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Role of interleukin-10 in the synovial fluid of the anterior cruciate ligament injured knee

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Abstract. – OBJECTIVE: This review assesses the roles of IL-10 in post ACL reconstruction OA, and highlights the potential therapeutic effects of this cytokine.

MATERIALS AND METHODS: We conducted a systematic review of the literature in order to consolidate evidence of IL-10 profiles in synovial fluid (SF) of patients with ACL tears. The review was conducted in accordance with the PRISMA statement. In total, 10 studies were found to be pertinent and were considered in depth. Seven studies reported on trends in IL-10 concentrations after an ACL tear; in addition, three studies described IL-10 concentrations after ACL reconstruction. In all studies, IL-10 levels were assessed using enzyme-linked immunosorbent assay.

RESULTS: IL-10 levels in SF were higher after ACL injury and ACL reconstruction compared to control knees. IL-10 levels were most elevated shortly after injury, but, decreased to more normal levels in chronic lesions. In contrast, the inflammatory cytokine TNF-α remained higher than controls immediately subsequent to, and, even 5 years post-injury.

CONCLUSIONS: IL-10 is a modulatory cytokine with an active role in antagonizing TNF-α in the knee joint environment. Consideration of the role of IL-10 in the knee has now shifted from simply a key biomarker to having active therapeutic potential in the prevention of OA after ACL injury.

Key Words:
Interleukin-10, ACL tear, ACL reconstruction, Cytokines, Knee, Osteoarthritis.

Introduction

Anterior cruciate ligament (ACL) injuries represent approximately 25% of all knee injuries with an annual incidence of at least 0.8 per 1000 persons aged between 10 and 64 years[1]. In particular, ACL tears account for 6.7% of all injuries in pediatric populations and 30.8% of all knee injuries in soccer players aged 5 to 18[2]. Compared with conservative treatment, surgical reconstruction (i) improves knee stability, (ii) reduces the incidence of further knee injuries such as meniscal and chondral lesions in adults and pediatric patients, and (iii) allows the patients' return to sporting activities with improved performance[3,4]. However, ACL reconstruction does not reduce the occurrence of posttraumatic osteoarthritis (OA)[5,6]; consequently, the appropriate management of this lesion is still the object of considerable debate[7]. Fifty to 60% of patients with ACL reconstructed knees have radiographic evidence of OA after just five years post-surgery[6,8]. OA is usually a slow progressive joint disorder, characterized by (i) joint pain, (ii) cartilage degeneration, and (iii) decreased joint function[9]. In addition to biomechanical factors, changes in biochemical profiles within the knee joint after injury and ACL reconstruction could have a role in causing joint degeneration. Several studies[10] have been conducted on the influence of many cytokines and other soluble factors in the injured knees of children and adults. However, the results are inconclusive, and some findings are contradictory. Elevated levels of varied inflammatory cytokines (including IL-6, IL-1ß and TNF-α) have been reported in acute and chronic ACL-injured knees, suggesting that inflammatory cytokines can promote cartilage catabolism through the synthesis of free radicals and metalloproteases (MMPs) and consequently contribute to OA development[8,11-13]. A second group of cytokines, endowed with anti-inflammatory properties (such as IL-10, IL 4 and IL-13) interacts with the inflammatory group thereby

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modulating cartilage catabolism; however, their exact role in OA pathogenesis, with respect to inter- and intracellular signaling pathways remains undefined, and is still under investigation[12,14,15]. Many attempts have been made to prevent OA development by inhibiting the synthesis and/or activity of inflammatory cytokines such as IL-1ß and TNF-α. It is generally accepted that TNF-α drives acute inflammation whereas IL-1ß has a primary role in sustaining the inflammation and promoting cartilage erosion[16,17]. An alternative approach was proposed which involved potentiating the activity of anti-inflammatory cytokines[18,19]. IL-10, in particular, has received considerable attention since its anti-inflammatory activity is well documented. IL-10 functions primarily to modulate inflammatory responses by regulating the activity of T cells, monocytes and macrophages. Several *in vitro* studies found that IL 10 can antagonize several inflammatory pathways[20,21].

