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Invited Review

The role of the immune system in tendon healing: a systematic review

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Abstract

Introduction: The role of the immune system in tendon healing relies on polymorphonucleocytes, mast cells, macrophages and lymphocytes, the 'immune cells' and their cytokine production. This systematic review reports how the immune system affects tendon healing.

Sources of data: We registered our protocol (registration number: CRD42019141838). After searching PubMed, Embase and Cochrane Library databases, we included studies of any level of evidence published in peer-reviewed journals reporting clinical or preclinical results. The PRISMA guidelines were applied, and risk of bias and the methodological quality of the included studies were assessed. We excluded all the articles with high risk of bias and/or low quality after the assessment. We included 62 articles assessed as medium or high quality.

Areas of agreement: Macrophages are major actors in the promotion of proper wound healing as well as the resolution of inflammation in response to pathogenic challenge or tissue damage. The immune cells secrete cytokines involving both pro-inflammatory and anti-inflammatory factors which could affect both healing and macrophage polarization.

Areas of controversy: The role of lymphocytes, mast cells and polymorphonucleocytes is still inconclusive.

Growing points: The immune system is a major actor in the complex mechanism behind the healing response occurring in tendons after an injury. A dysregulation of the immune response can ultimately lead to a failed healing response.

Areas timely for developing research: Further studies are needed to shed light on therapeutic targets to improve tendon healing and in managing new way to balance immune response.

Key words: tendinopathy, immune cells, macrophages, monocytes, cytokines

Introduction

Tendons are highly specialized structures composed mainly of tenocytes surrounded by an abundant extracellular matrix (ECM).[1,2] The specialized fibroblasts include tenoblasts and tenocytes which are more than 90% of the cellular components in tendons.[3] The ECM is a complex collagen-based structure based on proteoglycans including glycosaminoglycans, and several other small molecules.[1,2] The standard mechanical and structural features of tendons depend on a complex and dynamic remodeling process.[1,4] The dysregulation of these features results in tendon inflammation, injury or tendinopathy,[5–7] resulting in considerable pain which impacts negatively the life of the patients restricting pain-free activities.[5–7]

Healing from acute tendon injury occurs through three progressive and partially overlapping phases: an acute inflammatory phase, a proliferative phase and a remodeling phase. While the role of inflammation is still being studied,[4,8] emerging evidence supports a major role of the immune system, both in the etiopathogenesis and treatment of the tendinopathy.[9,10] The first inflammatory phase lasts 3–7 days from the injury and is characterized by the presence of monocytes and macrophages at the site of injury.[4,8] Mechanical stimuli are an integral part of this process, and type III collagen is upregulated within the tendon cells and its extracellular matrix.[11] This is followed by the proliferation phase with the release of vascular endothelial growth factor to allow neovascularization and stimulate the progression of granulation tissue.[3,4] In the final remodeling phase, the tissue proceeds to reorganize its structure quantitatively (i.e. morphology, macroscopic features, tissue composition) and qualitatively (i.e. cytokines profile, molecular signaling, metabolic status).[3] This process can take up to 2 years to complete healing.

The dysregulation of tendon healing probably results from a chronic low-grade inflammation which finds major actors in the dual relationship between inflammatory and growth factors and immune cells such as polymorphonucleocytes, mast cells, macrophages and lymphocytes which have been recently showed to be well represented in tendons.[12–14] Controversially, it is increasingly evident that, even when absent or poorly present, this does not equate to the absence of inflammation.[9] Also, tendon injuries are accompanied and preceded by the secretion of several molecular actors of inflammation by tendon cells such as pro-inflammatory

and anti-inflammatory cytokines, as well as several growth factors including 'TNF-a, IL-1b, IL-6, IL-10, VEGF, TGF-b, COX-2 and PGE2.'[11,15,16] Although the inflammation driven by the cytokines might be involved in the healing process, its disruption, its role on the onset, progression, healing and resolution of tendinopathy, tendon healing after rupture and other inflammatory processes remain controversial.[17,18]

This systematic review reports the most up-to-date evidence on the role of immune cells on tendon healing with a focus on its clinical relevance.

Methods

Literature search strategy

This systematic review was conducted according to the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)[19] and MOOSE guidelines.[20] A comprehensive search was performed on three electronic medical databases (PubMed, Embase and Cochrane Library) by two independent authors (E.C. and W.S.K.) from their inception to 10 June 2019. Our main aims were to (1) understand the role of inflammation and immune response in tendon healing, (2) identify factors associated with anti-inflammatory intervention, (3) evaluate their effects through the review of animal and *in vitro* studies and (4) critically summarize the evidence available. To achieve the maximum sensitivity of the search strategy, we combined the terms 'tendon', as well as some common terms of tendon conditions such as 'tendon injury OR (tendon damage) OR tendonitis OR tendinopathy OR (chronic tendonitis) OR tendinosis OR (chronic tendinopathy) OR enthesitis),' AND 'healing' AND '(immune response) OR (macrophages) OR (immune cells) OR (monocytes) OR (lymphocytes) OR (immunology)' as either keywords or MeSH terms. The reference lists of all included articles, previous literature reviews on the topic and top hits from Google Scholar were reviewed for further identification of potentially relevant studies. To avoid overlapping with other ongoing investigations, we first searched PROSPERO site for any similar review and then prospectively registered our study (registration number: CRD42019141838).

Selection criteria

Eligible studies included those investigating inflammation and immune response in tendon healing. Primary screening of the titles and abstracts was performed by adding studies of any level of evidence published in peer-reviewed journals reporting clinical or preclinical results in English. Also, Italian, French, Spanish and Portuguese articles were included since the senior author was able to evaluate them (N.M.). Moreover, articles discussing the effect of several cytokines and immune response actors, both pathologically and physiologically, were reviewed. Exclusion criteria included studies investigating the treatment response of tendon to regenerative treatments, including platelet-rich plasma (PRP), mesenchymal stem cells (MSCs), etc. or new drugs related to healing of the tissue. Additionally, we excluded studies in which data were not accessible, missing, without an available full text or not well reported. We also eliminated duplicates, and the studies with poor scientific methodology assessed as described below. Abstracts, case reports, conference presentations, reviews, editorials and expert opinions were excluded. Two authors (E.C. and W.S.K.) performed the search and evaluated the articles independently. A researcher experienced in systematic reviews (N.M.) solved cases of doubt. At the beginning of the procedure, each investigator read the abstracts of all the articles, selected the relevant ones according to both inclusion and exclusion criteria and then compared the results with the other investigators. After 4 weeks, the same studies were reread to establish the agreement of the investigators about articles' selection. No disagreement was observed among the investigators. One investigator extracted the data from the full-text articles to Excel spreadsheet structured tables to analyze each study descriptively (Tables 1–3). Another investigator independently double-checked the extraction of primary data from all the articles. Doubts and inconsistencies are solved by discussion.

Table 1 Quality assessment of the included studies.

