Serum and Tissue Cytokines and Chemokines Increase with Repetitive Upper Extremity Tasks

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Received 24 August 2007; accepted 22 February 2008
Published online 7 May 2008 in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/jor.20674

ABSTRACT: We investigated inflammation in rats performing a low repetition, negligible force (LRNF) or high repetition, negligible force (HRNF) task of reaching and retrieving food pellets at target rates of two or four reaches/min for 2 h/day, for 6–8 weeks. Serum was assayed for 11 cytokines and chemokines; forelimb tissues for four cytokines. Macrophages were counted in forelimb tissues of LRNF rats to add to results from our previous studies of HRNF rats. In HRNF rats, serum IL-1α, IL-1β, TNFα, MIP2, MIP3a, and RANTES were elevated in weeks 6 and 8. In contrast, only MIP2 and MIP3a increased in serum of LRNF rats. In 8 week HRNF reach limb tissues, IL-1α, IL-1β, TNFα, and IL-10 increased in distal bones, IL-1α and -β in muscles, and TNFα in tendons. Only IL-10 increased in LRNF reach limb muscles in week 8. Serum IL-1α and MIP2 correlated with macrophages in LRNF loose connective tissues, serum MIP3a and MIP2 correlated negatively with grip strength, while serum TNFα, MIP3a, and MIP2 correlated positively with total number of reaches. Thus, several tissue and circulating cytokines/chemokines increase in an exposure dependent manner following short-term performance of repetitive reaching tasks and correlate with macrophage infiltration and decreasing grip strength. © 2008 Orthopaedic Research Society. Published by Wiley Periodicals, Inc. J Orthop Res 26:1320–1326, 2008

Keywords: cytokines; chemokines; macrophages; repetitive motion injury; WMSD

Repetitive motion injuries (RMI) of the wrist and hand are painful, potentially disabling, and costly. Recent work suggests that RMIs induce injury in several tissue types in animal models,[1–6] as well as an early inflammatory response at the tissue level.[2,3,7–9] Increases in four circulating inflammatory mediators, C-reactive protein, interleukin 1 beta (IL-1β), IL-6, and tumor necrosis factor alpha (TNFα) have also been shown to increase in patients with upper extremity overuse disorders.[10] The factors triggering underlying pathophysiological responses are still under investigation, which impedes progress toward their primary and secondary prevention.

Using a rat model, we have reported that repetitive reaching causes injury and wide-spread increases in macrophage influx into musculotendinous tissues and peripheral nerves, extraneural fibrosis, decreased nerve conduction, and decreased grip strength.[2–4,7,9] The macrophage response is associated with increased inflammatory cytokines in the median nerve,[9] and increased serum IL-1α following HRNF task performance.[2]

A variety of inflammatory mediators including cytokines and chemokines are released by injured cells and infiltrating macrophages.[9,11] Although serum cytokine and chemokine response patterns to exercise of varying intensities[12–15] and multiple organ trauma and fractures have been studied,[16–18] exposure to more chronic, lower levels of injury are not yet well characterized.

Here, we examine several cytokines and chemokines in forelimb musculoskeletal tissues and serum to identify their response profiles, as well as potential cellular sources, after the performance of a voluntary repetitive task at two exposure levels: high repetition, negligible force (HRNF) and low repetition, negligible force (LRNF). In serum, we chose 11 cyotokines and chemokines to examine, including key pro-inflammatory cytokines (IL-1α/β and TNFα), a highly pleiotropic cytokines with both pro- and anti-inflammatory properties (IL-6), a potent anti-inflammatory cytokine (IL-10), several inflammatory chemokines known to mediate chemotaxis of monocytes/macrophages and T cells (RANTES, macrophage inflammatory protein 3 and 2a, interferon gamma and fractalkine), and a chemokine that mediates chemotaxis of neutrophils and growth of fibroblasts (growth-related oncogene keratinocyte-derived cytokine). We examine macrophage infiltration into flexor forelimb tissues of LRNF rats to add to results from our previous studies of HRNF rats[2,3,7,9] and correlate their numbers with serum levels of inflammatory cytokines and chemokines. In addition, we compare performance parameters, reach rate, and grip strength, with the inflammatory response. We hypothesize that performance of the HRNF task will have a greater effect on serum and tissue concentrations of cytokines and chemokines than the LRNF task, and a negative impact on motor performance.