Recently, several research groups have focused their attention on the potential of IL-10 as a therapeutic tool for OA therapy and prevention. One very novel approach examined the therapeutic potential of overexpression of IL-10 induced by gene therapy in a three-dimensional micro-mass model of the human synovial membrane; the consequent overexpression of IL-10 reduced production of inflammatory cytokines, suggesting a possible application in prevention of OA[22].

IL-10 protects against blood-induced joint damage due to its anti-inflammatory properties and therefore ought to be evaluated as candidate for inhibiting the tissue damaging effects of joint hemorrhages[23]. A fusion protein made up of IL-10 and IL-4 prevented blood induced cartilage damage in hemophilic mice and reduced the inflammatory response[24].

The purpose of this review was to summarize published evidence regarding the anti-inflammatory properties of IL-10 in synovial fluid of ACL injured knees

Materials and Methods

Focused Question Based

Based on the Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) guidelines, a specific question was constructed. The focused question was ‘‘What are the synovial IL-10 trends in patients with and without an ACL injury?”

Eligibility Criteria

The following inclusion criteria were used to determine study eligibility: (i) original clinical studies, (ii) patients with ACL injury, (iii) inclusion of case control and cohort study, and (iv) intervention: patients with and without ACL injury. Letters to the editor, historic reviews, case reports, case-series and unpublished articles were excluded.

Search Strategy and Study Selection

We conducted a comprehensive literature search using PubMed/Medline (National Library of Medicine, Washington, DC), EMBASE and Scopus from 1965 up to and including October 2017. The literature search terms included the following combination of keywords: (a) “IL-10” AND “ACL injury”, (b) “IL-10” AND “ACL tear”, (c) “IL-10” AND “ACL”, (d) “IL-10” AND “synovial”, (e) “IL-10” AND “synovial” AND “ACL injury”, (f) “IL-10” AND “synovial” AND “ACL tear”, as well as (g) “IL-10” AND “synovial” AND “ACL”. We also searched with the following combination of MeSH terms: (a) “Interleukin-10” AND “Anterior Cruciate Ligament Injuries”, (b) “Interleukin-10” AND “Anterior Cruciate Ligament”, (c) “Interleukin-10” AND “Synovial Fluid”, (d) “Interleukin 10” AND “Anterior Cruciate Ligament Injuries” AND “Synovial Fluid”, as well as (e) “Interleukin-10” AND “Anterior Cruciate Ligament” AND “Synovial Fluid”.

Titles and abstracts of studies identified using the above-described protocol were screened by two authors (NZ and MT) and checked for agreement. Full-texts of studies judged by title and abstract to be relevant were read and independently evaluated for the stated eligibility criteria. Reference lists of potentially relevant original articles were hand-searched in order to identify any studies that could have remained unidentified in the previous step. A search for “similar article” was done for the papers selected. Once again, the articles were checked for disagreement via discussion among the authors following a structured algorithm (Figure 1). The initial search yielded 1031 studies. One thousand and twenty-one studies, which did not fulfill the eligibility criteria, were excluded (Figure 1). In total, 10 studies[13,22-30] were included and processed for data extraction (Tables I and II).

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Methodological Study Quality Assessment

The Newcastle-Ottawa Scale[34] (NOS) was used to grade the methodological quality of

