British Medical Bulletin, 2019, **00**:1–20
doi: 10.1093/bmb/ldz035

Invited Review

Tendon healing in presence of chronic low-level inflammation: a systematic review

Emanuele Chisari[1], Laura Rehak[2], and Wasim S. Khan[3] and Nicola Maffulli[4,5,6,7,*]

[1]University of Catania, Departmento of General Surgery and Medical Specialities, Via Santa Sofia 78, Catania 95123, Italy, [2]Athena Biomedical Innovations, Viale Europa 139, Florence, 50126, Italy, [3]Division of Trauma and Orthopaedics, Addenbrooke's Hospital, University of Cambridge, Hills Rd, Cambridge CB2 0QQ, United Kingdom, [4]Department of Musculoskeletal Disorders, Via Salvador Allende, 43, 84081 Baronissi SA, Italy, Salerno, Italy., [5]Clinica Ortopedica, Ospedale San Giovanni di Dio e Ruggi D'Aragona, Largo Città di Ippocrate, Salerno, 84131, Italy, [6]Barts and the London School of Medicine and Dentistry, Centre for Sports and Exercise Medicine, Mile End Hospital, Queen Mary University of London, 275 Bancroft Road, London E1 4DG, England., and [7]School of Medicine, Institute of Science and Technology in Medicine, Guy Hilton Research Centre, Keele University, Thornburrow Drive, Hartshill, Stoke-on-Trent ST4 7QB, England.

*Correspondence address. E-mail: n.maffulli@qmul.ac.uk Via Salvator Allende 23, Baronissi, 89100 Salerno, Italy

Received 28 August 2019; Revised 19 October 2019; Accepted 21 October 2019

Abstract

Background: Tendinopathy is a common musculoskeletal condition affecting subjects regardless of their activity level. Multiple inflammatory molecules found in *ex vivo* samples of human tendons are related to the initiation or progression of tendinopathy. Their role in tendon healing is the subject of this review.

Sources of data: An extensive review of current literature was conducted using PubMed, Embase and Cochrane Library using the term '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 '(inflammation OR immune response)' as either key words or MeSH terms.

Areas of agreement: An environment characterized by a low level of chronic inflammation, together with increased expression of inflammatory cytokines and growth factors, may influence the physiological tendon healing response after treatment.

Areas of controversy: Most studies on this topic exhibited limited scientific translational value because of their heterogeneity. The evidence associated with preclinical studies is limited.

Growing points: The role of inflammation in tendon healing is still unclear, though it seems to affect the overall outcome. A thorough understanding of the biochemical mediators of healing and their pathway of pain could be used to target tendinopathy and possibly guide its management.

Areas timely for developing research: We require further studies with improved designs to effectively evaluate the pathogenesis and progression of tendinopathy to identify cellular and molecular targets to improve outcomes.

Key words: tendinopathy, inflammation, growth factors, failed healing response

Introduction

Human tendons, together with ligaments, muscles and bones, are a complex group of tissues with several fundamental roles ranging from structural to supporting movement.[1,2] Tendons, interposed between muscles and bones, contain highly specialized cells and have a unique structure composed of long type I collagen fibrils (70–80%) and few elastic fibres. The most representative group of cells is the highly metabolically active fibroblasts termed tenoblasts, which mature into less metabolically active tenocytes.[3] Together, tenoblasts and tenocytes constitute 90–95% of the cellular elements in tendons.[3] Other cell types include chondrocytes at the bone attachment and insertion sites, synovial cells and vascular cells.[3] In addition, an abundant extracellular matrix (ECM) composed of proteoglycans, glycosaminoglycans and several other small molecules surrounds collagen and tenocytes.[1,2]

Physiologically, tendon homeostasis is maintained by a dynamic remodelling process involving matrix metalloproteases (MMPs) and their inhibitors.[1,4] Mechanical load, cytokines and systemic conditions play a crucial role within this dynamic environment.[1,4] Disruption of this balance results in tendon inflammation, tendinopathy or tendon injury, all common causes of musculoskeletal pain.[5–7] These injuries peak in ageing individuals and young athletes.[5–7] Chronic tendinopathy can restrict activities of daily living and produces long lasting physical and psychological effects.[5–7]

In acute tendon injuries, which are usually traumatic and include ruptures and partial tears, three phases define the healing process: an acute inflammatory state, a proliferative phase and a remodelling phase.[4,8] Inflammation commences 3–7 days from the injury, is usually accompanied by pain and is characterized by the prevalence of inflammatory cells, such as monocytes and macrophages.[8] During this phase, platelet activation leads to hematoma formation. Immune cells form a granuloma, and other cells types start migrating from the peritendinous epitenon and endotenon tissue (tendon sheath) to the injured site. The proliferative phase starts about 3 weeks after the injury. The cells continue to migrate to the injured site while intense proliferation and differentiation begin, with migrated fibroblasts producing mostly type III collagen. Elastic deformation and mechanical stimuli are an integral part of the process. Type III collagen is increasingly produced within the tendon and its ECM, allowing repair callus to enlarge and tendon strength to increase.[9] Tenocytes become the main cell type during this phase, and collagen synthesis lasts for the next 5–8 weeks. The release of vascular endothelial growth factor (VEGF) allows neovascularization and stimulates the

formation of granulation tissue. In this stage, continuous, intermittent or activity-related pain occurs.[3,4] In tendinopathic patients, this phase is prolonged because of pain, and patients often have to limit their activities to accommodate their symptoms.[3,4] In the remodelling phase, maturation and remodelling of the ECM results in better tissue organization and increasing cross-linking, producing more and better quality tissue. The transverse area of the callus progressively decreases, and the mechanical properties of the repair tissue improve. Remodelling takes up to 2 years to complete healing, but full tendon regeneration is never achieved. Hypercellularity, altered collagen diameter and fibrils thinning result in reduced mechanical strength and resistance of the tendon.[3]

