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Short Communication

Improvement in muscle strength after an anterior cruciate ligament injury corresponds with a decrease in serum cytokines

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ABSTRACT

The purpose of this communication was to identify if a decrease in serum cytokine concentrations associates with an improvement in muscle strength after an anterior cruciate ligament (ACL) injury. To establish groups with contrasting serum cytokine concentrations, subjects scheduled for ACL reconstructive surgery were separated into one of two groups (gender matched) based on their time from injury occurrence: (1) Early (<21-d from injury occurrence; $n = 22$) or (2) Late ($\geqslant$21-d from injury occurrence; $n = 22$). Before surgery, each subject provided a fasting blood sample and performed single-leg peak isometric force testing on the injured (INJ) and non-injured (NI) limbs. Compared to the NI limb, peak isometric force in the INJ limb was decreased ($p < 0.05$) in both groups (Early, ~35%; Late, ~18%). The deficit in peak isometric force, however, was increased ($p < 0.05$) in the Early compared to Late group. Similarly, serum granulocyte macrophage colony-stimulating factor (GM-CSF), interleukin (IL)-6, and IL-13 were increased (all $p < 0.05$) in the Early group. These unique findings show a concurrent increase in muscular weakness and serum cytokine concentrations shortly after (<21-d) an ACL injury. Importantly, muscular weakness persisted thereafter ($\geqslant$21-d) but at an attenuated level and parallel to a decrease in circulating cytokine concentrations. We conclude that a decrease in serum cytokines associates with a reduction in muscular weakness after an ACL injury.

1. Introduction

The anterior cruciate ligament (ACL) is one of the most commonly injured ligaments in the knee. Reconstructive surgery restores knee joint stability after an ACL injury, but muscular (quadriceps) weakness persists and is predisposing factor for knee osteoarthritis years later. Interestingly, an increase in muscular weakness prior to surgery [1] and surgery shortly after (<21-d) an ACL injury [2] enhance muscular weakness after surgery, thereby demonstrating the influence of pre-surgical variables on post-surgery outcomes.

Cytokines are small peptides that regulate host defenses and cell signaling. Following trauma, cytokines expressed by the initial T-helper (Th) 1 response assist with the removal of necrotic tissue or cellular debris and aid in regeneration. The Th1-type cytokines are subsequently replaced by Th2-type cytokines that attenuate the initial inflammatory response and promote tissue repair. Although not widely studied, a variety of diverse cytokines increase locally and systemically following an ACL injury [3,4]. The local cytokine increase immediately after an ACL injury is not sustained as concentrations gradually decrease [5,6]. Unlike the local response, it is unknown if the circulating cytokine response is temporally dependent following an ACL injury, and as demonstrated in experimental animals [7], influential on skeletal muscle function. Understanding the association between circulating cytokines and muscular weakness following an ACL injury could be paramount in determining when to undergo surgery and consequentially govern post-surgery outcomes.

Therefore, we sought to identify if a decrease in serum cytokine concentrations associates with an improvement in muscle strength after an ACL injury. We hypothesized that a decrease in serum cytokines corresponds with a reduction in muscular weakness after an ACL injury and prior to reconstructive surgery.

2. Materials and methods

Subjects with a ruptured ACL that required reconstructive surgery were separated into one of two groups; Early or Late. Early was defined as sustaining an ACL injury within 21-d of data collection, while an injury $\geqslant$21-d from data collection was designated as

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Late. As designed, days from injury occurrence to data collection was significantly ($p < 0.05$) different between the Early (mean (SD); 9 (6) d) and Late (92 (112) d) groups. Groups were gender-matched.

It should be noted that for the purpose of this investigation, only the baseline gender-matched data are presented from a randomized, double blind, placebo-controlled study. Data from the intervention portion of this study will be presented in a later manuscript that is currently in preparation.

Modestly active (i.e., 30 continuous minutes of activity at least 3 times per week prior to sustaining an ACL injury) adults between 18 and 45 years of age who required and voluntarily elected for ACL reconstructive surgery were recruited for participation. Subjects were excluded from participation if: they suffered a previous lower extremity injury that required the use of crutches for more than 1 week within the past year, had a history of any condition requiring medical attention, pregnant, or morbidly obese (body mass index > 40 kg/m^2). Subjects were also excluded from participation if they were using warfarin or other anti-coagulants, cholesterol lowering medication, a daily dietary supplement or vitamin during the previous year, or any non-steroidal anti-inflammatory drugs or physician guided medication. Subjects were informed of and provided written and verbal consent to the experimental protocol and procedures. The Urban Central Region Institutional Review Board at Intermountain Healthcare (Salt Lake City, UT USA) approved this study.

2.1. Study protocol

Each subject reported to the Physiology Research Laboratory at The Orthopedic Specialty Hospital. At this visit, each subject provided a fasting blood sample (between 07:00 and 11:30 a.m. and within 1 h of waking), completed patient-reported survey's, and performed single-leg peak isometric force testing on the injured (INJ) and non-injured (NI) limbs. All subjects were scheduled to undergo ACL surgery by one orthopedic surgeon (RHT) at The Orthopedic Specialty Hospital.