Author (year)	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*Score*
Abraham (2019)	x	-	x	-	x	X	x	x	x	x	8
Ackerman (2017)	x	-	x	x	x	X	x	x	x	x	9
Aktas (2017)	x	x	x	-	x	-	x	x	x	x	8
Alaseirlis (2005)	x	x	-	x	-	X	x	-	x	x	7
Andersson (2012)	x	x	-	x	-	-	x	-	-	x	5
Bedi (2010)	x	-	-	-	-	X	x	-	x	x	5
Blomgran (2016)	x	-	-	-	-	X	x	-	x	x	5
Chamberlain (2011)	x	x	x	x	x	-	x	x	x	x	9
Chamberlain (2017)	x	-	x	-	-	-	x	-	x	x	5
Chamberlain (2019)	x	x	x	x	x	-	x	x	x	x	9
Cui (2019)	x	x	x	x	x	-	x	x	x	x	9
Dagher (2009)	x	-	x	-	x	X	x	x	x	x	8
Dakin (2012)	x	x	x	x	x	X	x	x	x	x	10
Dayan (2011)	x	x	-	-	x	X	x	x	x	x	8
De La Durantaye (2014)	x	-	x	x	-	X	x	x	x	x	8
Eliasson (2012)	x	x	x	x	x	-	x	x	x	x	9
Gelberman (2017)	x	-	x	-	-	-	x	-	x	x	5
Godbout (2006)	x	-	x	-	-	-	x	-	x	x	5
Godbout (2010)	x	x	x	-	x	X	x	x	x	x	9
Hammerman (2014)	x	-	x	-	x	X	x	x	x	x	8
Hammerman (2017)	x	x	x	-	x	-	x	x	x	x	8
Hammerman (2018)	x	x	-	-	x	X	x	x	x	x	8
Hays (2008)	x	x	-	x	-	X	x	-	x	x	7
Kawamura (2005)	x	x	-	x	-	X	x	x	-	x	7
Khan (2005)	x	x	x	-	-	-	x	-	x	x	6
Komatsu (2018)	x	x	-	x	-	-	x	-	-	x	5
Lucas (2010)	x	x	-	-	x	-	x	-	-	x	6
Manning (2014)	x	-	x	-	x	X	x	-	x	x	7
Marsolais (2001)	x	x	-	-	-	X	x	x	x	x	7
Marsolais (2006)	x	x	x	x	x	-	x	x	x	x	9
Németh (2009)	x	x	x	-	x	-	x	x	x	x	8
Peters (2005)	x	x	x	x	-	X	x	x	x	x	9
Shen (2016)	x	-	-	-	-	X	x	-	x	x	5
Stålman (2015)	x	-	-	x	-	X	x	x	x	x	7
Sugg (2014)	x	x	-	-	x	X	x	x	x	x	8
Tarafder (2017)	x	x	-	x	-	-	x	x	x	x	7
Tellier (2018)	x	-	x	-	x	X	x	x	x	x	8
Virchenko (2004)	x	-	x	-	x	X	x	x	x	x	8
Wojciak (1993)	x	x	-	x	-	X	x	-	x	x	7
Wong (2009)	x	-	x	-	-	X	x	-	x	-	5

(1) Publication in a peer-reviewed journal; (2) statement of temperature control; (3) random allocation to groups; (4) allocation concealment; (5) blinded assessment of outcome; (6) use of anesthetic without significant internal protection of blood vessel; (7) appropriate animal model (aged, healthy, diabetic or hypertensive); (8) sample size calculation; (9) compliance with animal welfare regulations; (10) statement of potential conflict of interests.

Table 2 SYRCLE bias risk assessment

Author (year)	*Selection bias 1*	*Selection bias 2*	*Selection bias 3*	*Performance bias 1*	*Performance bias 2*	*Detection bias 1*	*Detection bias 2*	*Attrition bias*	*Reporting bias*	*Other potential bias*
Ackerman (2017)	?	x	✓	x	?	✓	✓	?	x	✓
Aktas (2017)	✓	x	x	x	?	✓	✓	?	✓	✓
Alaseirlis (2005)	?	x	x	x	?	✓	✓	✓	✓	x
Andersson (2012)	✓	x	x	x	?	✓	✓	✓	✓	✓
Bedi (2010)	✓	x	x	x	x	✓	✓	✓	✓	✓
Blomgran (2016)	✓	x	x	x	x	✓	✓	?	✓	✓
Chamberlain (2011)	✓	x	x	x	x	✓	✓	✓	✓	✓
Chamberlain (2017)	✓	x	x	x	x	✓	✓	✓	✓	✓
Chamberlain (2019)	?	x	x	x	?	X	✓	?	?	✓
Cui (2019)	?	x	x	✓	x	X	x	?	x	✓
Dagher (2009)	?	x	x	x	?	✓	✓	✓	✓	✓
Dakin (2012)	✓	x	x	x	?	✓	✓	✓	✓	✓
Dayan (2011)	✓	x	x	x	x	✓	✓	✓	✓	✓
De La Durantaye (2014)	?	x	✓	x	?	✓	✓	x	✓	✓
Eliasson (2012)	?	x	x	x	?	✓	✓	✓	✓	
Gelberman (2017)	✓	x	x	x	?	✓	✓	?	?	✓
Godbout (2006)	✓	x	x	x	?	✓	✓	✓	✓	✓
Godbout (2010)	?	x	✓	x	?	✓	✓	✓	✓	✓
Hammerman (2014)	?	x	✓	x	?	✓	✓	✓	✓	✓
Hammerman (2017)	?	x	✓	x	?	✓	✓	✓	x	✓
Hammerman (2018)	?	x	x	x	?	X	✓	✓	✓	✓
Hays (2008)	✓	x	x	x	x	✓	✓	✓	x	✓
Kawamura (2005)	?	x	✓	x	?	✓	✓	✓	✓	✓
Khan (2005)	?	x	x	x	?	X	✓	✓	✓	✓
Komatsu (2018)	?	x	x	x	?	✓	✓	x	✓	✓
Lucas (2010)	?	x	x	x	?	✓	✓	✓	✓	✓
Manning (2014)	?	x	x	x	?	X	✓	?	✓	✓
Marsolais (2001)	✓	x	x	x	?	✓	✓	?	✓	✓
Marsolais (2006)	?	x	✓	x	?	✓	✓	✓	✓	✓
Németh (2009)	✓	x	x	x	?	✓	✓	✓	✓	✓
Peters (2005)	?	x	✓	x	?	✓	✓	x	✓	✓
Shen (2016)	?	x	✓	x	?	✓	✓	✓	x	✓
Stålman (2015)	✓	x	x	x	x	✓	✓	x	✓	✓
Sugg (2014)	✓	x	x	x	x	✓	✓	?	✓	✓
Tarafder (2017)		?	x	?	x	✓	x	?	?	x
Tellier (2018)	✓	?	x	✓	x	X	x	x	?	✓
Virchenko (2004)	?	x	x	x	?	X	✓	x	✓	✓
Wojciak (1993)	?	x	x	x	?	X	✓	✓	x	x
Wong (2009)	?	x	✓	x	?	✓	✓	✓	✓	✓

(1) ✓ = Adequate randomization; ? = randomized but no details; x = no evidence of randomization. (2) ✓ = Baseline characteristics given; x = baseline characteristics not given. (3) ✓ = Evidence of adequate concealment of groups; x = no evidence of adequate concealment of groups. (4) ✓ = Evidence of random housing of animals;? = unknown housing arrangement. (5) ✓ = Evidence of caregivers blinded to intervention; x = no evidence of caregivers blinded to intervention. (6) ✓ = Evidence of random selection for assessment; x = no evidence of random selection for assessment. (7) ✓ = Evidence of assessor blinded; x = no evidence of assessor blinded. (8) ✓ = Explanation of missing animal data; x = no explanation of missing animal data. (9) ✓ = Free of selective reporting based on methods/results; ? = insufficient reporting; x = selective reporting. (10) ✓ = Free of other high bias risk; ? = insufficient data to determine risk of other bias.