Inflammation and its dysregulation play a role in the early initiation of tendon pathologies.[10] Inflammation begins earlier than fibrotic and other degenerative tendon changes.[11] Tendon injuries are accompanied and preceded by inflammation, with intrinsic expression of various chemical inflammatory mediators by tenocytes. These mediators include pro-inflammatory and anti-inflammatory cytokines and several growth factors including TNF-α, IL-1β, IL-6, IL-10, VEGF, TGF-b and 25 COX-2 and PGE2.[9,12,13]

Cytokines and mechanical load act on cell maturation, tissue metabolism[14–18] and gene expression[17] in healthy tendons. Even though inflammation driven by cytokines might play a role in the healing process, its role in the development, healing and complete resolution of tendinopathy, tendon rupture and other inflammatory processes remains controversial.[14,19] Mechanical loads play a key role in both the maintenance and recovery of tissue homeostasis.[20] Sedentary individuals have higher levels of pro-inflammatory factors such as TNF-α, IL-1 β and VEGF and low levels of COL-I; this improves inflammation and preludes to an increased MMPs (MMP-2, -9 and -13) activity with the onset of a low state of inflammation that results in a higher risk of tendon rupture.[8] Without initial inflammation, the healing process and the subsequent changes that characterize chronic tendinopathies (>12 weeks) cannot take place.[21]

This systematic review reports the most up-to-date evidence on the effects of low state of inflammation on tendon healing with a focus on its clinical relevance.

Methods

Literature search strategy

We first searched the PROSPERO database to ensure that similar/overlapping systematic reviews on the topic where not already in progress. At that point, we registered the present systematic review in PROSPERO (number CRD42019141608). This systematic review was conducted according to the guidelines of the preferred reporting items for systematic reviews and meta-analyses (PRISMA)[22] and MOOSE guidelines[23] (Fig. 1). A comprehensive search was performed on three electronic databases (PubMed, Embase and Cochrane Library) by two independent authors (E.C. and W.S.K.) from their inception to June 10, 2019. Our main aims were to (i) understand the role of inflammation and immune response in tendon healing, (ii) identify factors associated with anti-inflammatory intervention and (iii) evaluate their effects through the review of animal and *in vitro* studies. To achieve the maximum sensitivity of the search strategy, we combined the term '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 '(inflammation OR immune response)' as either key words or MeSH terms. We also reviewed the reference list of all the included scientific articles and top hits from Google Scholar to identify further potentially relevant investigation, which were then assessed using the inclusion and exclusion criteria.

Selection criteria

Eligible studies included those investigating the inflammation and immune response in tendon healing. We screened the titles and abstracts of all investigations regardless of their level of evidence

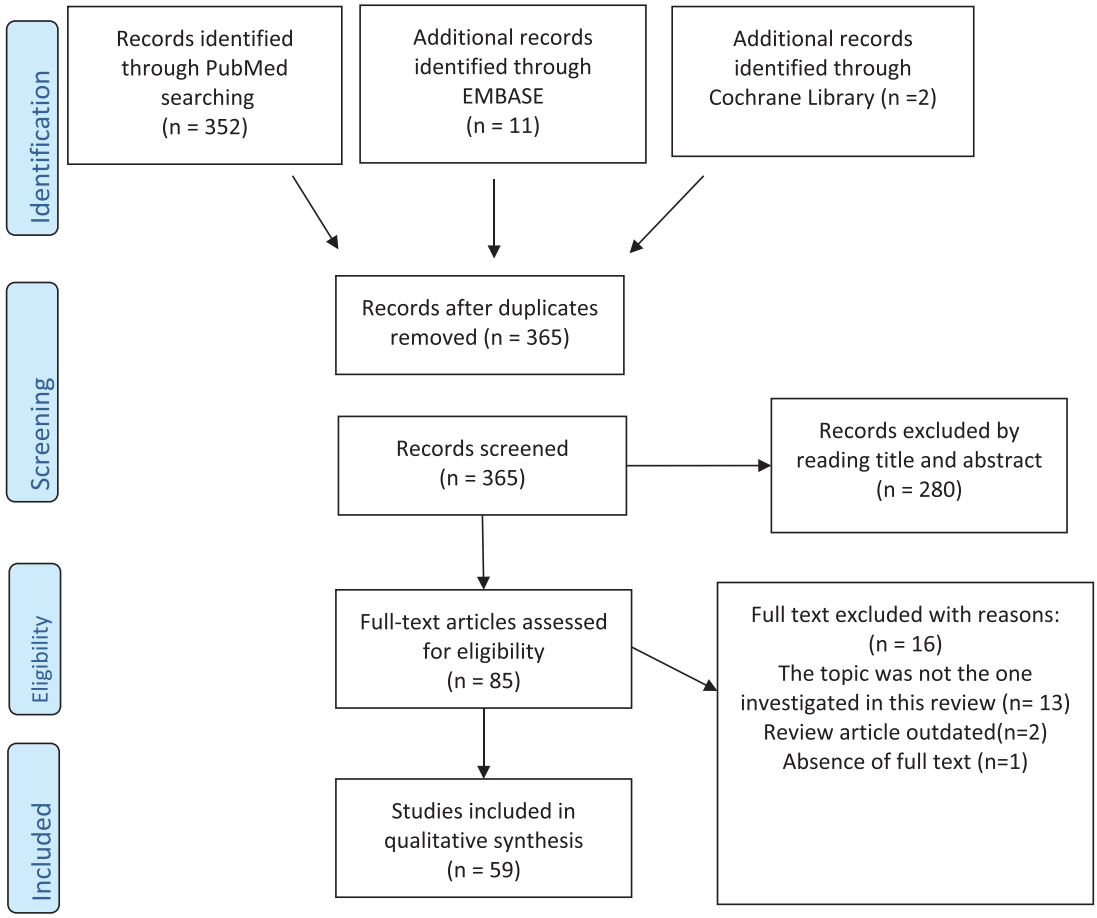

Fig. 1 PRISMA Flow diagram.