MATERIALS AND METHODS

Experimental Animals

The Temple University Institutional Animal Care and Use Committee approved all experiments in compliance with NIH guidelines for the humane care and use of laboratory animals. Studies were conducted on 57 young adult, female Sprague-Dawley rats (285–310 g; 14–16 weeks of age at onset of task training), which were housed in the central animal facility in separate cages in a 12-h light:dark cycle with free access to water. Thirty-six rats were trained to reach and retrieve a 45-mg pellet of food at a low reach rate (LRNF; target rate of

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two reaches/min), or at a high reach rate (HRNF; target rate of four reaches/min). Both tasks were performed for 2 h/day, in 30-min sessions, separated by 1.5-h breaks, 3 days/week for 6 or 8 weeks. Twenty-one rats were used as controls: seven as age-matched normal controls, nine as age- and weight-matched controls that were food restricted but did not undergo task shaping, and five as age- and weight-matched controls that were food restricted and went through the initial task shaping period of 7–10 days (see below) but did not proceed to the task regimen. These latter rats are considered as trained control rats. These three control groups were used to explore the effects of weight restriction and/or initial training on cytokine response. Control rats were euthanized at points that matched the 6- to 8-week task endpoints.

Behavioral Apparatus and Repetitive Movement Task

The rats were placed in operant test chambers for rodents (Med. Associates, Albans, VT) with a tube located in one end from which they had to reach, grasp, and retrieve a 45-mg pellet of food. Food pellets (Bioserve) were dispensed (Pellet dispenser, Med. Associates) either every 15 s (HRNF task) or every 30 s (LRNF task) during the task session. Further details are as described previously.[2,3] Experimental and trained control rats learned to reach for the food during an initial 7–10-day shaping period in which access to food was restricted to motivate them to learn the task. Some animals may have undergone a short period (no more than 7 days) of weight reduction to 80% of the weights of the age-matched control group that did not undergo food restriction. Once the animals learned the task, they rapidly gained weight and were maintained at ±5% of age-matched control rats' weights. Rats were weighed twice weekly and food adjusted accordingly.

Behavioral Analysis

Reach rate was defined as the average number of reaches performed per minute on the last day of each task week and was determined by direct observation of reaches meeting preset criteria: the forepaw was extended and then withdrawn beyond a line 0.5 cm inside the elevated tube. The number of actual reaches was recorded on a hand counter. Total number of reaches was defined as the sum of the reaches/week (product of reach rate, minutes per day of task performance and days per week) across all weeks of task performance. Grip strength was determined using methods described previously.[4]

Collection of Serum

Twenty-seven rats were euthanized at 6 or 8 weeks after task onset and serum collected: HRNF 6 weeks ($n = 8$), HRNF 8 weeks ($n = 6$), LRNF 6 weeks ($n = 7$), and LRNF 8 weeks ($n = 6$); and the 21 control rats. Following euthanasia (Nembutal, 120 mg/kg body weight) 18–36 h after completion of the final task session, blood was collected by cardiac puncture using a 23-gauge needle and centrifuged immediately at $1000 \times g$ for 20 min at 4°C. Serum was collected, flash frozen, and stored at −80°C until analyzed.

Measurement of Serum Cytokines and Chemokines

The following cytokines and chemokines were analyzed in serum using a customized multiplexed sandwich ELISA system (SearchLight; Pierce, Rockford, IL): IL-1α, IL-1-β, IL-6, TNFα, interferon gamma (IFNγ), macrophage inflammatory protein 3 (MIP3a), macrophage inflammatory protein 2 (MIP2), Regulated on Activation, Normal T-cell Expressed and Secreted (RANTES), growth-related oncogene keratinocyte-derived cytokine (Gro/KC), and fractalkine. IL-10 was examined using a commercially available ELISA kit (Biosource International, Carmaillo, CA). All samples were analyzed in duplicate in a blinded fashion, and batched to reduce potential interassay variability. Data are presented as pg/mL serum.

Measurement of Musculoskeletal Tissue Cytokines using ELISA

Rats were euthanized with an overdose of sodium pentobarbital (Nembutal; 120 mg/kg body weight). Forearm bones and flexor muscles and tendons were collected from rats that had performed the HRNF task for 6 ($n = 6$) or 8 ($n = 5$) weeks, or the LRNF task for 6 ($n = 3$) or 8 ($n = 3$) weeks, and 15 controls. The forearm bones were further divided into distal and proximal portions (distal part consisting of radius/ulna metaphyses and epiphyses and carpal bones; the proximal part consisting of diaphyses). Tissues were flash frozen and stored at −80°C until homogenization. All tissue samples were homogenized unpooled with 0.5 to 1.0 mL RIPA buffer ((NaCl, KCl, NaH_2PO_4, KH_2PO_4, and $DDH_2O + NaOH$) plus EDTA free complete protease inhibitor cocktail tablets (Roche Diagnostics, GMPH, Germany) using a PowerGen 125 Homogenizer. Tissue homogenates were centrifuged at 14,000 rpm for 15 min at 4°C. Total protein was determined using BCA-200 protein assays (Bicin Choninic Acid; Pierce). Tissue lysates were analyzed for IL-1α, IL-1β, TNFα, and IL-10 using commercially available ELISA kits according to manufacturer's protocols (BioSource International). Each sample was run in duplicate and data (pg cytokine protein) normalized to μg total protein.