Data extraction and criteria appraisal

All data were extracted from article text, tables, and figures. Data were obtained using the Population, Intervention, Comparison and Outcome (PICO) framework and included title, year of publication, study design, sample size, study population, patient characteristics, intervention and comparator (where applicable), outcomes, funding and conclusions. Two investigators independently reviewed each article (E.C. and L.R.). Discrepancies between the two reviewers were resolved by discussion and consensus. The final results were reviewed by another experienced investigator (N.M.).

Risk of bias assessment

The assessment of the risk of bias of all *in vivo* selected full-text articles was performed according to the SYRCLE's risk of bias tool[21] for preclinical studies and the Cochrane Collaboration's risk of bias tool[22] for clinical studies (Table 2). This assessment

Table 3 Modified Coleman score for human studies

Author and year	*Coleman score*	*Study size*	*Mean follow-up*	*Surgical approaches*	*Type of study*	*Diagnostic certainty*	*Description of surgical technique*	Description of postoperative rehabilitation	Outcome criteria	Procedures for assessing outcomes	Description of subject selection process
Schepull (2013)	56	4	4	7	15	5	10	5	2	4	0
Robertson (2012)	55	10	0	5	0	5	5	5	10	0	15
Yin (2014)	55	10	3	5	0	5	5	0	8	9	10

used 'Low,' 'Moderate' and 'High' as judgment keys: 'Low' indicated a low risk of bias, 'Moderate' suggested that the risk of bias was moderate and 'High' showed a high risk of bias. The assessment was performed by two authors (E.C. and L.R.) independently. Inter-rater agreement was 92%. Any discrepancy was discussed with the senior investigator (N.M.) for the final decision. In addition for the human clinical studies included, the modified Coleman methodology score[23] was used for bias risk assessment (Table 3).

Study quality assessment

The quality of evidence was assessed according to the Collaborative Approach to Meta-Analysis and Review of Animal Data from Experimental Studies (CAMARADES) checklist with supporting guidance from the CAMARADES website,[24] giving one point for each of (1) publication in a peer-reviewed journal, (2) statement of temperature control, (3) random allocation to groups, (4) allocation concealment, (5) blinded assessment of outcome, (6) use of anesthetic without significant internal protection of blood vessel, (7) appropriate animal model (aged, healthy, diabetic or hypertensive), (8) sample size calculation, (9) compliance with animal welfare regulations and (10) statement of potential conflict of interests. Each study was assessed and scored on a scale from 0 (lowest) to 10 (highest) points. The assessment was performed by two authors (E.C. and L.R.) independently. Inter-rater agreement was 94%. Any discrepancy was discussed with the senior investigator (N.M.) for the final decision.

Results

A total of 225 studies were identified from the databases according to the inclusion and exclusion criteria mentioned above. Overall, 112 articles were screened through abstract and title reading after removal of duplicates. Eventually, after full-text reading and reference list check, we selected 62 articles to include in the present manuscript. A PRISMA[19] flowchart of the selection process and screening is provided (Fig. 1).

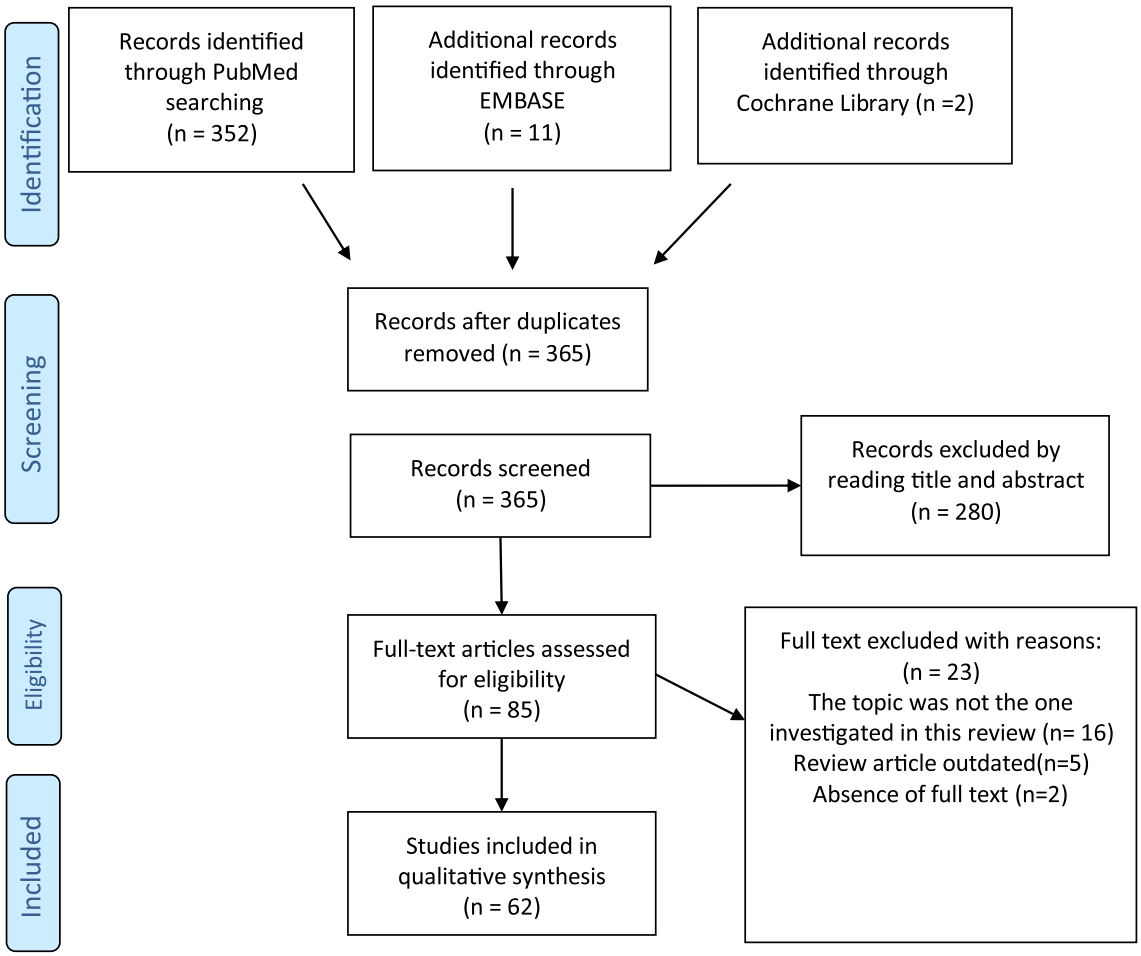

Fig. 1 PRISMA flowchart of the included studies.

We ultimately included 62 articles[12,24–85] after applying the relevant search strategy, inclusion and exclusion criteria. The articles included investigate the role of immune cells, the pathway triggered by their action and other immune mediators involved in the healing response of tendons after an injury. In particular, 40 included articles were based on animal model of tendon injury,[25–34,36,38,40,43,44,46,47,52,58,60,61,63,64,68,70–73,75–81,83–87] 4 were previous review on similar topic,[39,41,45,50] 4 were *in vitro* human studies,[42,48,49,51] 11 *in vitro* animal studies,[12,53–56,59,62,66,67,82,88] and 3 human clinical studies.[37,57,74] All the studies were assessed as medium or high quality with an average of 7275 under CAMARADES guideline.