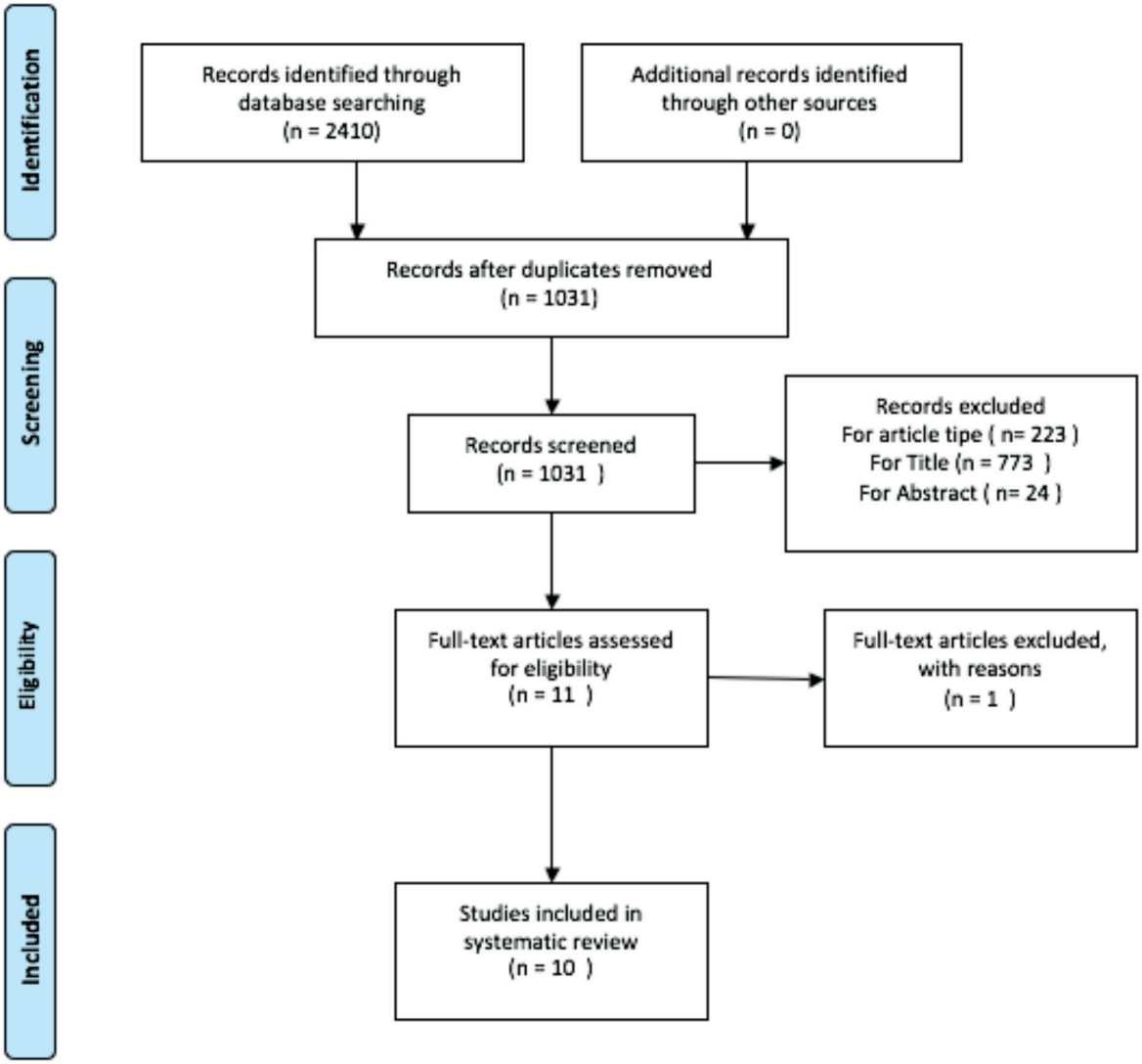

Figure 1. Review algorithm

each study assessed in the present systematic review (Table III). In summary, the NOS scale uses a systematic approach based on 3 specific criteria: Selection (S), Comparability (C) and Exposure (E), which are subdivided into 9 criteria: (S1) adequate case definition, (S2) representativeness of the cases, (S3) selection of control, (S4) definition of control, (C1) comparability of cases, (C2) controls on the basis of the analysis, (E1) ascertainment of exposure, (E2) same method of ascertainment for cases and controls, as well as (E3) non-response rate. Each criterion returned an answer that could be either "Yes", "No", or "cannot tell". Each study could have a maximum score of 9.

Results

General Characteristics of Included Studies

Five studies were case-control[13,23,25-27] and four were cohort studies[11,24,28,30].