Immunohistochemical Analysis and Quantification of Tissue Macrophages

To examine for possible cellular sources of tissue cytokines, immunohistochemical analysis was performed on forelimb tissues collected from 16 rats: normal controls ($n = 10$) and HRNF 8 week ($n = 6$). Also, 14 control and 8 LRNF rats were examined for ED1-positive macrophages: normal controls ($n = 10$), trained controls ($n = 4$), LRNF 6 week ($n = 4$), and LRNF 8 week ($n = 4$). Tissues were prepared, immunostained, and quantified as described previously.[2,7,9]

Statistical Analyses

To test for differences in serum cytokines/chemokines between the 3 control groups, a one-way ANOVA was performed for each cytokine/chemokine. Because there were no differences between the three control groups (see Results section), all control rats were combined into one group. To determine the effect of reach rate and length of performance on serum markers, two-way ANOVAs were used for serum analyses with the factors week (control, which was considered as week 0, as well as 6 and 8 weeks) and task (HRNF and LRNF), followed by Bonferroni post hoc analysis. To determine the effect of reach rate and length of performance on tissue cytokines, four-way ANOVAs were used for each cytokine with the factors week, task, limb (preferred or nonpreferred) and tissue, followed by Fisher's least significant post hoc analysis. To determine the effect of length of performance on ED1+ macrophages in LRNF and control rat tissues, mixed model two-way ANOVAs were used with the factors week and limb, and with the three microscopic observations/tissue used as a blocking factor, followed by Bonferroni post hoc analysis. Grip strength was compared using two-way ANOVAs with the factors week and task, followed by Bonferroni post hoc analysis. Pearson's correlation tests (r) were used for correlation analyses.

RESULTS

Weight Restriction and Initial Training Did Not Affect Serum or Tissue Cytokine/Chemokine Levels

One-way ANOVA comparing the levels of serum biomarkers in three control groups (age-matched controls, age- and weight-matched controls, and trained controls) showed no significant differences for the serum cytokines and chemokines examined (data not shown). Also, there were no significant differences in musculoskeletal tissue cytokine levels in trained controls compared to normal controls (data not shown). Therefore, the controls were combined and termed "C" hereafter.

Serum Cytokine and Chemokines Increase with Repetition

For IL-1α, there were significant differences by week ($p = 0.0367$) and task ($p = 0.0481$). For TNFα, there were significant differences by week ($p = 0.0238$) and task ($p = 0.0481$). For MIP2, there were significant differences by week ($p < 0.0001$), and for MIP 3a, there were significant differences by week ($p < 0.001$) and task ($p = 0.0274$). Significant post hoc results are shown in Figure 1A–F. IL-1β and RANTES were significantly different by task ($p = 0.0250$ and $p = 0.0408$, respectively; Fig. 1E and F), with no significant post hoc findings for either. No significant serum increases were found for IL-6, IFNγ, Gro/KC, or fractalkine (data not shown).