The onset and progression of tendinopathy are related to an imbalance of inflammatory factors, immune system cells and chemical mediators, hormones, mechanical stimuli and other yet unknown agents. Morita et al.[89] described over 20 cytokines as actors of the immune and inflammatory process involved in tendon healing. While emerging evidence supports their role in every physiological phase of healing, their imbalance can ultimately lead to a failed healing response.[37] Chemokines such as CCL5, CCL2, CCL3 and CXCL10 are involved in the pathogenesis of tendinopathy inducing inflammation,[38] even after mechanotransduction.[8,39] The most investigated pro-inflammatory molecules such as IL-1β, IL-6 and TNF-α are also able to elicit the

immune response.[89] The immune cells are reported to be the main actor of all the processes mentioned above both producing mediating factors and acting through cell-mediated processes.

Mast cells

Mast cells exert pro-inflammatory response on human tendon-derived cells *in vitro*.[42] Despite the lack of studies on their role in tendinopathy and tendon healing, and given their role in immune system and neuroimmunity for other physiological and pathological changes, future studies should investigate the topic in greater details.

Macrophages

Macrophages are immune cells with a major role in both inflammatory and healing processes.[40,41] After the acute injury, macrophages secrete proinflammatory cytokines, such as IL-1β, TNF- and PGE2, reactive oxygen species, and other proteases.[43–45] These molecules act as essential starters of the tendinopathic pathogenesis,[46,48] and this can ultimately upregulate matrix metalloproteinase (MMP) action and the catabolism of the matrix.[48,49] They can be divided into two wide subpopulations including the M1 (classically activated) and the M2 (alternatively activated) macrophages.[41,50] Although the M1 and M2 (and its subset such as M2a, M2b, M2c and M2d) dichotomy seems to be insufficient to illustrate their diverse biology and molecular action,[41,50] M-polarized macrophages show a pro-inflammatory response pattern, while M2 macrophages regulate healing response by downregulating inflammatory cytokines through immunosuppressing molecules such as 'IL-1 receptor antagonist, IL-10, IL-4, and IL-13.'[41,51]

The literature suggests that tenocytes are able to influence the macrophage polarization during the early phase of inflammation through soluble factors.[69,82] This was shown in a study which found significant changes in macrophage subsets and epithelial-to-mesenchymal transition gene expression following Achilles tenotomy and subsequent repair repair.[26] In an equine tendon repair model, a polarization favoring M2 subset macrophage along with reduced expression for the lipoxin A4 receptor was seen in chronic injury suggesting incomplete inflammation resolution.[69] Emerging evidence supports the role of macrophages as critical players in tendon homeostasis and tendon repair.[40,50,69,86] In particular, the downregulation of inflammation of the M2 subset on classically activated M1 limits their action, promoting tissue repair.[41,50]

In animal models, rodents with surgically transected tendon have been studied to evaluate the presence of inflammatory cells.[40,52] In a rat Achilles tendon injury model, the temporary presence of neutrophils in the early phases after the injury was shown, followed by an augmented presence of macrophages in the tissue 1–28 days post-injury.[40] This was further reported in another study[52] where time-dependent presence of inflammatory cell subsets was found. In particular, the study reported a peak of neutrophils and macrophages 1–5 days and 21 days after the injury, respectively.[52] The need of macrophages for normal healing response is further confirmed by wound repair studies in murine macrophage knockout models, where impaired healing responses were reported in macrophage-deficient wounds.[43–45]

The complex network of factors influencing macrophage phenotypes, in preclinical studies, can be affected by MSCs, raising the possibility of a regenerative medicine solution for tendon healing.[34,53–57,59–62] In animal models of tendon injury, MSC therapy upregulated M2 macrophages and their associated anti-inflammatory action, which followed an improved healing response.[27,63,64] MSC-stimulated macrophages seem to have marked anti-inflammatory properties compared with wild-type control macrophages, with higher levels of IL-10 and IL-6 and lower levels of IL-12 and TNF-α expression.[34,65] The M2-like stimulated macrophages, in particular, can modulate an improved and faster tendon healing with better mechanical and histological features.[34]

MSCs seem to stimulate the monocyte to macrophage transition, inhibiting naive macrophages to an M1 subset polarization, controlling already activated M1 macrophages and ultimately upregulating M2 activation.[66] Even though the exact molecular pathway behind MSC and macrophage interaction across different activation stages is not fully understood, Németh et al.[61] suggested a role by inflammation signaling factors such as PGE2 and its receptors EP2 and EP4.[61] Other studies have noted metabolic changes in the expression of IDO1, SIRUTIN1, AMPK and GLUT1.[66] It seems that both M1 and M2 are crucial for the proper regulation and balance of satisfactory repair response as well as the downregulation of inflammation after injury. Additional studies are still needed to further investigate the MSC modulated macrophage polarization.

Overall, the role of macrophages on tendon healing is not clear. However, some evidence shows that macrophage absence is associated with a better healing response,[25,86] together with a diminished mass of granulation tissue.[86] Although these studies focused on the effects of the depletion of macrophages through the whole process of healing response, studies where macrophages were specifically inhibited during early inflammation,[79,81] e.g. with NSAIDs,[78] demonstrated a positive impact.

Lymphocytes

The possible role of lymphocytes in tendon healing and tendinopathy is still not understood. Although their presence in healthy and tendinopathic tendons has been reported,[12,67] further studies are needed to explore and define the role of lymphocytes in tendinopathy.

Mechanical load and immune cells

Mechanical pressure appears to influence the metabolism and healing of tendons.[28,47,68,70–76,86] It upregulates both anabolic and catabolic pathways through regulation of inflammation and immune reaction. In animal studies, loading prolonged the early inflammatory response and increased the cross-sectional area in tendons.[28,68,77] Other effects included macrophage polarization (M1 > M2) with a delayed regeneration phase-type of inflammation featured by higher M2 number along with and Treg cells.[28] Studies on macrophage polarization reported that the mechanical stress also influenced the immune cell differentiation and action.[28,47,75,76] Animals whose tendons were loaded by free cage activity demonstrated an augmented strength of the affected tendon associated to the increased activity tendon.[68] This probably resulted from a greater cross-sectional area of the tendon, but it did not translate into improvement in its mechanical properties.

Discussion

There are conflicting data on the presence and role of immune cells in tendon healing inflammation. This review systematically analyzed the current evidence on the presence and possible role of both pro-inflammatory and anti-inflammatory cytokines in tendon healing.

Macrophages seem to be strictly involved in the immune response following tendon injury. Their role might be related to their repair properties, but there is evidence of an involvement of adipose tissue.[15,90] This abundant tissue is able to produce immune cells as well as other proteins with specific role in immune and inflammatory process called adipokines.[15,90] One of them is the MCP-1 which is able to stimulate macrophage translocation in adipose tissue. These cells are activated and able to release pro-inflammatory cytokines, thus significantly increasing their TNF-a expression.[15,90] The increased production of pro-inflammatory cytokines influences the expression of adiponectin, an anti-inflammatory adipokine, ultimately leading to a dysregulation of the balance between pro- and anti-inflammatory cytokines and macrophages.[15] Because of the local infiltration and activation of macrophages, the adipose mass is augmented, coming to be able to influence the interaction between leptin and suppressor T cells.[15,90] Leptin, an adipokine responsible for the central control of

energy balance, also seems to inhibit the proliferative capacity of suppressor T cells.[90]

Macrophage polarization and action seem to be influenced by mechanical loading.[28,47,75,76,91] In particular, emerging evidence supports their dualistic role setting the basis for a U curve interpretation of their function, where overloading the tendon will result in a failed healing response and reinjury and underloading in a less effective measure.