published in peer-reviewed journals reporting clinical or basic sciences results. Articles in English, French, Italian, Portuguese and Spanish were considered, as the senior author (N.M.) was able to evaluate them. Articles discussing the effect of several cytokines and immune response actors, both pathologically and physiologically were also reviewed. Exclusion criteria included studies investigating the treatment response of tendon to regenerative treatments (PRP, MSCs etc.) or new drugs related to healing of the tissue. Additionally, we excluded studies in which data were not accessible, missing or poorly reported. We did not include studies without an available full text. We also excluded all the remaining duplicates, and the investigations with poor scientific methodology (see below). Abstracts, case reports, conference proceedings, editorials and expert opinions were excluded. At the start of the present investigation, each of two investigators (E.C. and W.S.K.) searched and evaluated all the articles; they 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 scientific articles were re-evaluated to establish the agreement of the two investigators about articles' selection, observing no disagreement. One investigator extracted the data from the full-text articles to Excel spreadsheet structured tables to allow descriptive analysis of each study. The other

investigator independently checked the extraction of primary data from all the articles. Doubts and inconsistencies were grouped and solved by the senior author, a researcher experienced in systematic reviews (N.M.).

Data extraction and criteria appraisal

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

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[24] for preclinical studies and the Cochrane Collaboration's risk of bias tool[25] for clinical studies (Supplementary Material Table 1 a and b). This assessment used 'Low', 'Moderate' and 'High' as categories to classify potential bias. The subcategories evaluated by the tool are selection bias, performance bias, detection bias, attrition bias, reporting bias and 'other potential risk of bias'. Two investigators (E.C. and L.R.) undertook the assessment independently, with a 95% inter-rater agreement. In case of discrepancy, the senior investigator (N.M.) made the final decision. The *in vitro* studies were not assessed because the nature of these studies does not allow one to assess the risk of bias.[26]

Study quality assessment

The Collaborative Approach to Meta-Analysis and Review of Animal Data from Experimental Studies (CAMARADES) checklist with supporting guidance from the CAMARADES website[27] was used to assess the quality of evidence, giving one point for each of (i) publication in a peer-reviewed journal, (ii) statement of temperature control, (iii) random allocation to groups, (iv) allocation concealment, (v) blinded assessment of outcome, (vi) use of anaesthetic without significant internal protection of blood vessel, (vii) appropriate animal model (aged, healthy, diabetic or hypertensive), (viii) sample size calculation, (ix) compliance with animal welfare regulations and (x) 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 investigators (E.C. and L.R.) independently, with a 94% inter-rater agreement. In case of discrepancy, the senior investigator (N.M.) made the final decision. If the total score was lower than 5, the study was excluded from the review ($n = 4$).

Results

We identified a total of 645 studies from the databases according to the inclusion and exclusion criteria specified above. After removal of duplicates, a total of 365 scientific articles were screened by reading their title and abstract. Eventually, after full-text reading and reference list check, we selected 59 articles for the purposes of the present manuscript.[10,11,13,14,16,18,21,28–77,78] Figure 1 provides the PRISMA[22] flow chart of the selection process and screening.

The articles included investigate the role of several cytokines, growth factors and immune mediators in the healing response of tendons during inflammation. Five included articles are previous review on similar topics that were reviewed to support the background of this investigation.[49–53] Thirty were human and animal *in vitro/ex vivo* studies.[10,13,16,21,28–48,72–76,79] Twenty-four were studies using an animal *in vivo* model.[11,15,16,18,36,54–71]

The risk of bias, assessed as previously described, was low to moderate for all the studies. The overall quality was assessed as moderate to high (mean CAMARADES score, 7.2). The main characteristics of the studies included are detailed in Tables 1 and 2.

Table 1 Main characteristics of the animal model *in vivo* studies

Author (year)	Study design	Tissue involved	Model of injury	Main outcomes
Abraham 2019[68]	Animal model of healing	Rotator cuff tendons	Tendinopathy induced in KO mouse for IKKβ/NF-κB pathway	Accordingly, targeting of the IKKβ/NF-κB pathway in tendon stromal cells may offer previously unidentified therapeutic approaches in the management of human tendon disorders.
Alaseirlis 2005[69]	Animal model of healing	Rat patellar tendons	Transection without repair	Decreasing the inflammatory response in the early stages of tendon wound healing enhances the quality of the healing tendon through increased collagen fibre diameter and better organization.
Alim 2017[70]	Animal model of healing	Rat Achilles tendon	Transection without repair	NMDA receptor-1
Asundi 2007[64]	Animal model of healing	Rabbit flexor profundis	Transection without repair	MMP-1, MMP-3, VEGF. CTGF, COX-2, IL-1beta, COL-III, FBRN
Best 2019[43]	Animal model of healing	Flexor tendon	NFKB1 deletion, transection and repair	NFKB1 deletion increased activation of both NF-κB and MAPK signalling
Blomgran 2016[71]	Animal model of healing	Rat Achilles tendon	Transection without repair	M1 and M2 macrophages
Eliasson 2012[79]	Animal model of healing	Rat Achilles tendon	Transection and then exercise	IL-1β
Fedorczyk 2009[11]	Animal model of healing	Rat flexor digitorum tendon	Tendinopathy model of repetitive strain injury	IL-1β
Gao 2013[65]	Animal model of healing	Rat flexor digitorum	Tendinopathy model based on high-repetition, low-force reaching task	TNF-α, IL-6, TGFB1, CTGF and MMP2
Hammerman 2018[59]	Animal model of healing	Rat Achilles tendon	Transection and then exercise	COL1A1, COL3A1, LOX, VCAM
Hammerman 2017[58]	Animal model of healing	Rat Achilles tendon	Transection and then exercise	CCL20, CCL7, IL-6, NFIL3, PTX3, SOCS1 and TLR2
Hosaka 2010[66]	Animal model of healing	Equine superficial digital flexor tendon	Tendinopathy model was based on the exposure of TNF-alpha	MPPs and TNF-alpha
Kietrys 2012[67]	Animal model of healing	Rat flexor digitorum and supraspinatus tendons	Upper extremity repetitive overuse model of tendinopathy	CTGF
Koshima 2007[62]	Animal model of healing	Rabbit rotator cuff tendon	Surgical created tear without repair	PGE2, COX-2, IL-1β