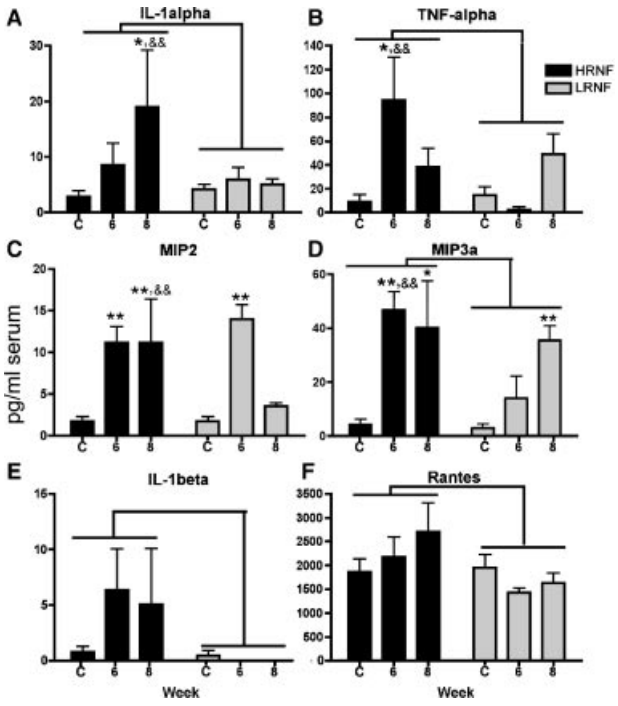

Figure 1. Serum cytokines and chemokines increase with performance of repetitive tasks compared to controls (C). (A,B) IL-1α and TNFα increase with the high repetition, negligible force (HRNF) task. (C,D) MIP2 and MIP3 increase in serum after performance of both the HRNF and the low repetition, negligible force (LRNF) task (E,F). IL-1beta and RANTES were significantly increased by task only (E,F). Mean + SEM shown. $^{*}p < 0.05$ compared to control rats; $^{**}p < 0.01$ compared to control rats; $^{\&\&}p < 0.01$ compared to same week LRNF rats; black horizontal bars indicate significant differences by task.

Tissue Cytokine Levels Increase with HRNF Task in Forelimb Musculoskeletal Tissues

For IL-1α, four-way ANOVA showed significant differences by week ($p = 0.0024$) and tissue ($p < 0.0001$). Post hoc results show that IL-1α increased significantly in HRNF flexor muscles and distal bones (distal radius/ulna and first row of carpal bones) of the preferred reach limb in week 8 (Fig. 2A), and in flexor tendons of the nonpreferred limb in week 8 (Fig. 2B). For IL-1β, there were significant differences by task ($p = 0.05$) and tissue ($p < 0.0001$). Similar post hoc results were found for IL-1β as for IL-1α (Fig. 2C and D). Neither IL-1 subtype increased significantly in LRNF tissues (Fig. 3A–D). For TNFα, there were significant differences by week ($p = 0.0019$) and tissue ($p < 0.0001$). Post hoc results show that TNFα increased significantly in week 8 HRNF distal bones, muscles, and tendons of the preferred limb (Fig. 2E), and in the nonpreferred limb in distal forelimb bone and tendons (Fig. 2F) ($p < 0.01$

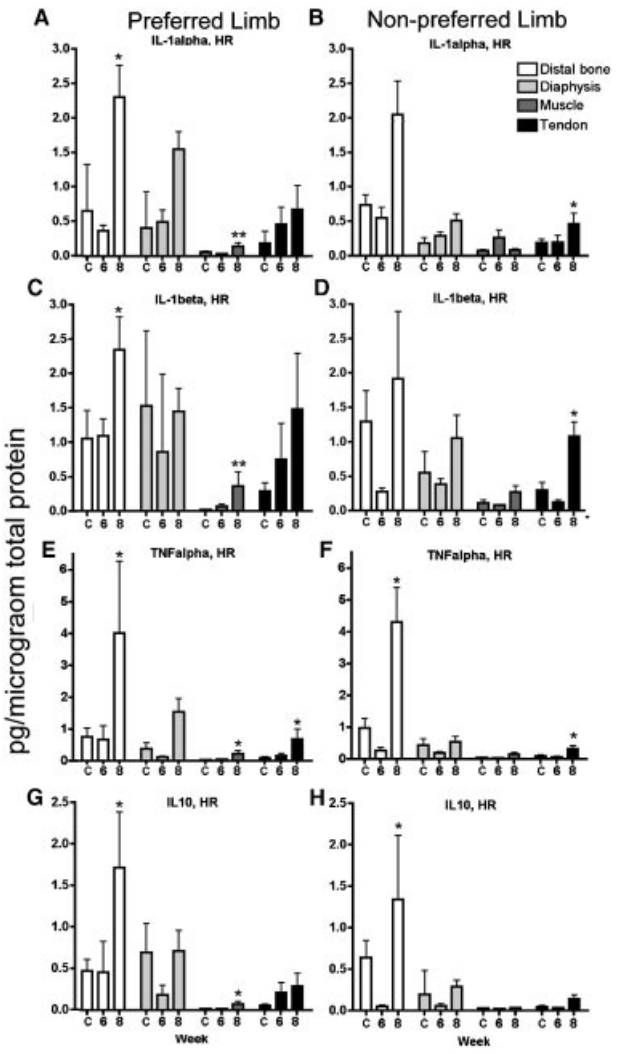

Figure 2. Cytokine expression in the high repetition, negligible force (HR) preferred and nonpreferred limb distal bones (distal radius/ulna and first row of carpal bones), radius/ulna diaphysis, and flexor forelimb muscles and tendons following performance for 6 or 8 weeks, compared to controls (C). Mean + SEM shown. $^{*}p < 0.01$ compared to control rats.