A better comprehension of the role of immune cell subpopulations in the setting and progression of tendinopathy and healing response is fundamental to clarify the role of potential therapeutic targets and improve future treatment options for patients. Preclinical animal studies on animal models of tendinopathy suggest that chronic inflammation may originate from inadequate resolution of inflammation and failed healing response.[69,87] Further studies might look at the suppression of chronic inflammation, perhaps targeting the early phase of the response.

Exercise still represents one of the best ways to positively influence tendon healing by negatively affecting the inflammatory environment, as reported in several preclinical studies focusing on the role of early mobilization of injured tendons.[70–74] In addition, immobilization of tendon tissue is often associated with pathological changes in the cytokine profile and deterioration of the tendon mechanical features.[84] While all this can ultimately lead to decreased resistance to mechanical stimulus, in the early stages seems to be reversible as shown in animal models.[71,74,75] The mechanism of cytokine expression is still not fully understood, but seems to rely on the stimulatory effect exerted by trauma leading to microdamage and vessel leakage.[70,71] On the other hand, rat models exposed to loading by free cage activity showed an increased strength of the healing tendon without a significant improvement in mechanical quality.[68] Even though there is no clear consensus on how much load is appropriate for tendon healing, early and progressive physical therapy after tendon injury, tendon surgery, and in tendinopathy should be advised.

Limitations

The main limitation of this systematic review is the heterogeneity and quality of the included studies. Most of the studies were preclinical studies, with no clinical randomized controlled trials. Despite applying strict methodological evaluation through quality and risk of bias tools, treatment variables including dose, drug delivery and population used differed across the included studies. The findings of our review will, however, hopefully help direct future investigations.

Conclusion

The immune system plays a major actor in the complex mechanism behind the healing response occurring in tendons after an injury. A dysregulation of the immune response can ultimately lead to the failed healing response typical of chronic tendinopathic lesions. Further studies are needed to shed light on therapeutic target to improve tendon healing.

References

1. Sakabe T, Sakai T. Musculoskeletal diseases–tendon. *Br Med Bull* [Internet] 2011 [cited 2019 Apr 12];99:211–25. Available from:. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3202301/
2. Maffulli N, Benazzo F. Basic science of tendons. *Sports Med Arthrosc* [Internet] 2000 [cited 2019 Apr 12];8:1–5. Available from: http://link.springer.com/10.1007/978-3-662-54181-4_21
3. Sharma P, Maffulli N, Maffulli N. Tendon structure biology of tendon injury: healing, modeling and remodeling [Internet]. *J Musculoskelet Neuronal Interact* 2006;6:[cited 2019 Apr 12]: Available from: http://www.ismni.org/jmni/pdf/24/14MAFFULLI.pdf
4. D'Addona A, Maffulli N, Formisano S, Rosa D. Inflammation in tendinopathy. *Surg* [Internet] 2017 [cited 2019 Apr 11];15:297–302. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1479666X17300884
5. Raikin SM, Garras DN, Krapchev PV. Achilles tendon injuries in a United States population [internet]. *Foot Ankle Int* 2013;34:[cited 2019 Apr 12]:475–80. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23386750

6. Järvinen TAH, Kannus P, Maffulli N, Khan KM. Achilles tendon disorders: Etiology and epidemiology. *Foot Ankle Clin* [Internet] 2005 [cited 2019 Apr 12];10:255–66. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1083751505000148
7. de Jong JP, Nguyen JT, Sonnema AJM, Nguyen EC, Amadio PC, Moran SL. The incidence of acute traumatic tendon injuries in the hand and wrist: a 10-year population-based study. *Clin Orthop Surg* [Internet] 2014 [cited 2019 Apr 12];6:196. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24900902
8. Abate M, Gravare-Silbernagel K, Siljeholm C, Di Iorio A, De Amicis D, Salini V, et al. Pathogenesis of tendinopathies: inflammation or degeneration? *Arthritis Res Ther* [Internet] 2009 [cited 2019 Apr 14];11:235. Available from: http://arthritis-research.biomedcentral.com/articles/10.1186/ar2723
9. Dean BJF, Gettings P, Dakin SG, Carr AJ. Are inflammatory cells increased in painful human tendinopathy? A systematic review [Internet]. *Br J Sports Med*. BMJ Publishing Group Ltd and British Association of Sport and Exercise Medicine; 2016;50:[cited 2019 May 26]:216–20. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26246419
10. Rees JD, Wilson AM, Wolman RL. Current concepts in the management of tendon disorders [internet]. *Rheumatology Narnia* 2006 [cited 2019 May 26];45:508–21. Available from: http://academic.oup.com/rheumatology/article/45/5/508/1788804/Current-concepts-in-the-management-of-tendon
11. Del Buono A, Battery L, Denaro V, Maccauro G, Maffulli N. Tendinopathy and inflammation: some truths. *Int J Immunopathol Pharmacol* [Internet] 2011 [cited 2019 Apr 14];24:45–50. Available from: http://journals.sagepub.com/doi/10.1177/03946320110241S209
12. Kragsnaes MS, Fredberg U, Stribolt K, Kjaer SG, Bendix K, Ellingsen T. Stereological quantification of immune-competent cells in baseline biopsy specimens from Achilles tendons. *Am J Sports Med* [Internet] 2014 [cited 2019 Apr 11];42:2435–45. Available from: http://journals.sagepub.com/doi/10.1177/0363546514542329
13. Scott A, Lian O, Bahr R, Hart DA, Duronio V, Khan KM. Increased mast cell numbers in human patellar tendinosis: correlation with symptom duration and vascular hyperplasia. *Br J Sports Med* [Internet] 2008 [cited 2019 May 27];42:753–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18308880
14. Millar NL, Hueber AJ, Reilly JH, Xu Y, Fazzi UG, GAC M, et al. Inflammation is present in early human tendinopathy. *Am J Sports Med* [Internet] 2010 [cited 2019 Apr 15];38:2085–91. Available from: http://journals.sagepub.com/doi/10.1177/0363546510372613
15. Battery L, Maffulli N. Inflammation in overuse tendon injuries [Internet]. *Sports Med Arthrosc Rev* 2011 [cited 2019 Apr 11];19:213–7. Available from: http://content.wkhealth.com/linkback/openurl?sid=WKPTLP:landingpage&an=00132585-201109000-00006
16. Yang G, Im H-J, JH-C W. Repetitive mechanical stretching modulates IL-1β induced COX-2, MMP-1 expression, and PGE2 production in human patellar tendon fibroblasts. *Gene* [Internet] 2005 [cited 2019 Apr 14];363:166–72. Available from: https://www.sciencedirect.com/science/article/pii/S0378111905005044
17. Dakin SG, Dudhia J, Smith RKW. Resolving an inflammatory concept: the importance of inflammation and resolution in tendinopathy. *Vet Immunol Immunopathol* [Internet] 2014 [cited 2019 Apr 11];158:121–7. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0165242714000087
18. Tang C, Chen Y, Huang J, Zhao K, Chen X, Yin Z, et al. The roles of inflammatory mediators and immunocytes in tendinopathy. *J Orthop Transl* [Internet] 2018 [cited 2019 Apr 13];14:23–33. Available from: http://www.ncbi.nlm.nih.gov/pubmed/30035030
19. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gotzsche PC, Ioannidis JPA, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. *BMJ* [Internet] 2009;339:b2700–0. Available from: http://www.bmj.com/cgi/doi/10.1136/bmj.b2700
20. Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis of observational studies in epidemiology (MOOSE) group. *JAMA* [Internet] 2000 [cited 2019 Jan 24];283:2008–12. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10789670
21. Hooijmans CR, Rovers MM, de Vries RB, Leenaars M, Ritskes-Hoitinga M, Langendam MW. SYRCLE's risk of bias tool for animal studies. *BMC Med Res Methodol* [Internet] 2014 [cited 2019 Jan 24];14:43. Available from: https://bmcmedresmethodol.biomedcentral.com/articles/10.1186/1471-2288-14-43
22. Sterne JA, Hernán MA, Reeves BC, Savović J, Berkman ND, Viswanathan M, et al. ROBINS-I: a tool for assessing risk of bias in non-randomised studies of interventions. *BMJ* [Internet] 2016 [cited 2018 Sep 11];355:i4919. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27733354