(Continued)

Table 1 Continued

Author (year)	Study design	Tissue involved	Model of injury	Main outcomes
Lin 2006[15]	Animal model of healing	Tendons	Healing after transection in KO mice for IL-4 and IL-6	IL-4, IL-6
Manning 2014[60]	Animal model of healing	Canine flexor tendons	Transection and then repair	IL-1β
Millar 2009[16]	Animal model and human model	Rat and human supraspinatus	Rat overuse tendinopathy model and torn supraspinatus samples from humans	MIF, IL-18, IL-15, IL-6, TNF-alpha, Caspase 3, Caspase 8
Pingel 2013[80]	Animal model of healing	Rat calcaneal tendon	High-intensity uphill running exercise model	Mast cells
Stalman 2015[72]	Animal model of healing	Rat Achilles tendon	Transection without repair	Chemokins (CCL5, CCL2, CCL3, CXCL10)
Sugg 2014[74]	Animal model of healing	Rat Achilles tendon	Transection and repair	IL-1β, 6, 10
Sun 2008[18]	Animal model of healing	Rat patellar tendons	Transection and then repair and exercise	IL-1β
Tarafder 2017[73]	Animal model of healing	Rat patellar tendons	Transection without repair	JNK and JAK-STAT3 e tendon stem progenitor cells
Uchida 2005[61]	Animal model of healing	Rat patellar tendons	Tendinopathy model of overuse injury	IL 1 beta and TNF-alpha

KO, knockout; IKK, inhibitor of nuclear factor kappa-B kinase; NF-kB, nuclear factor kappa-B; NMDA, N-methyl-D-aspartate; MMP, metalloproteinases; COX, cyclooxygenase; IL, interleukin; TIMP, tissue inhibitors of metalloproteinases; TNF, tumour necrosis factor; TGF, transforming growth factor; CTGF, connective tissue growth factor; COL, collagen; CCL, chemokine ligands; PGE, prostaglandin E; JNK, c-Jun N-terminal kinase; JAK, janus kinase; STAT, signal transducer and activator of transcription; PDGF, platelet-derived growth factor.

The healing of an injured tendon is a complex process requiring inflammation, immune system mediators, growth factors, mechanical stimuli and other yet unknown factors. A total of 20 pro-inflammatory and anti-inflammatory cytokines have been reported to be involved in the process of tendon healing: 'IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-6, IL-8, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-17, IL-18, IL-27, TNF-α, IFN-γ and TGF-β'.[80] Their role seems crucial in every phase of healing and, although controversial and often tissue dependent, they appear to be capable of inducing a failed healing response. Several other actors play a role including chemokines; chemoattraction at the injured site is mostly performed by CCL5, CCL2, CCL3 and CXCL10, and they act as the main mediators of immune response, inflammation[69] and mechanical load[8,20]. Among the pro-inflammatory cytokines, IL-1β, IL-6 and TNF-α are the most potent inducers of chemokines and immune response.[80] Repetitive mechanical overloading and microtrauma, which ultimately results in hypoxic injury, have been suggested as one of the causes of tendinopathy, leading to elevated inflammatory markers[51,81]. In addition, the inflammation present in tendon injury, both acute and chronically induced, may alter the expression of MMPs such as MMP-1 and MMP-9, which may ultimately lead to a diminished ability to heal or a 'failed healing response'.[74]

Interleukin family

Interleukin-1β (IL-1β), a pro-inflammatory cytokine involved in several cellular physiological responses, is produced in conditions such as infection and injury.[52] Previously thought to be produced only by monocytes and macrophages, IL-1β is now known to be produced by some other cells in the connective

Table 2 Main characteristics of the *in vitro/ex vivo* studies

Author (year)	Study design	Tissue involved	Model of injury	Main outcomes
Ackermann 2013[21]	Human *in vitro* study	Achilles tendon	Tear repair post-op	IL-1β, 6, 8, 10; TNF-α
Ahn 2017[29]	Human *in vitro* study	Rotator cuff tendons	Tear	The downregulation of inflammatory response genes and the upregulation of cell differentiation genes in torn rotator cuffs at the time of surgery are related to rotator cuff healing.
Arnold 2007[50]	Human *in vitro* study	Skeletal muscle	Strain	Injured skeletal muscle recruits monocites exhibiting inflammatory profiles that operate phagocytosis and rapidly convert to anti-inflammatory cells that stimulate myogenesis and fibre growth.
Campbell 2014[30]	Human *in vitro* study	Rotator cuff tendons	Samples harvested at surgery	IL-21R and IL-21
Chaudhury 2016[31]	Human *in vitro* study	Rotator cuff tendons	Tear	MMP 3, 10, 12, 13, 15, 21 and 25; a disintegrin and metalloproteinase (ADAM) 12, 15 and 22; and aggrecan
Chamberlain 2011[48]	Animal *in vitro* study	Rat medial collateral ligaments	Tear	Our results suggest that an early macrophage response, which is necessary for debridement of damaged tissue in the wound, is also important for cytokine release to mediate normal repair processes.
Chamberlain 2019[114]	Human *in vitro* study	Achilles tendon	Tendon rupture	Treatment with MSC-derived EVs alone was less effective functionally but stimulated a biological response as evidenced by an increased number of endothelial cells and decreased M1/M2 ratio.
Courneya 2010[32]	Human *in vitro* study	Achilles, flexor digitorum and flexor hallucis longus tendons	Rupture	IL-4 or IL-13
Dakin 2012[45]	Animal *in vitro* study	Equine tendon	Tendon injury	The data suggest that although tenocytes are capable of mounting a protective mechanism to counteract inflammatory stimuli, this appears to be of insufficient duration and magnitude in natural tendon injury, which may potentiate chronic inflammation and fibrotic repair, as indicated by the presence of M2.
Dakin 2015[82]	Human *in vitro* study	Rotator cuff tendons	Tendinopathy	NF-κB and IFN target genes

