammatory diseases appear to engage distinct molecular pathways of myofiber protein degradation [4]. We have also recently described that female Wistar rats with CIA presented a reduction in myofiber CSA of 30 %, while animals immobilized with copper boot reduced 60 % (submitted). This study also observed the involvement of distinct molecular pathways between the experimental groups, suggesting that locomotion is not an isolated factor of muscle loss. Hartog et al. [13] have described reduced weight of different types of muscles (soleus, gastrocnemius, tibialis anterior, and exterior digitorum longus) and spontaneous locomotion in CIA model, demonstrating generalized muscle loss. In a related experiment, regular forced exercise of CIA mice could only partially prevent the muscle mass loss.

5 Conclusion

In summary, our results point to a progressive development of muscle wasting, with an early onset, which seems to be related to the severity of arthritis and with increased serum levels of inflammatory cytokines, especially IL-6. These

observations are relevant to understand the development of muscle wasting, as well as for the design of future studies on this animal model trying to understand the mechanisms involved. Accordingly, to study the initiation of muscle wasting, we recommend using early phases of the arthritis, while to study the molecular pathways involved in well-established muscle atrophy, we recommend more advanced phases of the disease.

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Conflict of interest The authors declare that they have no conflict of interest

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