23. Coleman BD, Khan KM, Maffulli N, Cook JL, Wark JD. Studies of surgical outcome after patellar tendinopathy: clinical significance of methodological deficiencies and guidelines for future studies. Victorian Institute of Sport Tendon Study Group. *Scand J Med Sci Sports* [Internet] 2000 [cited 2019 Aug 29];10:2–11. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10693606
24. Macleod MR, O'Collins T, Howells DW, Donnan GA. Pooling of animal experimental data reveals influence of study design and publication bias [Internet]. *Stroke* 2004;35:[cited 2019 Jan 24]:1203–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15060322
25. Alaseirlis DA, Li Y, Cilli F, Fu FH, Wang JH-C. Decreasing inflammatory response of injured patellar tendons results in increased collagen fibril diameters. *Connect Tissue Res* [Internet] 2005 [cited 2019 Apr 11];46:12–7. Available from: http://www.tandfonline.com/doi/full/10.1080/03008200590935501
26. Sugg KB, Lubardic J, Gumucio JP, Mendias CL. Changes in macrophage phenotype and induction of epithelial-to-mesenchymal transition genes following acute Achilles tenotomy and repair. *J Orthop Res* [Internet] 2014 [cited 2019 Apr 11];32:944–51. Available from: http://doi.wiley.com/10.1002/jor.22624
27. Aktas E, Chamberlain CS, Saether EE, Duenwald-Kuehl SE, Kondratko-Mittnacht J, Stitgen M, et al. Immune modulation with primed mesenchymal stem cells delivered via biodegradable scaffold to repair an Achilles tendon segmental defect. *J Orthop Res* [Internet] 2017 [cited 2019 May 8];35:269–80. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27061844
28. Blomgran P, Blomgran R, Ernerudh J, Aspenberg P. A possible link between loading, inflammation and healing: immune cell populations during tendon healing in the rat. *Sci Rep* [Internet] 2016 [cited 2019 Apr 11];6:29824. Available from: http://www.nature.com/articles/srep29824
29. Gelberman RH, Linderman SW, Jayaram R, Dikina AD, Sakiyama-Elbert S, Alsberg E, et al. Combined administration of ASCs and BMP-12 promotes an M2 macrophage phenotype and enhances tendon healing. *Clin Orthop Relat Res* [Internet] 2017 [cited 2019 May 28];475:2318–31. Available from: http://link.springer.com/10.1007/s11999-017-5369-7
30. Tarafder S, Chen E, Jun Y, Kao K, Sim KH, Back J, et al. Tendon stem/progenitor cells regulate inflammation in tendon healing via JNK and STAT3 signaling. *FASEB J* [Internet] 2017 [cited 2019 Apr 11];31:3991–8. Available from: http://www.fasebj.org/doi/10.1096/fj.201700071R
31. Tellier LE, Krieger JR, Brimeyer AL, Coogan AC, Falis AA, Rinker TE, et al. Localized SDF-1α delivery increases pro-healing bone marrow-derived cells in the supraspinatus muscle following severe rotator cuff injury. *Regen Eng Transl Med* [Internet] 2018 [cited 2019 May 28];4:92–103. Available from: http://link.springer.com/10.1007/s40883-018-0052-4
32. Abraham AC, Shah SA, Golman M, Song L, Li X, Kurtaliaj I, et al. Targeting the NF-κB signaling pathway in chronic tendon disease. *Sci Transl Med* [Internet] 2019 [cited 2019 Apr 11];11:eaav4319. Available from: http://stm.sciencemag.org/lookup/doi/10.1126/scitranslmed.aav4319
33. Cui H, He Y, Chen S, Zhang D, Yu Y, Fan C. Macrophage-derived miRNA-containing Exosomes induce Peritendinous fibrosis after tendon injury through the miR-21-5p/Smad7 pathway. *Mol Ther Nucleic Acids* [Internet] 2019 [cited 2019 May 28];14:114–30. Available from: https://linkinghub.elsevier.com/retrieve/pii/S2162253118303007
34. Chamberlain CS, Clements AEB, Kink JA, Choi U, Baer GS, Halanski MA, et al. Extracellular vesicle-educated macrophages promote early Achilles tendon healing. *Stem Cells* [Internet] 2019 [cited 2019 Apr 11];37:652–62. Available from: http://doi.wiley.com/10.1002/stem.2988
35. Ackerman JE, Geary MB, Orner CA, Bawany F, Loiselle AE. Obesity/Type II diabetes alters macrophage polarization resulting in a fibrotic tendon healing response. Zhao C, editor. *PLoS One* [Internet]. 2017 [cited 2019 May 28];12:e0181127. Available from: http://dx.plos.org/10.1371/journal.pone.0181127
36. Wojciak B, Crossan JF. The accumulation of inflammatory cells in synovial sheath and epitenon during adhesion formation in healing rat flexor tendons. *Clin Exp Immunol* [Internet] 1993 [cited 2019 May 28];93:108–14. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8324895
37. Robertson CM, Chen CT, Shindle MK, Cordasco FA, Rodeo SA, Warren RF. Failed healing of rotator cuff repair correlates with altered collagenase and gelatinase in supraspinatus and subscapularis tendons. *Am J Sports Med* [Internet] 2012 [cited 2019 Apr 11];40:1993–2001. Available from: http://journals.sagepub.com/doi/10.1177/0363546512456519
38. Stålman A, Bring D, Ackermann PW. Chemokine expression of CCL2, CCL3, CCL5 and CXCL10 during early inflammatory tendon healing precedes nerve regeneration: an immunohistochemical study in the rat. *Knee Surgery Sport Traumatol Arthrosc* [Internet] 2015

[cited 2019 Apr 11];23:2682–9. Available from: http://link.springer.com/10.1007/s00167-014-3010-9