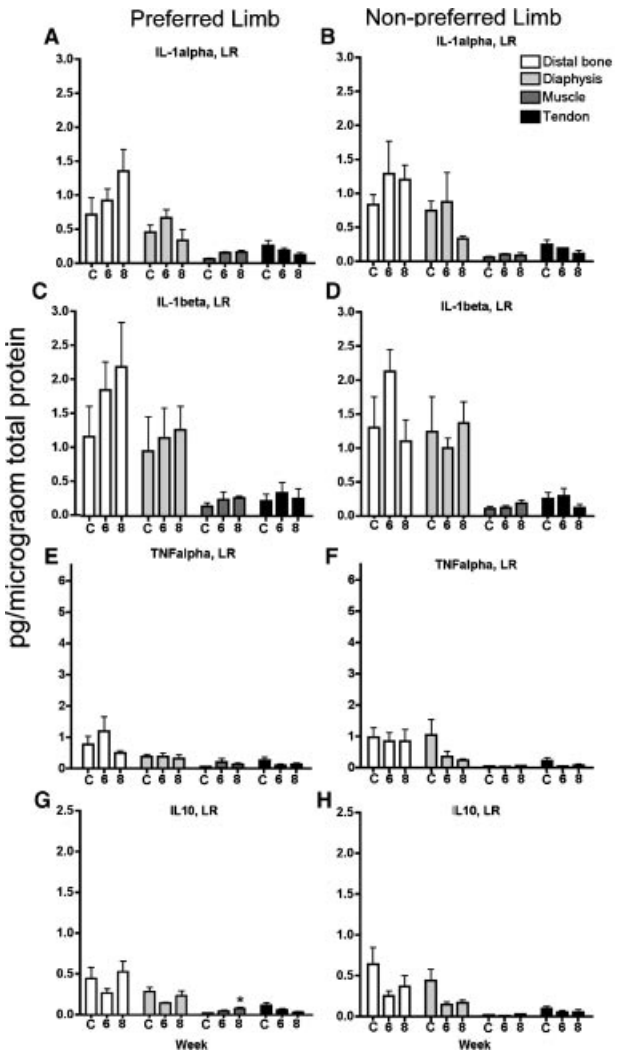

Figure 3. Cytokine expression in the low repetition, negligible force (LR) preferred and nonpreferred limb distal bones (distal radius/ulna and first row of carpal bones), radius/ulna diaphysis, and flexor forelimb muscles and tendons following performance for 6 or 8 weeks, compared to controls (C). Mean + SEM shown. $^{*}p < 0.01$ compared to control rats.

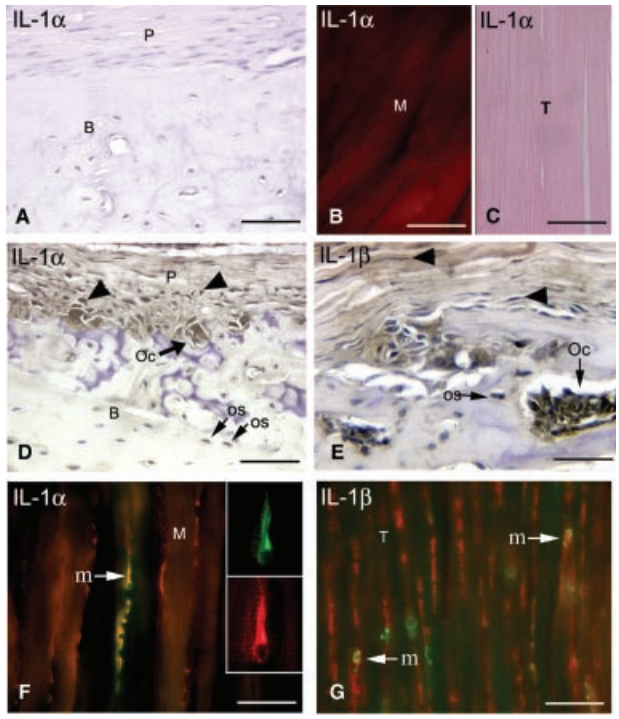

Figure 4. Immunohistochemistry of IL-1α/β. (A–C) IL-1α is expressed in few to no cells in control rat forelimb flexor periosteum (P), cortical bone (B), muscle (M), or tendon (T). (D–G) Forelimb musculoskeletal tissues of an 8 week HRNF rat. *(D,E)* Osteoclasts (Oc; larger arrow) and osteocytes (os; small arrows) in cortical bone (B) and mononucleated cells (arrowheads) in the periosteum (P) express IL-1α and IL-1β. (F) IL-1α (red) is expressed in myofiber (M) and by ED-1+ macrophages (m; yellow, because merged image is shown). Insets show enlargement of a green macrophage indicated with arrow in F; that also immunoexpress IL-1α (red). (G) A tendon (T) section showing IL-1β positive fibroblasts (red only) and ED-1+ macrophages (m; green and yellow). Scale bars = 50 μm.

each), but not in LRNF tissues (Fig. 3E and F). For IL-10, there were significant differences by week ($p = 0.0019$) and tissue ($p < 0.0001$). Post hoc results show that IL-10 increased significantly in week 8 in the HRNF distal bone and flexor muscles in the preferred limb (Fig. 2G), in the nonpreferred limb in distal bone (Fig. 2H) ($p < 0.01$ each), and in the LRNF preferred limb muscles ($p < 0.01$; Fig. 3G).

