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Immune Modulation to Improve Tissue Engineering Outcomes for Cartilage Repair in the Osteoarthritic Joint

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Osteoarthritis (OA), the most common form of arthritis, is a disabling degenerative joint disease affecting synovial joints and is associated with cartilage destruction, inflammation of the synovial membrane, and subchondral bone remodeling. Inflammation of the synovial membrane may arise secondary to degenerative processes in articular cartilage (AC), or may be a primary occurrence in OA pathogenesis. However, synovial inflammation plays a key role in the pathogenesis and disease progression of OA through the production of pro-inflammatory mediators, and is associated with cartilage destruction and pain. The triggers that initiate activation of the immune response in OA are unknown, but crosstalk between osteoarthritic chondrocytes, cartilage degradation products, and the synovium may act to perpetuate this response. Increasing evidence has emerged highlighting an important role for pro-inflammatory mediators and infiltrating inflammatory cell populations in the progression of the disease. Tissue engineering strategies hold great potential for the repair of damaged AC in an osteoarthritic joint. However, an in-depth understanding of how OA-associated inflammation impacts chondrocyte and progenitor cell behavior is required to achieve efficient cartilage regeneration in a catabolic osteoarthritic environment. In this review, we will discuss the role of inflammation in OA, and investigate novel immune modulation strategies that may prevent disease progression and facilitate successful cartilage regeneration for the treatment of OA.

Introduction

Osteoarthritis (OA) is a complex disease of synovial joints that is associated with chronic pain and reduced joint mobility. It is an age-related condition, with known risk factors for disease development including high body mass index, particularly in young adults, and previous joint injury.[1–3] OA is characterized by cartilage breakdown, but the disease process affects various joint structures involving inflammation of the synovial membrane and subchondral bone remodeling. Therefore, it has been suggested by Loeser *et al.* and others that OA should be considered a disease of the entire joint as an organ.[4]

An imbalance of cellular homeostasis is an important feature of OA, with mechanical stress and pro-inflammatory cytokines postulated to contribute to this change. Cell proliferation and enhanced matrix remodeling in bone and cartilage are also major features, and the formation of new bone at the joint margins.[5,6] Articular chondrocytes increase expression of matrix molecules, and catabolic factors including matrix metalloproteinases (MMPs), ADAMTs (a disintegrin and metalloproteinase with thrombospondin motifs), and pro-inflammatory cytokines. Chondrocytes suffer a loss of characteristic phenotype due to loss of extracellular matrix (ECM) components and structure, and undergo hypertrophy and terminal differentiation.[5] Decreased accumulation of sulphated proteoglycans and collagen type II has been observed in osteoarthritic cartilage compared to healthy.[6] Increasing evidence highlights the importance of factors produced by inflamed synovium to the initiation and progression of the disease and inflammation of the synovial membrane with increased vascular density and cellular infiltration is a prominent feature of OA pathogenesis.[7] Additionally, pro-inflammatory mediators detected in synovial fluid of OA joints are known to stimulate degradation of cartilage and inhibit matrix synthesis.[8–13] Inflammation of the synovial membrane may be a primary occurrence in disease pathogenesis, with thickening of the synovial membrane identified by MRI in patients with early stage and mild OA.[14] Alternatively, synovial inflammation may be secondary to degenerative processes in articular cartilage (AC), with the release of cartilage degradation products activating immune and synovial cells and initiating an inflammatory response (Fig. 1).

Tissue engineering strategies can be harnessed to promote repair of damaged AC in an osteoarthritic joint. However, it is clear that interplay between joint tissues and pro-inflammatory