All studies reported numbers of study participants, which ranged from 25 to 134 subjects, with a mean age ranging between 19 and 64.8 years. In all studies the SF was collected by arthrocentesis without saline infiltration. Five studies[13,23,25-27] included a control group, two studies[23,26] used SF collected from healthy contralateral knees, two studies[13,27] used SF from chronic ACL lesioned knees, and one study[28] used SF from a knee with OA.

Table I. Characteristic of the studies included.

Authors	Study Groups	Measure of cytokine levels	Cytokines studied	Outcomes of study
Bigoni et al[12]	Group 1: ACL (n=8) Group 2: Control (n=17)	ELISA	IL-1ß, IL-6, IL-8, IL-10, TNF alfa	IL-6 -8 -10 significantly higher in acute and post-surgery compared to chronic group. Similar level in pre-surgery group compared to chronic lesion.
Bigoni et al[25]	Group 1: ACL (n=48) Group 2: Literature Control	ELISA	IL-1ß, IL-1ra, IL-6, IL-8, IL-10, TNF alfa	IL-1 ß, IL-6, IL-8 levels higher compared to healthy knee, IL-1ra levels lower compared to healthy knees. Control values not available for IL-10.
Kaplan et al[29]	Group 1: ACL or MI or CI (n=30 – 35 – 5) Group 2: Control (n=32)	ELISA	MMP-3; MMP-13; TIMP 1; TIMP-2; TIMP-3; TIMP-4; FGF 2; IL-10; PDGF; IL-1ra; IL-1b; IL-6; MCP-1; MIP 1a; MIP-1b; RANTES	In ACL subgroup, significantly higher levels of IL-6 and MMP-3 compared to healthy group. No difference in IL-10 pre-arthroscopy.
Inoue et al[27]	Group 1: early ACL surgery (<60 days) Group 2: late ACL surgery (>60 days)	ELISA	TNF-Alpha, IL1-beta, IL2, IL6, IL8, IL10, IFN- gamma	Pro inflammatory and anti-inflammatory cytokine were significantly higher in patients with delayed surgical treatment compared to acute treatment. Worst recovery in delayed surgery.
Irie et al[28]	Group 1: ACL (n=36) Group 2: Control (OA) (n=7)	ELISA	TNF-a, IL-1b, IL-6, IL-8, IL-1ra, IL-10	All cytokines were significantly higher after ACL injury compared to control with different pattern of decrease.
Kaplan et al[29]	Group 1: ACL (n=34) Group 2: ACL + cartilage (n=28) Group 3: Control (n=72)	ELISA	MMP-3, MMP-13, TIMP-1, TIMP-2, TIMP-3, TIMP-4, (FGF-2), eotaxin, IFNg, IL-10, PDGF, IL-1Ra, IL-1b, IL-6, MCP-1, MIP-1a, MIP-1b, TNFa, VEGF	6 cytokines rose significantly in knee with ACL injury compared to healthy contralateral knee (MMP-3, IL-6, MIP-1ß, TIMP 1, TIMP 2, FGF). No statistically significant difference for IL-10.
Larsson et al[27]	Group 1: early ACL recon (n=59) Group 2: delay ACL rec (n=30) Group 3: rehab (n=29)	ELISA	IL-6, IL-8, IL-10, IFNg, (TNF), ARGS- CTX-II, NTX-I	Early ACL reconstruction had higher cytokine concentrations at 4 months (IL-6, IL-8, IL-10, TNF), 8 months (IL-6 and TNF) and at 5 years (IFNγ) compared to rehabilitation group. Delayed ACL reconstruction within 5 years, had higher synovial fluid concentrations of IL-6 at 5 years compared to those treated with rehabilitation alone.
Martinez et al[28]	Group 1: Mi (32) Group 2: ACLi (17) Group 3: Ci (13)	ELISA	IL-1, IL-2, IL-6, IL-10, TNF-alpha, IGF-1, TGF ß	Rise of inflammatory cytokine. IL-10 higher in ACL injury compared to meniscal and chondral injury.

Table continued

Table I. *(Continued).* Characteristic of the studies included.