39. Wang JH-C, Thampatty BP, Lin J-S, Im H-J. Mechanoregulation of gene expression in fibroblasts. *Gene* [Internet] 2007 [cited 2019 Apr 13];391:1–15. Available from: https://www.sciencedirect.com/science/article/pii/S0378111907000339
40. Marsolais D, Côté CH, Frenette J. Neutrophils and macrophages accumulate sequentially following Achilles tendon injury. *J Orthop Res* [Internet] 2001 [cited 2019 May 27];19:1203–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11781025
41. Mantovani A, Biswas SK, Galdiero MR, Sica A, Locati M. Macrophage plasticity and polarization in tissue repair and remodelling [Internet]. *Journal of Pathology* 2013 [cited 2019 May 8];229:176–85. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23096265
42. Behzad H, Sharma A, Mousavizadeh R, Lu A, Scott A. Mast cells exert pro-inflammatory effects of relevance to the pathophyisology of tendinopathy. *Arthritis Res Ther* [Internet] 2013 [cited 2019 May 27];15:R184. Available from: http://arthritis-research.biomedcentral.com/articles/10.1186/ar4374
43. Peters T, Sindrilaru A, Hinz B, Hinrichs R, Menke A, Al-Azzeh EAD, et al. Wound-healing defect of CD18−/− mice due to a decrease in TGF-β1 and myofibroblast differentiation. *EMBO J* [Internet] 2005 [cited 2019 May 27];24:3400–10. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16148944
44. Lucas T, Waisman A, Ranjan R, Roes J, Krieg T, Muller W, et al. Differential roles of macrophages in diverse phases of skin repair. *J Immunol* [Internet] 2010 [cited 2019 May 27];184:3964–77. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20176743
45. Werner S, Grose R. Regulation of wound healing by growth factors and cytokines. *Physiol Rev* [Internet] 2003 [cited 2019 May 27];83:835–70. Available from: http://www.physiology.org/doi/10.1152/physrev.2003.83.3.835
46. Khan MH, Li Z, Wang JH-C. Repeated exposure of tendon to prostaglandin-E 2 leads to localized tendon degeneration. *Clin J Sport Med* [Internet] 2005 [cited 2019 May 27];15:27–33. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15654188
47. Kawamura S, Ying L, Kim HJ, Dynybil C, Rodeo SA. Macrophages accumulate in the early phase of tendon-bone healing. *J Orthop Res* [Internet] 2005 [cited 2019 Jul 4];23:1425–32. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16111854
48. Tsuzaki M, Guyton G, Garrett W, Archambault JM, Herzog W, Almekinders L, et al. IL-1β induces COX2, MMP-1, −3 and −13, ADAMTS-4, IL-1β and IL-6 in human tendon cells. *J Orthop Res* [Internet] 2003 [cited 2019 May 27];21:256–64. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12568957
49. Thampatty BP, Li H, Im HJ, Wang JH-C. EP4 receptor regulates collagen type-I, MMP-1, and MMP-3 gene expression in human tendon fibroblasts in response to IL-1β treatment. *Gene* [Internet] 2007 [cited 2019 May 27];386:154–61. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17046175
50. Murray PJ. Macrophage polarization. *Annu Rev Physiol* [Internet] 2017 [cited 2019 May 8];79:541–66. Available from: http://www.annualreviews.org/doi/10.1146/annurev-physiol-022516-034339
51. Mantovani A, Sozzani S, Locati M, Allavena P, Sica A. Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. *Trends Immunol* [Internet] 2002 [cited 2019 Apr 15];23:549–55. Available from: https://www.sciencedirect.com/science/article/pii/S1471490602023025
52. Wong JKF, Lui YH, Kapacee Z, Kadler KE, Ferguson MWJ, McGrouther DA. The cellular biology of flexor tendon adhesion formation. *Am J Pathol* [Internet] 2009 [cited 2019 May 27];175:1938–51. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19834058
53. Da Silva CL, Gonçalves R, Crapnell KB, Cabral JMS, Zanjani ED, Almeida-Porada G. A human stromal-based serum-free culture system supports the ex vivo expansion/maintenance of bone marrow and cord blood hematopoietic stem/progenitor cells. *Exp Hematol* [Internet] 2005 [cited 2019 May 8];33:828–35. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15963859
54. Eslani M, Putra I, Shen X, Hamouie J, Tadepalli A, Anwar KN, et al. Cornea-derived mesenchymal stromal cells therapeutically modulate macrophage immunophenotype and angiogenic function. *Stem Cells* [Internet] 2018 [cited 2019 May 8];36:775–84. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29341332
55. Cantu DA, Hematti P, Kao WJ. Cell encapsulating biomaterial regulates Mesenchymal stromal/stem cell differentiation and macrophage Immunophenotype. *Stem Cells Transl Med* [Internet] 2012 [cited 2019 May 8];1:740–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23197666

56. Selleri S, Bifsha P, Civini S, Pacelli C, Dieng MM, Lemieux W, et al. Human mesenchymal stromal cell-secreted lactate induces M2-macrophage differentiation by metabolic reprogramming. *Oncotarget* [Internet] 2016 [cited 2019 May 8];7:30193–210. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27070086
57. Yin F, Battiwalla M, Ito S, Feng X, Chinian F, Melenhorst JJ, et al. Bone marrow mesenchymal stromal cells to treat tissue damage in allogeneic stem cell transplant recipients: correlation of biological markers with clinical responses. *Stem Cells* [Internet] 2014 [cited 2019 May 8];32:1278–88. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24452962
58. Hays PL, Kawamura S, Deng X-H, Dagher E, Mithoefer K, Ying L, et al. The role of macrophages in early healing of a tendon graft in a bone tunnel. *J Bone Joint Surg Am* [Internet] 2008 [cited 2019 May 28];90:565–79. Available from: https://insights.ovid.com/crossref?an=00004623-200803000-00014
59. Gómez-Aristizábal A, Kim KP, Viswanathan S. A systematic study of the effect of different molecular weights of hyaluronic acid on mesenchymal stromal cell-mediated immunomodulation. Almeida-Porada GD, editor. *PLoS One* [Internet] 2016 [cited 2019 May 8];11:e0147868. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26820314
60. Dayan V, Yannarelli G, Billia F, Filomeno P, Wang X-H, Davies JE, et al. Mesenchymal stromal cells mediate a switch to alternatively activated monocytes/macrophages after acute myocardial infarction. *Basic Res Cardiol* [Internet] 2011 [cited 2019 May 8];106:1299–310. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21901289
61. Németh K, Leelahavanichkul A, Yuen PST, Mayer B, Parmelee A, Doi K, et al. Bone marrow stromal cells attenuate sepsis via prostaglandin E 2-dependent reprogramming of host macrophages to increase their interleukin-10 production. *Nat Med* [Internet] 2009 [cited 2019 May 8];15:42–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19098906
62. King SN, Hanson SE, Chen X, Kim J, Hematti P, Thibeault SL. In vitro characterization of macrophage interaction with mesenchymal stromal cell-hyaluronan hydrogel constructs. *J Biomed Mater Res - Part A* [Internet] 2014 [cited 2019 May 8];102:890–902. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23564555
63. Shen H, Kormpakis I, Havlioglu N, Linderman SW, Sakiyama-Elbert SE, Erickson IE, et al. The effect of mesenchymal stromal cell sheets on the inflammatory stage of flexor tendon healing. *Stem Cell Res Ther* [Internet] 2016 [cited 2019 May 8];7:144. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27677963
64. Chamberlain CS, Saether EE, Aktas E, Vanderby R. Mesenchymal stem cell therapy on tendon/ligament healing. *J cytokine Biol* [Internet] 2017 [cited 2019 May 8];2. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28670649
65. Kim J, Hematti P. Mesenchymal stem cell–educated macrophages: a novel type of alternatively activated macrophages. *Exp Hematol* [Internet] 2009 [cited 2019 May 8];37:1445–53. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19772890
66. Vasandan AB, Jahnavi S, Shashank C, Prasad P, Kumar A, Jyothi Prasanna S. Human Mesenchymal stem cells program macrophage plasticity by altering their metabolic status via a PGE 2 -dependent mechanism. *Sci Rep* [Internet] 2016 [cited 2019 May 8];6:38308. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27910911
67. Schubert TEO. Achilles tendinosis is associated with sprouting of substance P positive nerve fibres. *Ann Rheum Dis* [Internet] 2005 [cited 2019 May 8];64:1083–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15958764
68. Andersson T, Eliasson P, Hammerman M, Sandberg O, Aspenberg P. Low-level mechanical stimulation is sufficient to improve tendon healing in rats. *J Appl Physiol* [Internet] 2012 [cited 2019 May 29];113:1398–402. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22936727
69. Dakin SG, Werling D, Hibbert A, Abayasekara DRE, Young NJ, Smith RKW, et al. Macrophage subpopulations and the Lipoxin A4 receptor implicate active inflammation during equine tendon repair. Frasch MG, editor. *PLoS One* [Internet] 2012 [cited 2019 Apr 11];7:e32333. Available from: https://dx.plos.org/10.1371/journal.pone.0032333
70. Hammerman M, Aspenberg P, Eliasson P. Microtrauma stimulates rat Achilles tendon healing via an early gene expression pattern similar to mechanical loading. *J Appl Physiol* [Internet] 2014 [cited 2019 Apr 11];116:54–60. Available from: http://www.physiology.org/doi/10.1152/japplphysiol.00741.2013
71. Eliasson P, Andersson T, Aspenberg P. Achilles tendon healing in rats is improved by intermittent mechanical loading during the inflammatory phase. *J Orthop Res* [Internet] 2012 [cited 2019 Apr 11];30:274–9. Available from: http://doi.wiley.com/10.1002/jor.21511
72. Hammerman M, Dietrich-Zagonel F, Blomgran P, Eliasson P, Aspenberg P. Different mechanisms activated by