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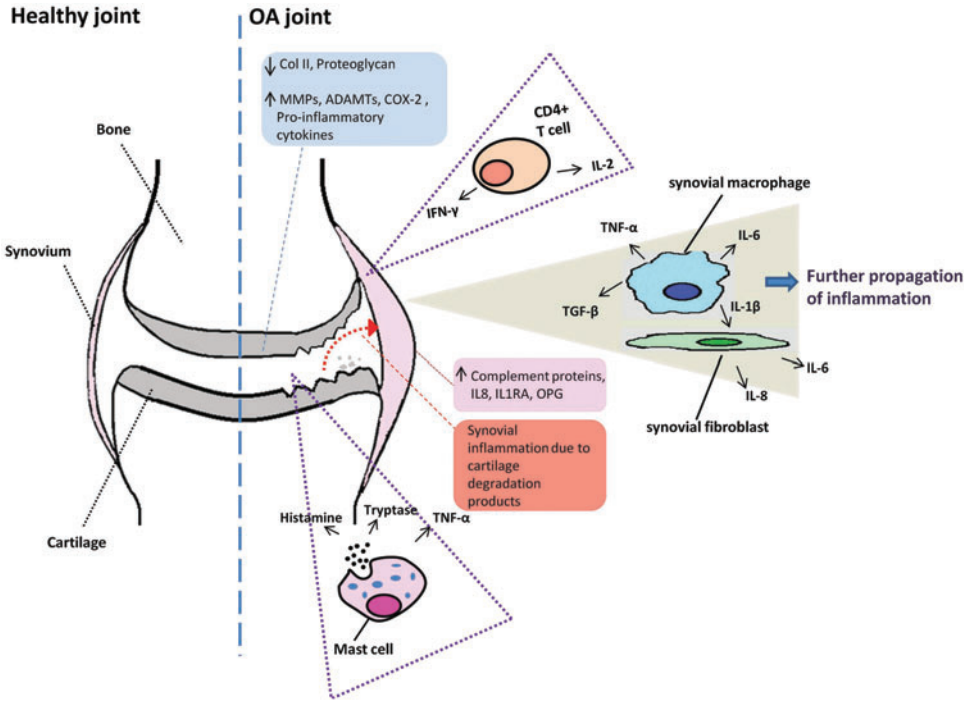

FIG. 1. Schematic representation of inflammatory processes associated with the pathogenesis of osteoarthritis (OA). Activation of resident synoviocytes by cartilage degradation products or pro-inflammatory mediators, and infiltration of the synovium by immune cells, may induce destructive processes in cartilage and drive disease progression. Color images available online at www.liebertpub.com/teb

mediators contributes to the loss of cellular homeostasis that is associated with disease progression. Therefore, inflammatory processes associated with OA need to be addressed to achieve successful cartilage repair in the osteoarthritic environment. In this review, we will examine the role of inflammation in OA and explore novel immune modulation approaches, which may halt disease progression and facilitate successful cartilage regeneration in the osteoarthritic environment.

Tissue Engineering Applications in OA

Current approaches for the treatment of AC damage resulting from OA include bone marrow stimulation techniques such as microfracture.[15] This method involves drilling or abrasion of subchondral bone and subsequent release of progenitor cells from the bone marrow to induce repair. However, generation of suboptimal reparative fibrocartilage may occur, which limits the effectiveness of this technique for repair of AC defects and restoration of function.[16] Regenerative procedures such as osteochondral autografting (mosaicplasty), autologous chondrocyte implantation (ACI), and matrix-induced autologous chondrocyte implantation (MACI) are considered favorable therapeutic strategies, given their potential for the generation of hyaline cartilage.[17–19] ACI involves the use of cultured autologous chondrocytes harvested from a nonweight bearing region of the knee, to resurface chondral defects.[18] In the case of MACI, chondrocytes are seeded on to a type I/III collagen scaffold for implantation.[19] Both ACI and MACI have been found to be superior to microfracture for the treatment of larger articular defects.[20,21] However, the current standard for treatment of severe joint injuries and advanced OA still involves partial or total joint replacements.