Authors	Study Groups	Measure of cytokine levels	Cytokines studied	Outcomes of study
Tourville et al[30]	Group 1: ACL (67) Group 2: Control	ELISA	ARGS neoepitope, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-12, IL-13, IL-15, IL-17. , MMP 1, 2, 3, 7, 9, 10, 12, and 13 and TIMP 1, 2, 3, and 4.	Rise of inflammatory cytokines in acute settings. No correlation of IL-10 with synovial ARGS.
Struglics et al[29]	Group 1: ACLi (121) Group 2: Control (21)	ELISA	MMP-3, MMP-13, TIMP-1, TIMP-2, TIMP-3, TIMP-4, (FGF-2), eotaxin, IFNg, IL-10, PDGF, IL-1Ra, IL-1b, IL-6, MCP-1, MIP-1a, MIP-1b, TNF- alpha, VEGF	Elevation of (IL-6, IL-8, IL-10, TNF) in the baseline visit. After 30 days, all the cytokines return to healthy control levels except for TNF that remains elevated with a statistically significant difference compared to healthy control for all the 5 years of follow up.

All studies[13, 23, 25-27] used the ELISA method to measure cytokines in synovial fluid

Synovial IL-10 in Acute ACL Injury

Five studies reported significantly increased levels of IL-10 in SF after ACL injury[13,22,25,28, 29] compared to healthy knees. Of note, IL-10 levels were higher when samples were obtained within a few days post-injury compared to samples obtained from chronic ACL injured and OA knees. Inflammatory cytokines, such as IL-6 and IL-8, increased in the SF of acute ACL injured knee[13,22,25,28-30]. To illustrate, IL-6 levels were only 1.050-fold higher compared to healthy knees, whereas the increase of other cytokines ranged from 6-fold (TNF-α) to 21- fold (IL-10)[32]. Again, in acute conditions (<72 hours after knee injury) IL-6 and 8 were elevated 15- and 11- fold, respectively, com-

Table II. Characteristic of the studies included.

Authors	No. of Patients	Mean age/SD o range in years	Gender (F/M)	ACL injury diagnosis method	Synovial fluid collection method
Bigoni et al[13]	25	Group 1: 24.5 ± 7.5 Group 2: 32.6 ± 9.05	All male	MRI	Arthrocentesis without lavage.
Bigoni et al[22]	48	NA	All male	MRI	Arthrocentesis without lavage.
Cuellar et al[23]	102	NA	NA	MRI	Arthrocentesis without lavage.
Inoue et al[24]	79	Group: 19 (12-62)	40/39	MRI	Arthrocentesis without lavage.
Irie et al[25]	43	Group 1: 27.2 (13-55) Group 2: 64.8 (49 – 80)	23/18	MRI	Arthrocentesis without lavage.
Kaplan et al[26]	134	Group 1: 34 ± 8.12 Group 2: 36.29 ± 9.04 Group 3: 41.06 ± 14.25	NA	MRI	Arthrocentesis without lavage.
Larsson et al[27]	118	Group 1: 26.6 ± 5.1 Group 2: 26.4 ± 4.9 Group 3: 25.2 ± 4.5	88/118	MRI	Arthrocentesis without lavage.
Martinez et al[28]	62	Group 1: 46.18 ± 9.23 Group 2: 34.05 ± 6.88 Group 3: 45.14 ± 8.16	5/57	MRI	Arthrocentesis without lavage.
Tourville et al[30]	67	Group 1: 30 ± 11.4	29/38	MRI	Arthrocentesis without lavage.
Struglics et al[29]	141	Group 1: 26 ± 4.9 Group 2: 28 ± 9.4 Group 1: 31/90 Group 2: 8/13.		MRI	Arthrocentesis without lavage.

Table III. Methodological quality assessment with Newcastle–Ottawa Scale.