(Continued)

Table 2 Continued

Author (year)	Study design	Tissue involved	Model of injury	Main outcomes
Dakin 2018[46]	Human *in vitro* study	Achilles tendon	Tendon rupture	Tissue and cells derived from tendinopathic and ruptured Achilles tendons show evidence of chronic (non-resolving) inflammation
Gaida 2012[33]	Human *in vitro* study	Achilles tendon	Tendinopathy	TNF-α, TNFR1 and TNFR2
Gotoh 1997[34]	Human *in vitro* study	Supraspinatus tendon	Tear (complete and incomplete)	IL-1β, MMP-1
Italiani 2014[51]	Human *in vitro* study	Monocytes	Inflammatory response	Transcriptomic profiling
John 2010[35]	Human *in vitro* study	Tendon inflammation model	In vitro induced injury	Both TNF-alpha and IL 6
Kragsnaes 2014[36]	Human *in vitro* study	Achilles tendon	Chronic tendinopathy	Macrophages and endothelial cells role
Langberg 2002[83]	Human *in vitro* study	Peritendinous tissue/ Achilles tendon	Exercise	Achilles tendon produces significant amounts of IL-6 in response to prolonged physical activity, which might contribute to the exercise-induced increase in IL-6 found in plasma.
Legerlotz 2012[10]	Human *in vitro* study	Achilles, tibialis posterior tendons	Tear/tendinopathy	IL-6; Oncostatin M; LIF
Li 2019[96]	Human *in vitro* study	Embryonal and post-natal tenocytes	*In vitro* induced injury	IL-1β
Liang 2012[75]	Human *in vitro* study	Tenocytes	Hypoxia-induced cell death	Hypoxia markedly upregulated VEGF-A mRNA, followed by increased VEGF protein secretion. However, treatment with VEGF did not improve tenocyte survival. As a protective strategy for tenocytes at risk of hypoxic death, we added prosurvival growth factors insulin or platelet rich plasma. Both agents strongly protected tenocytes from hypoxia-induced death over 48 hours, suggesting possible efficacy in the acute postrupture tendon or integrating graft.
Matthews 2006[38]	Human *in vitro* study	Rotator cuff tendon	Chronic tear	CD68, CD45 expression
Millar 2009[16]	Animal model and human model	Rat and human supraspinatus	Tendinopathy and torn supraspinatus	MIF, IL-18, IL-15, IL-6, TNF-alpha, Caspase 3, Caspase 8
Millar 2010[39]	Human *in vitro* study	Subscapularis tendon	Early tendinopathy	CD34 expression and macrophage role
Millar 2015[44]	Human *in vitro* study	Tenocytes	Tendon injury	These data provide a molecular mechanism of miRNA-mediated integration of the early pathophysiologic events that facilitate tissue remodelling in human tendon after injury.

(Continued)

Table 2 Continued

Author (year)	Study design	Tissue involved	Model of injury	Main outcomes
Noah 2019[49]	Human *in vitro* study	Human tendon samples	Tendon injury	The lymphatic vasculature is present in the epitenon and superficial regions of Achilles tendons. Dendritic cells and CD4+ T cells peaked 2 weeks after injury, while B cells and CD8+ T cells demonstrated a progressive increase over time
Oliva 2011[115]	Human *in vitro* study	Rotator cuff tendon	Calcific tendinopathy	A significantly increased expression of tissue transglutaminase (tTG)2 and its substrate, osteopontin, was detected in the calcific areas compared to the levels observed in the normal tissue from the same subject with calcific tendinopathy.
Pingel 2013[63]	Human *in vitro* study	Tenocytes	Exercise	The mRNA for TGF-β, collagen-I and collagen-III were significantly higher expressed, and decorin, CTGF, IL-6 and IL-10 were significantly lower expressed in the tendinopathic versus healthy tendon area. Only IL-10 was lower in expression in experiments with NSAID administration, while all other determined parameters were unaffected by NSAID.
Riley 2002[76]	Human *in vitro* study	Rotator cuff tendon	Tendinopathy	After tendon rupture, there was increased activity of MMP-1, reduced activity of MMP-2 and MMP-3, increased turnover and further deterioration in the quality of the collagen network. Tendon degeneration is shown to be an active, cell-mediated process that may result from a failure to regulate specific MMP activities in response to repeated injury or mechanical strain.
Robertson 2012[28]	Human *in vitro* study	Subscapularis tendon, supraspinatus tendon, glenohumeral synovium, and subacromial bursa	Failed healing	MMP-1 and MMP-9
Skutek 2001[77]	Human *in vitro* study	Human patellar tendon	Acute stretch stress	TNF-alpha, IL-6, PDGF, FGF
Thankam 2016[41]	Human *in vitro* study	Rotator cuff tendon	Tear	TREM-1, HMGB1 and RAGE
Yang 2005[13]	Human *in vitro* study	Patellar tendon	*In vitro* induced injury	IL 1 beta, COX-2, MMP-1, PGE2

IL, interleukin; NF-kB, nuclear factor kappa-B; TNF, tumour necrosis factor; MMP, metalloproteinases; VEGF, vascular growth factor; PDGF, platelet-derived growth factor; PGE, prostaglandin E.

tissue.[52] Tenocytes have a role in the upregulation of inflammatory molecules such as cyclooxygenase-2, prostaglandin E2 and MMP-1, which are common mediators of accelerated degradation of tendon ECM impairing the mechanical properties of tendon.[13]