New tissue engineering/regenerative medicine (TERM) strategies such as direct intra-articular delivery of progenitor cells, progenitor cell delivery on scaffolds or cell-free scaffolds coated with biological factors for recruitment of endogenous cells have been investigated for AC repair.[22–24] The use of cell-based therapeutics such as mesenchymal stem cells (MSCs) has been extensively studied for efficacy in OA.[25] MSCs are considered a favorable cell source for therapeutic applications in joint repair due to their chondrogenic differentiation capacity and paracrine effects on host cells.[26,27] Intra-articular injection of autologous MSCs in to the knee joints of patients with OA has been well tolerated and a significant reduction in pain has been observed, with paracrine anti-inflammatory activity of MSCs postulated to contribute to this outcome.[28–30] Furthermore, treatment of patients undergoing partial meniscectomy surgery with an intra-articular injection of allogeneic MSCs has resulted in meniscal regeneration as well as a significant reduction in pain.[31] To achieve successful regeneration with tissue engineering approaches in an osteoarthritic joint, an in-depth understanding is required of how OA-associated inflammation impacts chondrocyte and progenitor cell behavior. Addressing inflammatory processes in OA with TERM strategies may not only facilitate successful repair but halt the progressive destruction of an OA joint.

Inflammation in OA

Inflammation of the synovial membrane is a prominent feature of OA pathogenesis (Fig. 1), and increasing evidence highlights the role of cells of the innate and acquired immune system, and other inflammatory components, in disease progression. The innate immune system functions as a frontline of defence, involving cells such as macrophages, dendritic cells, and neutrophils, which act to recognize invading pathogens and elicit an antimicrobial and pro-inflammatory response.[32] The interaction between these antigen-presenting cells of the innate immune system and T lymphocytes is vital for the activation of T cell-dependent responses of the acquired immune system, which function to eliminate infected cells or further propagate inflammation.[33] The synovial membrane is characterized by a lining layer

containing macrophages and fibroblasts, termed synoviocytes, and $CD68^+$ macrophages and T lymphocytes, generally described as $CD3^+$ T cells, have been identified as the most abundant immune cells in infiltrates present in OA synovium.[34] An in-depth understanding of the role played by each cell type implicated in the propagation of inflammation in OA may allow for the development of therapeutic strategies for modulation of the pro-inflammatory milieu and facilitate successful tissue regeneration.

Synovitis

The synovial membrane is an area of high functional importance within the joint, responsible for nourishing chondrocytes and removing metabolites. Inflammation of the synovium results in synovitis, which is believed to reflect structural progression of OA.[35] Synovial hypertrophy and hyperplasia are associated with synovial inflammation, and synovitis may contribute to the catabolism of cartilage through pro-inflammatory mediator production. Joint injury has been reported to increase the risk of developing OA and traumatic knee meniscal injuries are associated with synovial inflammation.[36,37] Long-term coculture models of synovial tissue and cartilage explants highlight the role of activated synoviocytes in cartilage destruction associated with OA.[38] The synovial tissue was found to secrete high levels of interleukin (IL)-6, IL-8, and osteoprotegerin, and to produce cytokines throughout the culture period. Furthermore, cartilage explant glycosaminoglycan (GAG) content was found to be significantly lower following a 21-day exposure to the synovial tissue compared with cartilage monocultures.[38] It is evident that pro-inflammatory mediators produced by inflamed synovium are capable of driving the progression of OA through induction of destructive processes in cartilage (Table 1), and the abundance of such catabolic factors in an osteoarthritic joint may greatly impede cartilage tissue engineering strategies.

Synovial macrophages

Synovial macrophages, which are localized to the lining and sublining synovial layers, are considered primary cellular mediators of synovial inflammation in an OA joint.[39] These cells are capable of phagocytosis and antigen presentation, and they play a prominent role in the production of the pro-inflammatory cytokines tumor necrosis factor-α (TNF-α), IL-1β, IL-6, and oncostatin M.[38,40–42] IL-1β and TNF-α induce destructive processes in chondrocytes associated with OA pathology including downregulation of collagen type II and proteoglycan, and upregulation of MMP-9 and cyclooxygenase-2 (COX-2).[43] The enzyme COX-2 functions in the production of prostaglandins, which are known to play a role in pain and inflammation, and COX-2 expression is elevated in multiple immune cell types.[44] Moreover, IL-1β induces COX-2 and prostaglandin E2 (PGE2) expression in human chondrocytes and is associated with cartilage matrix calcification.[45]