	Selection				Comparability		Exposure			No.
Study	S1	S2	S3	S4	C1	C2	E1	E2	E3	of star
Bigoni et al[13]	X	X	X		X		X	X	X	7
Bigoni et al[22]	X		X	X	X		X	X		6
Cuellar et al[23]	X	X	X	X	X	X	X	X		8
Inoue et al[24]	X	X	X		X		X	X		6
Irie et al[25]	X	X	X	X	X		X	X	X	8
Kaplan et al[26]	X	X	X	X	X		X	X	X	8
Larsson et al[27]	X	X	X	X	X	X	X	X	X	9
Martinez et al[28]	X	X			X		X	X		5
Tourville et al[30]	X		X	X	X		X	X		6
Struglic et al[32]	X	X	X	X	X	X	X	X	X	9

pared to chronic (> 3 months) ACL knees[15], whereas IL-10 levels increased to a smaller extent. All these differences were found while measuring synovial cytokine levels in acute/sub-acute settings with a time range of measurement from 6 h[15] to 6 weeks[32] post-ACL injury. In contrast, two studies[23, 26] reported that synovial IL-10 levels in ACL injured knees were not significantly greater than in healthy knees; however, the first study[26] did not specify the time elapsed from ACL injury, while the second study[29] reported only that the mean time elapsed from ACL injury was 5.5 weeks, with a range from 3 to 12 weeks. Four studies[13,22,25,29] measured cytokine levels at different time intervals following ACL-injury. These studies reported a temporally downward trend of IL-10 and other cytokine concentrations progressing from acute to chronic injury states. To illustrate the consequence of time of sampling, initial elevations of IL-10 associated with acute ACL injury dropped to levels comparable with those in chronic ACL injuries (and healthy knees) within 30 days[15]. Furthermore, some authors[22,25] reported that intra-articular levels of IL-10 remained elevated for the first two weeks after trauma and then subsequently returned to control levels[15]. In sharp contrast, TNF-α levels remained elevated in all ACL injured knees for up to five years[25].

Synovial IL-10 After ACL Reconstruction

A few studies[13,24,27] investigated IL-10 trends after ACL reconstruction. These three studies reported a rise in both inflammatory and anti-inflammatory cytokines after ACL reconstructive surgery compared to control knees. Bigoni et al[15] found that one month after ACL reconstruction, IL-10, IL-6- and IL-8 levels were significantly elevated compared to a chronic ACL injury group. The increased levels of pro-inflammatory cytokines (IL-6 and IL-8) were similar to those reported in an acute ACL injury (72 h after trauma), whereas IL-10 levels were three-fold lower compared to the same acute ACL injury group. The observed increases in TNF-α and IL-10 were greater when surgery was delayed compared to those levels observed in patients undergoing early reconstructive surgery[27]. In any case, levels of all cytokines were higher compared to normal values found in the literature. Patients subjected to early ACL reconstruction had higher cytokine concentrations at 4 months (IL-6, IL-8, IL-10, TNF-α), 8 months (IL-6 and TNF-α) and at 5 years (IFN-γ) post-reconstruction, compared to those from an ACL injured group treated with rehabilitation alone[30]. Furthermore, patients experiencing delayed ACL reconstruction had greater levels of IL 6 at 5 years compared with patients conservatively treated only with rehabilitation.

Discussion

IL-10 in Acute ACL Injury

Synovial fluid levels of IL-10 were greater in ACL-injured knees compared with those in intact knees in 70% of the studies we analyzed[13,22,25,28,29]. By comparison, SF levels of IL-10 were not elevated in ACL-injured knees in only two of the studies considered[23,26], most likely due to the extended delay between injury and SF sampling. Time of sampling post-injury must be considered when trying to understand how IL-10 levels change after trauma. The time interval between injury and SF collection for cytokine measurement was not specified in one study[19] and a mean delay of 5.5 weeks (range 3 to 12 weeks) was reported in the second study[22]. Indeed,

two independent groups[29,32] reported a significant reduction of IL-10 levels 30 days post-injury. The temporal variations of IL-10 (and other cytokines) concentrations in SF necessitate implementation of a near immediate and standardized sampling paradigm. The rapid increase of IL-10 levels in SF suggests the existence of inter- and intra-signaling mechanisms which may modulate and limit the inflammatory response driven by IL-6, IL-8, and TNF-α. Moreover, it is important to consider the differential changes in cytokine species concentrations; for example, levels of IL-6 and IL-10 were 1.050 fold and 21 fold greater, respectively, in injured knees compared to healthy knees[32]. It is clear that an intra articular pro inflammatory environment is established following ACL injury. The duration of this inflammatory state appears variable. In fact, Struglics et al[32] analyzed the KANON trial, a randomized controlled study that has a greater sample size, a longer follow up, and the highest study quality of all the studies considered in this review. They found that all cytokine levels returned to normal 30 days post-injury, with the exception of TNF-α levels, which remained elevated, compared to healthy knees for all five years follow-up.