Some musculoskeletal cellular sources for IL-1α and IL-1β are shown in Figure 4. Few to no cells in control tissues express IL-1α (Fig. 4A–C) or IL-1β (data not shown). In forelimb tissues of 6-week HRNF rats, these two cytokines are expressed by osteoclasts and osteocytes in the distal radius and ulna bones (Fig. 4D and E), mononucleated cells in the periosteum (Fig. 4D and E), tendon fibroblasts (red cells in Fig. 4G), and ED-1-positive macrophages (Fig. 4F and G).

Presence of Phagocytic Macrophages in LRNF Rat Tissues and Correlations with Serum Cytokines

In LRNF loose connective tissues, which include tendon synovial tissues, there were significant differences in ED1+ macrophages by week only ($p < 0.0001$). Post hoc analysis showed increased ED1+ macrophages in week 6, bilaterally, compared to controls ($p < 0.01$; Fig. 5A). In LRNF tendons, ED1+ macrophages were not present above control levels (Fig. 5B), nor were they in distal bones or flexor muscles after task performance (data not shown). For the bone histological analyses, we examined sites of bone adjacent to distal flexor tendon and pronator quadratus attachments. The number of ED1+ macrophages in LRNF forelimb loose connective tissues correlated strongly with serum levels of IL-1α ($r = 0.79$; $p = 0.0284$) and MIP2 ($r = 0.82$; $p < 0.0120$) in these same rats.

Behavioral Results and Their Correlation of Serum Cytokines and Chemokines

The mean reach rate for the LRNF rats across all weeks of task performance was 3.25 ± 0.70 (mean ± SD) reaches per minute. The weekly variation in reach rate in these same LRNF rats was as follows: 3.3 ± 0.3 reaches per minute in week 1, 3.3 ± 0.2 in week 6, and

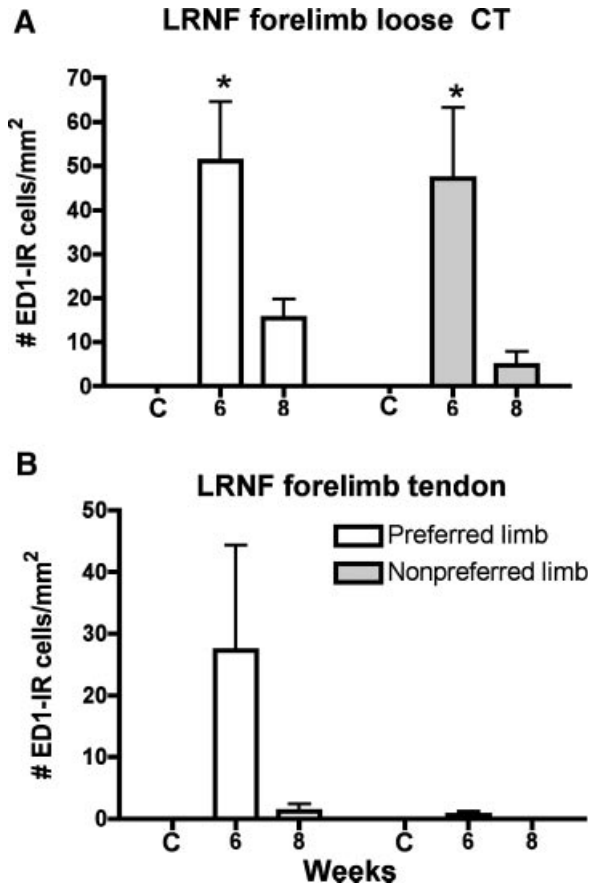

Figure 5. ED-1-immunopositive macrophages were quantified in forelimb flexor loose connective tissues (CT) and tendon in rats performing a low repetition, negligible force (LRNF) task for 6–8 weeks and compared to control rats (C). Mean + SEM shown. $^*p < 0.01$ compared to control rats.