In human tissues harvested from injured or torn rotators cuff tendons during surgery, gene expression of IL-1β was downregulated while protein expression was upregulated; the expression remained unchanged in the Achilles tendon after repair, exercise or tendinopathy.[21,28,31,60] Human studies on the role of IL-1β in tendon pathology are not conclusive. In animal models of tendon injury, gene and protein expression increased in the early stages of injury or healing for 2 weeks after the insult.[57,59,71] Similarly, exercise induces early gene and protein expression of IL-1β in the early stages.[11,18,54,58,61,62,64,82,83]

The interleukin-6 (IL-6) cytokine family is composed of IL-6 itself, leukaemia inhibitory factor, IL-11, ciliary neurotrophic factor, oncostatin M and cardiotrophin-1.[10] IL-6 is produced by Th2 subset of lymphocytes T and influences immune function. It has recently been shown to be involved in the process of tendon healing.[15,79] The production of IL-6 family cytokines is upregulated in tendon pathologies.[10] Human tendon fibroblasts subjected to an increased level of mechanical stretching secrete IL-6.[79] Gene and protein expression of IL-6 increased in both rotator cuff and Achilles tendon tear samples,[10,16,71,84] even 2 weeks following surgical repair.[21] This was not however the case in Achilles, rotator cuff or posterior tibialis tendinopathy.[10,29,84]

In healthy tendons, prolonged exercise increased protein expression[85] and effect not evident in Achilles tendinopathy.[60] In models of tendon injury in animals, IL-6 gene and protein expression increased 2 hours to 4 weeks after the intervention.[62,71] Even though the quality of the studies was moderate to high, with the exception of two studies[61,62] assessed as of medium quality, the effect of exercise on interleukins is controversial and inconclusive in animal models.[16,43,61,62,68] Tenocytes exposure to IL-6 did not produce any change in tendon ECM gene and protein expression.[32]

The pro-inflammatory effects of the aforementioned cytokines also act through the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway, which is implicated in chronic and acute tendon injuries.[65] This pathway is crucial in animal models of chronic tendinopathy, where its knockout protects against the development of the condition.[65] The deletion of NFKB1 increases the activity of both NF-κB and MAPK, resulting in stiffer and stronger tendons 4 weeks after repair from augmented matrix deposition, increased recruitment of macrophages and increased presence of myofibroblasts.[40] Moreover, its deletion upregulated the expression of Col1a1, Col3a1, Adgre1, Ccl2, Acta2 and pro-inflammatory cytokines Tnf-α. Therefore, the higher activity of NF-κB and MAPK via deletion of NFKB1 enhances macrophage and myofibroblast content at the site of repair, enhancing the deposition collagen resulted in more favourable biomechanical properties.

Another possible target of the interleukin family cytokines can be via ECM involvement through a higher expression of proteins associated with HMGB-1 and upregulation of the NLRP3 inflammasome pathway, TLR4, TLR2, TREM-1, RAGE, ASC and Caspase-1.[86] IL-10 activity *in vitro* was reported only in tendon samples previously challenged with IL-1β and CTGF. In particular, their expression was significantly higher in CD146$^+$ TSCs than CD146$^-$ tenocytes, especially for what concerns TIMP-3 expression.[70] Further signalling studies based on specific inhibition of these factors and subsequent western blot analysis reported that connective tissue growth factor-induced expression of IL-10 and TIMP-3 in CD146$^+$ TSCs are regulated by JNK/signal transducer and activator of transcription 3 signalling.[70] Overall, these findings support the anti-inflammatory action of CTGF-stimulated TSCs, which are probably associated with improved tendon healing. The expression of IL-10 was however inconsistent in clinical samples[28,31,60,84] and animal injury models[71]. In humans or animals, exercise had no effects on the expression of IL-10.[62,64,83]

The endogenous alarmins S100A8 and S100A9 are constitutively expressed in immune cells

(monocytes and neutrophils), which are released in response to environmental changes and cellular damage; their expression is upregulated during inflammation. Tendinopathy is an alarmin-mediated pathology:[41,77,78] alarmin modulates the stromal microenvironment, influencing the inflammatory profile observed in tendinopathy, triggering a positive feedback mechanism, which involves enhanced recruitment of leukocytes and release of pro-inflammatory cytokines from tenocytes.

The quality of the evidence is moderate to high with a low to moderate risk of bias.

TNF-α

Gene and protein expression of TNF-α do not show significant changes in biopsies from rotator cuff and Achilles tendinopathy and tears.[16,21,30,84] To our knowledge, no studies have focused on the effects of exercise and mechanical stimulus on the expression of TNF-α in non-pathological human tendons.[30] In animal injury models, the expression of TNF-α gene was raised from 2 hours to 9 days following the procedure, declining only 2 weeks following the intervention,[57] whereas protein expression increased after 4 days.[57] In animal models, the effect of exercise on TNF-α gene and protein expression was inconclusive.[11,58,62,64,83] The effects of TNF-α on tenocytes could not be ascertained, as the genes of interest differed in the studies included in this systematic review.[32,63] The quality of most studies supporting the role of TNF-α on tenocytes was moderate to high, with only one of the studies assessed of medium quality.[63]

M1 and M2 subsets of macrophages population

Macrophages are immune cells with a crucial role in injury and repair response. They have at least two functional phenotypic states that differ in their cell surface receptors, effector function and cytokine expression.[39] M1 (classically activated) macrophages are mainly pro-inflammatory, whereas M2 (alternatively activated) macrophages, which may play a role in fibrosis, usually counteract inflammation producing immune-regulating factors such as IL-1RA, IL-10 and IL-4.[39] Chemotactic cytokines, which promote the delivery of different cell populations to the site of repair, are released from the tendon ECM in response to structural damage. Phagocytic neutrophils and M1 macrophages reach the site within 24 hours after injury and are then followed by a reparative shift in function correlated with increases in M2 macrophages.[3,71]