Animal studies[36] have shown that elimination of synovial cytokines occurs within hours of injury. Therefore, increased TNF-α levels that last for years indicate a prolonged over-production of TNF-α and correlate directly with chondral damage[37-39].

IL-10 is an immunoregulatory, anti-inflammatory cytokine that modulates the production of pro inflammatory cytokines such as TNF-α. Furthermore, IL-10 inhibits the release of pro inflammatory mediators including TNF-α, IL-1, IL-6, IL-8, IL-12 from monocytes/macrophages[40-43], and stimulates type II collagen and proteoglycan expression which protects against chondrocyte apoptosis[43].

Different targets in these inflammatory pathways are under investigation with the aim of preventing the development of OA after ACL injury. Among them, IL-1Ra[44] has been studied by a number of researchers, but the potential role of increasing IL-10 activity for the prevention of OA development after ACL injury has not been fully investigated. In the wide field of biological therapies[45] we propose that IL-10 should be further investigated as a means for prevention of OA after ACL injury. Recently, platelet rich plasma (PRP) was reported to have anabolic effects on cartilage, increasing anti-inflammatory cytokines like IL-10 and decreasing levels of metalloproteinases (MMP)[46]. Autologous conditioned serum (ACS) is a cell-free treatment, obtained by incubating venous blood for 6-9 h in a specialized modified syringe. *In vitro* studies have demonstrated that exposure of blood to the syringe's internal surfaces induces blood cells to produce increased amounts of several anti-inflammatory cytokines [including an interleukin IL-1 receptor antagonist (IL-1Ra), IL-4 and IL-10[47] and regenerative growth factors such as TGF-β. The post-incubation serum is recovered by a single centrifugation step and injected into affected joints, usually in a series of 3 to 6 intra articular injections, given twice a week for 3 weeks. Recently, two randomized control trials showed an improvement in clinical outcomes in OA patients treated with intra-articular ACS[18,48]. Further studies will be required to understand if PRP or ACS is effective in modulating inflammatory intra-articular patterns and improving chondro-protection.

IL-10 After ACL Reconstruction

All the studies considered in this review have described elevated levels of inflammatory and anti inflammatory cytokines in injured knees compared to those of intact control groups. Bigoni et al[15] reported that 1 month after ACL reconstruction, the concentrations of IL-6 and IL-8 were comparable to those measured in an acute ACL injury group sampled 72 hours after injury; by contrast, IL-10 levels were three-fold lower compared to those in the acute ACL injury group. ACL reconstruction behaves as a second trauma in an already damaged knee; in fact, SF levels of inflammatory cytokines increased to levels comparable with those in an acute ACL injury. The only exception was the levels of IL-10, which did not appreciably change, thereby indicating that in this setting its chondro-protective and anti-inflammatory properties are blunted. Notably, pro-inflammatory cytokines levels were higher in the ACL reconstructed group compared to the ACL injured group treated with rehabilitation only[30]. It is clinically important that the levels of IL-6 were significantly elevated even five years after injury in those subjects for which surgery was delayed.

Conclusions

ACL injury is a traumatic event, which causes a rise in synovial inflammatory cytokines in acute, as well as chronic settings with a sustained inflammatory state that could last for years.

ACL reconstruction restores knee stability, improves knee biomechanics and allows patients to resume sporting activities with better performance compared with patients receiving only con-

servative rehabilitative treatment. Nonetheless, reconstructive surgery is a traumatic event that may induce a secondary increase in inflammatory cytokines, which may not be effectively modulated by anti-inflammatory cytokines such as IL-10.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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