3.6 ± 0.2 in week 8 (mean weekly reach rate ± SEM.) In contrast, the mean reach rate for the HRNF rats across all weeks of task performance was 5.42 ± 2.18 (mean ± SD) reaches per minute. The weekly variation in reach rate in these same HRNF rats was as follows: 6.53 ± 0.8 reaches per minute in week 1, 5.03 ± 0.7 in week 6, and 4.48 ± 0.3 in week 8 (mean weekly reach rate ± SEM). The total number of reaches, defined as the sum of the reaches performed per week, was 9446 ± 0.1673 (mean ± SEM) for the LRNF rats, and 16979 ± 6059 (mean ± SEM) for the HRNF rats.

The total number of reaches performed by the HRNF and LRNF rats correlated moderately in week 6 with serum levels of IL-1β ($r = 0.56$, $p = 0.0450$), strongly in week 6 with serum TNFα ($r = 0.80$, $p = 0.0088$) and MIP3a ($r = 0.82, p = 0.0068$), and strongly in week 8 with serum MIP2 ($r = 0.90$, $p = 0.0027$). For grip strength, there were significant differences by week ($p = 0.0001$), with grip strength decreasing significantly in week 6 HRNF rats ($p < 0.05$) and 8 ($p < 0.001$), and in week 6 LRNF rats ($p < 0.05$; Fig. 6A), compared to control rats. There were moderate but significant negative correlations between grip strength and serum levels of TNFα, MIP 2, and MIP3 in the HRNF/LRNF task and control

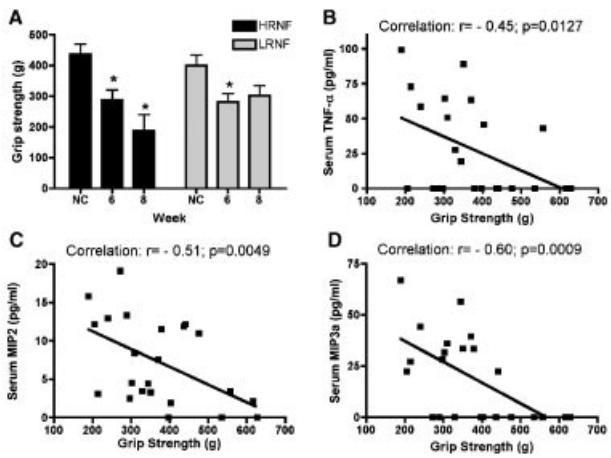

Figure 6. Grip strength and correlation with serum cytokines and chemokines. (A) Grip strength measurements in grams (g) in the reach limb of rats performing either the high repetition negligible force (HR) or low repetition negligible force (LR) task for 6 to 8 weeks, and age-matched normal control (NC) rats. Mean + SEM shown. $^*p < 0.01$ compared to controls. (B–C) Graphs showing Pearson's correlations between grip strength and three serum cytokines and chemokines.

rats (Fig. 6B–D), but not for the other cytokines or chemokines examined in serum (data not shown).

DISCUSSION

We show a dose–response relationship between reach rate and inflammatory and motor responses to repetitive reaching and grasping tasks in rats. Although the increase of some serum cytokines and chemokines was transient, others were prolonged with continued task performance. The greatest tissue cytokine increases were observed in the preferred limb distal bones in HRNF week 8, with smaller but still significant increases in preferred limb flexor muscles and tendons. Increases of tissue IL-1α matched its increase in serum temporally, while tissue IL-1β and TNFα levels did not match their serum levels. Our observed strong correlations between tissue macrophages and serum IL-1α and MIP2 levels, negative correlations between serum TNFα, MIP2, and MIP3a and grip strength, and positive correlations between serum cytokines/chemokines and total number of reaches performed suggest that increases in these serum protein are indicative of an underlying tissue inflammatory response that is affecting grip strength as task exposure increases.

Although several studies have found increased serum biomarkers of injury, inflammation, or collagen turnover in patients with repetitive motion injury or following performance of repetitive tasks,[2,10,19,20] this is the first study to report the presence of chemokines in serum following performance of repetitive upper extremity tasks and to examine such a large panel of cytokines and chemokines in RMI. We have previously reported that performance of the HRNF task leads to increased serum IL-1α, results confirmed here.[2] We have also observed a positive association between serum C-reactive protein, IL-1β, TNFα, and IL-6 and severity of symptoms in patients with short-term upper extremity

overuse injuries.[10] In contrast to our human study, we did not find increased serum IL-6 in this study, a result consistent with findings of a repetitive pinching task in a nonhuman primate model[6] and a study examining patients with carpal tunnel syndrome.[19] However, there was no detectable serum TNFα in those two studies[6,19] results differing from ours. Species differences in cytokine response profiles, the detection system used, and inherent differences in the injury induced may account for these varying results. For example, here, the rats flex their wrists as well as extend their elbow and shoulder, increasing the involvement of more regions than in the pinch task.[6]