Inflammation is present in the early phases of tendinopathy.[36] Human tissue biopsies from large rotator cuff tears exhibit significant inflammatory infiltrate, including macrophages, compared to smaller tears.[35] The macrophages, which usually are absent in healthy tendons, are able to polarize and move to tendon massively in depending on the stage of inflammation of the tissue. [14]. Although M1 macrophages predominated in the subacute phase completely different M2 macrophages abound in chronic injured tendons.[39] This is in accordance with the current understanding of tendinopathy: inflammation is present in the early stage of tendinopathy, and, as the condition progresses, inflammation gradually subsides with residual degenerative changes. This was further confirmed in animal injury models.[16,42,71] Tendon cells from biopsies of patients affected by either mid-portion Achilles tendinopathy or rupture showed upregulation of pro-inflammatory and stromal fibroblast activation markers compared with healthy tenocytes harvested from hamstrings.[43] Tendinopathic and ruptured Achilles tendons highly expressed CD14+ (monocytes) and CD68+ (macrophage) cells together with a complex inflammation signature (NF-κB, interferon and STAT-6), increased PTGS2 and interleukin-8 expression, as well as highly expressed interferon markers IRF1 and IRF5. Tissue and cells from tendinopathic and ruptured Achilles show evidence of chronic (non-resolving) inflammation.

Most tendons are surrounded by tendon sheets with thin layers of cells: these tissue layers may well provide a source of fibroblasts to repair injured tendons, as these cells can trans-

differentiate into fibroblasts through activation of epithelial-to-mesenchymal transition (EMT) to regenerate damaged ECM. This is a common even in other regenerative processes in the human body,[87] and it was reported how macrophage phenotype and activation of EMT-related programmes can likely contribute to the degradation and subsequent repair also in injured tendons.[71]

The healing process in tendinopathy is severely impaired, eventually resulting in failure.[4,8] The failed healing response concept is gaining research interest and is related to both extrinsic and intrinsic factors on a background of low-grade inflammation. The relationship between these factors and the underlying tendinopathy is not fully understood.[4,8] It is unclear whether early intervention after clinical presentation of tendinopathy can improve the overall outcome.[66]

Evidence supports a role of macrophages and their polarization on tendon healing. The quality of evidence was assessed as moderate to high with the exception of one study,[71] assessed as of medium quality.

The role of exercise

Exercise was studied in preclinical studies involving animal models of both tendinopathic and healthy tendons.[11,18,54–56] Exercise and tendon loading in a rat running model significantly altered the collagen structure and cell presence of both tenocytes and immune cells such as mast cells compared to non-runner controls ($P < 0.05$).[82] This was not however found in a human study involving 27 tendinopathic (over 6 months) tendons after a 1 hour running session.[60] Achilles tendon biopsies from painful and healthy regions of the same tendon 2 hours after 1 hour of running. Gene expression was analysed examining mRNA in the sampled biopsies. In the tendinopathic region, the expression of TGF-β, collagen-I, collagen-III and decorin was significantly higher, and the expression of CTGF, IL-6 and IL-10 was significantly decreased compared with healthy tendon ($P < 0.05$).[60]

Inflammation appears to be strongly influenced by exercise in animal models of tendinopathy.[11,18,54–56] Hammerman *et al.*[55,56] investigated the expression of several interleukins and inflammatory factors after exercise in a rat tendinopathy model. The expression of CCL20, CCL7, IL-6, NFIL3, PTX3, SOCS1 and TLR2 was significantly higher ($P < 0.05$), as was the presence of leucocytes assessed through histological analysis. This was further supported by other studies.[11,18,54] Based on the current evidence, it is difficult to establish the right threshold for a beneficial effect of exercise without raising the risk of a potential harm in human patients. Further prospective studies might elucidate this and guide clinicians.

Overall, the methodological quality of evidence is moderate to high for animal studies reporting an influence on inflammation from exercise in the acute and chronic setting.

An immune-centric approach: new tools in regenerative medicine

Regenerative medicine has greatly evolved in the past decade: the role of the innate immune system, and especially the role of macrophages, in the host response to regenerative medicine interventions has recently been extensively considered.[88,89,90] Macrophage phenotype and functions may be critical factors leading to emerging regenerative medicine approaches, which should promote the involvement of the immune system to produce positive outcomes. Macrophage and regulatory T cells (Treg) emerged also as potent regulators of stem cells in both physiological and tissue repair condition.[91,92] Monocytes and macrophages can exacerbate inflammation, induce tissue repair and fibrosis or drive regeneration: they should be primary targets when designing regenerative strategies.[93] Loading seems to delay the switch to an M2 type of inflammation, with more Treg cells[68] in a rat model. A prolonged M1 phase following loading might increase the quantity of tendon regenerate, suggesting an earlier switch to M2 as a new strategy to regenerate tendons.[44] Non-specific inhibition of macrophages resulted in poorer early

ECM formation and ligament strength.[45] M2-like macrophages generated using extracellular vesicles (EVs) isolated from MSCs in a mouse Achilles tendon rupture model improve tendon healing *in vivo* by modulation of tissue inflammation and endogenous macrophage immunophenotypes. Moreover, M2-like macrophages in the wound induced a functional and regenerative healing response significantly greater than with MSC-derived EVs alone.[44] Recently, Noah *et al.* [46] showed that the lymphatic vasculature is present in the epitenon and superficial regions of Achilles tendons: using flow cytometry and histology after tendon injury, a robust adaptive immune cell response was observed. One week after injury there was marked accumulation of monocytes, neutrophils and macrophages that declined thereafter. This was followed by dendritic cells and CD4+ T cells 2 weeks after injury, while B cells and CD8+ T cells progressively increased over time. Immune cells of the draining popliteal lymph node demonstrated a similarly coordinated reaction to the injury. The cellular components of the adaptive immune cell population as well as innate immune cell response are recruited following tendon injury and may play a role in the regulation of tendon healing.[46]