2.2. Analytical procedures

Serum cytokine concentrations were determined using the multiplex technology of Luminex (MAGPix; Austin, TX USA) with a high sensitivity kit (EMD Millipore, Billerica, MA USA). Metabolic and lipid measurements were performed on plasma samples (ARUP Laboratories, Salt Lake City, UT USA).

Single-leg peak isometric force testing was conducted (performed in triplicate, NI limb CV = 5.64%; INJ limb CV = 8.55%) on a horizontal Plyo-Press (Athletic Republic, Park City, UT USA), as described [8]. Peak isometric force was defined as the highest resultant force from the three isometric contractions on each leg.

Each subject completed the International Knee Documentation Committee (IKDC) subjective knee form, and the pain and physical function subsections of the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) questionnaire.

2.3. Statistical analysis

Data were checked for normality with a Shapiro–Wilk test. For normally distributed data, statistical significance between groups were assessed with separate *t* tests. Statistical significance of non-normally distributed data were checked with a Mann–Whitney *U* test. Analysis of variance was used to assess the statistical significance of peak isometric force between legs and groups. A Chi-Square test was performed to identify the association between injury status (Early vs Late) and the percent deficit in peak isometric force in the INJ compared to the NI limb. Normally distributed data are presented as mean (SD) and non-normally distributed data are presented as mean ± SEM. Statistical significance was set at $p < 0.05$ and all statistical analyses were performed with SYSTAT software (version 13.1, Chicago, IL USA).

3. Results

Subject characteristics, patient-reported outcomes, clinical chemistries, and peak isometric force in the NI limb were not significantly different between groups (Table 1). Compared to the NI limb, peak isometric force in the INJ limb was significantly ($p < 0.05$) decreased in the Early (~35%) and Late (~18%) groups. The peak isometric force deficit in the INJ limb, however, was significantly ($p < 0.05$) greater (~23%) in the Early compared to Late group (Table 1). Parallel to an increase in muscular weakness, serum GM-CSF, IL-6, and IL-13 were significantly (all $p < 0.05$) increased, and IFN-γ ($p = 0.08$) and IL-10 ($p = 0.06$) tended to increase in the Early group (Table 2).

4. Discussion

This study provides the first evidence that serum cytokine concentrations increase shortly after an ACL injury. In elderly, a pro-inflammatory cytokine (e.g., IL-6) increase in the circulation associates with muscle atrophy [9], while experimental animal results clearly establish the catabolic influence of a pro-inflammatory cytokine on skeletal muscle morphology [10]. Muscle atrophy

Table 1
Subject characteristics, chemistries, and force data.

	Early	Late	*p*-Value
Gender (m:f)	22 (14:8)	22 (14:8)	
Age, y	32 (8)	30 (7)	0.32
Height, cm	174 (11)	172 (8)	0.60
Body mass, kg	82.5 (15.9)	84.8 (15.7)	0.63
BMI, kg/m^2	27.0 (4.1)	28.4 (4.6)	0.27
IKDC score	22.9 (15.9)	21.8 (21.2)	0.86
WOMAC pain	7.60 (4.42)	6.86 (3.98)	0.58
WOMAC function	23.6 (13.1)	26.7 (15.4)	0.49
Cholesterol, mmol/L	4.83 (0.82)	4.88 (0.84)	0.86
Triglycerides, mmol/L	1.33 (0.45)	1.40 (0.68)	0.66
HDL, mmol/L	1.17 (0.36)	1.18 (0.33)	0.91
VLDL, mmol/L	0.61 (0.21)	0.64 (0.31)	0.68
LDL, mmol/L	2.99 (0.69)	3.06 (0.80)	0.77
Sodium, mmol/L	135 (6)	136 (4)	0.49
Potassium, mmol/L	4.02 (0.21)	3.93 (0.30)	0.25
Chloride, mmol/L	97.1 (5.0)	97.8 (4.7)	0.62
CO2, mmol/L	19.4 (2.5)	20.3 (2.3)	0.23
AP, U/L	65.0 (16.2)	58.4 (18.1)	0.21
ALT, U/L	19.4 (23.6)	17.2 (13.9)	0.71
AST, U/L	24.2 (10.8)	29.4 (35.6)	0.51
Glucose, mmol/L	4.78 (0.62)	4.72 (0.42)	0.69
BUN, mmol/L	5.07 (1.00)	4.89 (1.00)	0.49
Creatinine, μmol/L	78.7 (11.5)	80.4 (16.8)	0.57
Total protein, g/L	69.1 (5.7)	67.9 (5.5)	0.49
Albumin, g/L	42.7 (3.6)	43.0 (3.5)	0.79
Total bilirubin, μmol/L	9.15 (5.93)	7.80 (3.56)	0.38
Piso, N/kg			
INJ	7.09 (2.26)	9.24 (3.11)	0.01
NI	10.9 (2.7)*	11.3 (2.6)*	0.64

Data presented as mean (SD).
AP, alkaline phosphatase.
ALT, alanine aminotransferase.
AST, aspartate aminotransferase.
BUN, blood urea nitrogen.
HDL, high density lipoprotein.
INJ, injured limb.
LDL, low density lipoprotein.
NI, non-injured limb.
Piso, peak isometric force.
VLDL, very low density lipoprotein.
* $p < 0.05$ vs INJ.