Prolonged, intensive cycling results in short-lived increases (immediate to 2 h postexercise) of several serum cytokines,[13,14] presumably after release from contracting muscles.[21] However, it is unlikely that muscle contractions contributed to our increased serum cytokines and chemokines because we collected serum at 18–36 h after completion of the final task session. Although high-intensity eccentric exercise can induce postexercise serum increases of IL-6 and IL-10 that last up to 144 h,[14,22] we did not observe such increases. TNFα does not increase in muscle or serum during exercise,[21] but with injury and its severity, increases that persist up to 14 days post trauma.[15,16,23]

Cytokines and chemokines are produced by many cell types in response to stress, exercise, or trauma.[9,15,21,23] Possible sources of the increased serum or tissue cytokines and chemokines include circulating monocytes, macrophages, bone cells, and fibroblasts, all shown to produce IL-1α/β, TNFα, and IL-10.[9,11,13,24] MIP2 and MIP3a are produced by leukocytes, endothelial cells, and fibroblasts.[25–27] Skeletal muscle is also a known source of cytokines, at least locally, during states of chronic inflammation and during exercise.[21]

There are many consequences of increased circulating cytokines. IL-1α and β, key immunoregulatory cytokines, induce synovial cells, lymphocytes, endothelial cells, and macrophages to produce inflammatory mediators.[11] These mediators, which include IL-1 and TNFα, induce fibroblast proliferation and increase permeability of blood vessel walls, thus increasing inflammatory cell infiltration.[28] Also, both IL-1α and TNFα tend to shift the balance of bone turnover towards bone resorption.[11,29] Carpal tunnel syndrome, a RMI, has been associated with decreased bone mineral densities in the distal radius–ulna and metacarpal bones in the affected limbs.[30] Although thenar muscle atrophy may be one cause of this bone loss, perhaps increased circulating cytokines are contributory.

MIP2 is a potent chemoattractor of several subsets of leukocytes[25,31] and demonstrates growth regulatory bioactivity that may contribute to wound healing.[32] Infiltrating mononuclear cells and synovial fibroblasts produce MIP3a in rheumatoid arthritis patients in response to IL-1 and TNFα.[33] MIP3a then recruits leukocytes such as memory T cells, naïve B cells and dendritic cells.[33] RANTES, a pro-inflammatory chemokine, is also stimulated by IL-1 and TNFα, produced by fibroblasts, and involved in trafficking leukocytes subsets into inflamed tissues.[34]

We found significantly increased numbers in distal forelimb loose connective tissues in LRNF rats, bilaterally, in week 6. Previously, we found increased ED1 positive macrophages in rats performing the HRNF task in several tissues throughout the entire upper extremity by week 3 and remaining significantly elevated through week 8.[2,3,7,9] The prolonged and widespread macrophage response in rats performing the HRNF task contrasts with the restricted and transient increase in rats performing the LRNF task, indicating a dose-dependent response. Using the LRNF macrophage data, we found strong positive correlations between serum MIP2 and IL-1α and macrophages numbers in loose connective tissues of these rats, tissues that include flexor tendosynovial sheaths. Because tendosynovial sheaths have been shown to undergo pathological changes in humans with long-term carpal tunnel syndrome,[35] these correlations suggest that the elevation of these two proteins in serum may be due to macrophage recruitment as a consequence of tissue injury.

Here, increases of several serum and tissue cytokines correlated with decreased grip strength. The grip strength changes may be due to either tissue injury or discomfort. The dose-dependent decreases in grip strength suggest the possibility that at lower thresholds, little to no injury or inflammation is stimulated. Thus, the lower task intensity allows adequate tissue recovery without disruption in motor behavior. However, at higher task intensities, in which tissue injury and inflammation induced by continued task performance apparently outpaces tissue recovery mechanisms, the serum and tissue inflammatory response is not only detectable, but is associated with declines in motor performance.

In conclusion, we found that several potent proinflammatory cytokines/chemokines are associated with performance of a HRNF task in both tissues and serum, and that fewer increase with a LRNF task. We also found that increases in circulating cytokines and chemokines correlated with macrophage numbers in affected tissues and decreased motor function. These findings suggest that measurement of these mediators might be important adjuncts to managing ongoing tissue inflammation occurring as a consequence of tissue injury.

ACKNOWLEDGMENTS

This work was supported by grants from CDC-NIOSH OH 03970 to M.F.B. and from NIAMS AR051212-01 to A.E.B. No authors have professional or financial affiliations that would bias this work.

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