mild versus strong loading in rat Achilles tendon healing. Wang JH-C, editor. *PLoS One* [Internet] 2018 [cited 2019 Apr 11];13:e0201211. Available from: https://dx.plos.org/10.1371/journal.pone.0201211

73. Hammerman M, Blomgran P, Dansac A, Eliasson P, Aspenberg P. Different gene response to mechanical loading during early and late phases of rat Achilles tendon healing. 2017 [cited 2019 Apr 11];123:800–815. Available from: http://www.physiology.org/doi/10.1152/japplphysiol.00323.2017
74. Schepull T, Aspenberg P. Early controlled tension improves the material properties of healing human achilles tendons after ruptures: a randomized trial. *Am J Sports Med* [Internet] 2013 [cited 2019 Apr 19];41:2550–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24005873
75. Bedi A, Kovacevic D, Fox AJ, Imhauser CW, Stasiak M, Packer J, et al. Effect of early and delayed mechanical loading on tendon-to-bone healing after anterior cruciate ligament reconstruction. *J Bone Jt Surg - Ser A* [Internet] 2010 [cited 2019 Jul 4];92:2387–401. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20962189
76. Godbout C, Ang O, Frenette J. Early voluntary exercise does not promote healing in a rat model of Achilles tendon injury. *J Appl Physiol* [Internet] 2006 [cited 2019 Jul 4];101:1720–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16916920
77. Komatsu DE, King L, Gurevich M, Kahn B, Paci JM. The in vivo impact of leukocyte injections on normal rat Achilles tendons: potential detriment to tendon morphology, cellularity, and vascularity. *Am J Orthop (Belle Mead NJ)* [Internet] 2018 [cited 2019 Apr 11];47. Available from: https://www.amjorthopedics.com/article/vivo-impact-leukocyte-injections-normal-rat-achilles-tendons-potential-detriment-tendon
78. Virchenko O, Skoglund B, Aspenberg P. Parecoxib impairs early tendon repair but improves later remodeling. *Am J Sports Med* [Internet] 2004 [cited 2019 May 29];32:1743–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15494342
79. Marsolais D, Côté CH, Frenette J. Pifithrin-α, an inhibitor of p53 transactivation, alters the inflammatory process and delays tendon healing following acute injury. *Am J Physiol Integr Comp Physiol* [Internet] 2006 [cited 2019 May 29];292:R321–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16902184
80. Godbout C, Bilodeau R, Van Rooijen N, Bouchard P, Frenette J. Transient neutropenia increases macrophage accumulation and cell proliferation but does not improve repair following intratendinous rupture of Achilles tendon. *J Orthop Res* [Internet] 2010 [cited 2019 May 28];28:1084–91. Available from: http://doi.wiley.com/10.1002/jor.21092
81. Chamberlain CS, Leiferman EM, Frisch KE, Wang S, Yang X, Van Rooijen N, et al. The influence of macrophage depletion on ligament healing. *Connect Tissue Res* [Internet] 2011 [cited 2019 May 29];52:203–11. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21117894
82. Stolk M, Klatte-Schulz F, Schmock A, Minkwitz S, Wildemann B, Seifert M. New insights into tenocyte-immune cell interplay in an in vitro model of inflammation. *Sci Rep* [Internet] 2017 [cited 2019 Sep 2];7:9801. Available from: http://www.nature.com/articles/s41598-017-09875-x
83. Ackerman JE, Best KT, O'Keefe RJ, Loiselle AE, O'Keefe RJ, Loiselle AE. Deletion of EP4 in S100a4-lineage cells reduces scar tissue formation during early but not later stages of tendon healing. *Sci Rep* [Internet] 2017 [cited 2019 Apr 11];7:8658. Available from: http://www.nature.com/articles/s41598-017-09407-7
84. Dagher E, Hays PL, Kawamura S, Godin J, Deng X, Rodeo SA. Immobilization modulates macrophage accumulation in tendon-bone healing. *Clin Orthop Relat Res* [Internet] 2009 [cited 2019 May 28];467:281–7. Available from: http://link.springer.com/10.1007/s11999-008-0512-0
85. Manning CN, Havlioglu N, Knutsen E, Sakiyama-Elbert SE, Silva MJ, Thomopoulos S, et al. The early inflammatory response after flexor tendon healing: a gene expression and histological analysis. *J Orthop Res* [Internet] 2014 [cited 2019 Apr 14];32:645–52. Available from: http://doi.wiley.com/10.1002/jor.22575
86. De La Durantaye M, Piette AB, Van Rooijen N, Frenette J. Macrophage depletion reduces cell proliferation and extracellular matrix accumulation but increases the ultimate tensile strength of injured Achilles tendons. *J Orthop Res* [Internet] 2014 [cited 2019 May 27];32:279–85. Available from: http://doi.wiley.com/10.1002/jor.22504
87. Dakin SG, Dudhia J, Werling NJ, Werling D, Abayasekara DRE, Smith RKW. Inflamm-aging and Arachadonic acid metabolite differences with stage of tendon disease. Hussain S, editor. *PLoS One* [Internet] 2012 [cited 2019 May 27];7:e48978. Available from: https://dx.plos.org/10.1371/journal.pone.0048978
88. Kim HKW, Bian H, Aya-ay J, Garces A, Morgan EF, Gilbert SR. Hypoxia and HIF-1α expression in the epiphyseal cartilage following ischemic injury to the immature femoral head. *Bone* [Internet] 2009 [cited

2018 May 25];45:280–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19345751

89. Morita W, Dakin SG, Snelling SJB, Carr AJ. Cytokines in tendon disease: a systematic review. *Bone Joint Res* [Internet] 2017 [cited 2019 Apr 12];6:656–64. Available from: http://online.boneandjoint.org.uk/doi/10.1302/2046-3758.612.BJR-2017-0112.R1
90. Matarese G, Procaccini C, De Rosa V, Horvath TL, La Cava A. Regulatory T cells in obesity: the leptin connection. *Trends Mol Med* [Internet] 2010 [cited 2019 Apr 20];16:247–56. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20493774
91. Killian ML, Cavinatto L, Galatz LM, Thomopoulos S. The role of mechanobiology in tendon healing. *J Shoulder Elbow Surg* [Internet] 2012 [cited 2019 Jul 4];21:228–37. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22244066