Cell therapy is proposed as an alternative or adjuvant in tendinopathy patients: several cell lines delivered into the region of tendon injury can directly differentiate into tenocytes,[94] stimulating local endogenous reparative mechanisms through a paracrine effect mediated by secretion of cytokines, growth factors and chemokines.[95] This was further highlighted in preclinical animal studies,[93,94] which showed how local delivery of MSCs can enhance recruitment of endogenous progenitors and thus improve regeneration of the injured site mainly through the beneficial recruitment of M2 macrophages, crucial for tissue repair.[92] Additionally, MSCs drive the polarization of macrophages into the beneficial M2 phenotype:[95] monocyte-derived M2 in their turn suppress T cell proliferation in an IL-10-independent manner, amplifying the immunosuppressive effect produced by MSC. Moreover, monocytes themselves, which are more abundant and easily accessible than MSCs from both adipose tissue and bone marrow, are able to shift M1 macrophage into M2 phenotype in different tissue and disease.[47,48,93,96]

Surprisingly, the immune system plays a major role in stem cell repair-mediated tissue regeneration, and a promising therapeutic strategy may consist of paracrine factors production by the stimulation of immune cells in damaged tissues.[88,90–92,97–106] However, the majority of these findings are based on preclinical studies which are still not confirmed in. Therefore, further studies on the regenerative approach to tendinopathy are necessary.

Discussion

There are conflicting data on the role of low-grade inflammation in tendon healing. This review systematically analyses the current published evidence on the presence and possible role of both pro- and anti-inflammatory cytokines in tendon healing. Even though the published data are mostly from preclinical studies, they are nonetheless of moderate to high quality, giving strength to their interpretation given in the present review.

The members of the IL-1 family (IL-1a, IL-1β, IL-1 and IL-33) are the most potent cytokines produced by innate immunity.[107] Synthesized as precursors, both IL-1a (IL-1F1) and IL-1β (IL-1F2) are cleaved by Calpain[108] and Caspase 1 (IL1b converting enzyme),[109] respectively. Intracellular signalling involves adapter proteins MyD88 (myeloid differentiation factor 88), IRAK (IL-1-receptor-associated-kinase) and TRAF6 (TNF-receptor-associated factor 6), leading to the activation of NFκB, JNK (c-Jun N-terminal-kinase), AMPK (AMP-activated protein kinase), TREM-1 (Triggering Receptors Expressed on Myeloid cells-1) and MAPK, which were reported as part of the pathological process in preclinical studies.[37,38,65,70] Alarmins, such as IL-1a and IL-33, are released after necrosis: they enhance inflammation by binding to different receptors of NF-κB.[65,108] Increased activation of NF-κB and MAPK via NFKB1 deletion increases the

content of macrophages and myofibroblasts at the repair site, with enhanced collagen deposition and biomechanical properties.[40]

The ECM plays a substantial role in low inflammation environment [86], and further studies should investigate the role of the inflammasome pathways involved.

Serum IL-6 levels are commonly raised during and following stress and in fever and hyperthermia.[107,110] IL-6 is a cytokine that induces the acute phase response, and TNF-α and IL-1b promote its production.[10,110] Although IL-6 plays a central role in inflammation and tissue injury, it also enhances the early healing phase in tendons by promoting an increase of COL1A1 expression.[10] Early intervention in this particular phase may improve the overall outcome of the treatment both quantitatively and qualitatively.[66]

Macrophage phenotype and functions are critical factors in tendon injury and repair acting on M2 beneficial polarization; they emerged as potent regulators of stem cells in both physiological and tissue repair condition.[91,92] Their role in tendons healing should be further investigated. Cellular components of the adaptive immune cell population are recruited following tendon injury and may regulate tendon healing and innate immune cells response, suggesting new promising fields to be investigated.[46] Macrophages and other immune cells are also derived from adipose tissue: there is a complex relationship between these cells and the adipocyte-derived pro-inflammatory cytokines.[12,111] MCP-1 induces macrophage infiltration of adipose tissue. Activated macrophages in turn release further pro-inflammatory cytokines, and TNF-α expression is markedly increased. [12,111] The expression of adiponectin, an adipocyte-derived anti-inflammatory hormone, is then reduced. The altered balance between macrophages and chemotactic mediators produces persistent local inflammation within the adipose tissue.[12] Increased adipose mass index changes the relationship between leptin and suppressor T cells; leptin, an adipocyte-derived hormone responsible for the central control of energy balance, seems to inhibit the proliferative capacity of suppressor T cells.[111] For these reasons, special attention should be paid when considering the use of adipose tissue cells concentrates, both SVF and MSCs, on chronic inflamed tissue as tendinopathy, especially in Type 2 diabetes patients or obese patients, as their therapeutic potential may be limited[112].

Several preclinical studies note that exercise still represents one of the best ways to positively influence tendon healing by negatively affecting the inflammatory environment.[55,56,81,113,114] This highlights the role of early mobilization in injured tendons. The mechanism by which cytokines influence this is still not fully understood but appears to be related to the stimulatory effect of trauma induced micro-damage and vessel leakage.[81,113] Thus, early physical therapy, based on therapeutic eccentric and concentric exercise after tendon injury or surgery, and in tendinopathy, should be strongly recommended.

The overall role of inflammation remains unclear. Further research should aim at better understanding the pathogenesis of tendinopathy and specifically identifying molecular and cellular target to improve treatment outcome.

Limitations

The main limitation of this systematic review is the heterogeneity of the included studies: the majority was preclinical, and no clinical randomized controlled trials were identified. We applied strict methodological evaluation through quality and risk of bias tools, but the treatment variables, including protocol of study and technical facilities, remain a major limiting factor; in addition, the populations studied differed across the included investigations. The findings of our review will however hopefully help direct future investigations.

Conclusion

The prolonged state of low-grade inflammation evident in chronic tendinopathy may be a risk factor for a 'failed healing response' following an acute

tendon insult, predisposing affected individuals to disrupted healing, even after surgery. Further studies evaluating the impact of lifestyle modification and anti-inflammatory or immune-modulatory therapies able to target molecular and cellular target of chronic inflammation are necessary to better understand and clarify the role of inflammation in tendinopathy.

Conflict of interest statement

The authors have no potential conflicts of interest.

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