In addition to pro-inflammatory cytokine production, macrophages secrete high mobility group box protein 1 (HMGB1) as a late mediator of inflammation following stimulation with TNF, IL-1β, and lipopolysaccharide (LPS).[46] Secretion of HMGB1 can occur in response to pro-inflammatory signals, resulting in pro-inflammatory cytokine-like activity of HMGB1, whereby it may further propagate inflammatory responses.[47] HMGB1 expression has been detected in both normal and osteoarthritic synoviocytes, and it was found to act in synergy with IL-1β to amplify the expression of pro-inflammatory mediators.[48,49] Additionally, HMGB1 has been shown to have synergistic activity on TNF, IL-8, IL-6, and MMP-3 production by OA synovial fibroblasts when added to cultures in preformed complexes with LPS and IL-1β. In addition to propagating inflammatory responses, HMGB1 may promote destructive effects in joint tissues. The release of HMGB1 by chondrocytes has been reported by Taniguchi *et al.* to act as a chemoattractant for osteoblasts and osteocytes, regulating endochondral bone formation.[50] The process of endochondral ossification is a feature of osteophyte formation in OA, and endochondral signaling resulting from HMGB1 secretion may play an additional role in progression of the disease.[51,52] Synovial macrophages have been identified as key players mediating osteophyte formation in OA, with a significant reduction in TGF-β-induced osteophyte formation observed following the depletion of synovial macrophages utilizing clondronate liposomes.[53,54]

Table 1. Inflammatory Mediators that Negatively Impact Joint Structures

Mediator	*Effects*	*References*
IL-1β	Catabolic effects on cartilage phenotype: decreases collagen type II and proteoglycan, increases PGE2, MMP-9, and COX-2	Wang *et al.*[45] Li *et al.*[12]
	Induces cartilage matrix calcification through the expression of transglutaminases	Johnson *et al.*[13]
TNF-α	Induces catabolic and pro-inflammatory enzymes: COX-2 and MMP-9	Shakibaei *et al.*[43]
	Decreases cartilage-specific gene expression: reduces expression of ECM genes and aggrecan	Dvir-Ginzberg *et al.*[10] Westacott *et al.*[11]
	Contributes to cartilage matrix degradation and focal loss of cartilage	
TGF-β	Induces osteophyte formation	Scharstuhl *et al.*[138]
	Associated with changes in subchondral bone architecture	Zhen *et al.*[139]
IL-6	Downregulation of collagen type II and aggrecan in articular chondrocytes	Legendre *et al.*[9]
HMGB1	Amplifies expression of pro-inflammatory mediators	Yang *et al.*[47]
	Synergistic activity with IL-1β on amplifying IL-6, IL-8, MMP-1, and MMP-3 by synoviocytes	Garcia-Arnandis *et al.*[48]

COX-2, cyclooxygenase-2; ECM, extracellular matrix; HMGB1, high mobility group box protein 1; IL, interleukin; MMP, matrix metalloproteinases; PGE2, prostaglandin E2; TNF-α, tumor necrosis factor-α.

Synovial fibroblasts

Inflammatory mediators produced by synovial macrophages may induce activation and further pro-inflammatory mediator production by neighboring synoviocytes. OA synovial fibroblasts have been reported to produce IL-6, IL-8, macrophage colony stimulating factor, and vascular endothelial growth factor (VEGF) in response to IL-1β stimulation.[55] Furthermore, the AC pericellular matrix protein Laminin-111 may bind to and activate synovial fibroblasts in the presence of TGF-β1, which was found to result in IL-6 and IL-8 secretion, a mechanism by which synovial fibroblasts may contribute to disease pathology outside periods of acute inflammation.[56,57] Synovial fibroblasts also upregulate expression of vascular cell adhesion molecule-1 (VCAM-1) in response to chemokine (C-C motif) ligand 2 (CCL2) and CCN family member 4, both of which are detected in synovial fluid of patients with OA. This process may facilitate the adhesion of mononuclear cells to the site of inflammation.[58,59] These findings indicate that synovial fibroblasts may contribute to synovial inflammation through propagating inflammatory responses following activation by pro-inflammatory mediators, or by facilitating the adhesion of infiltrating immune cells. However, the contribution of synovium to the progression of OA appears to be primarily mediated by inflammatory cells including resident macrophages and infiltrating immune populations.

Mast cells

Mast cells are also considered to play a role in contributing to inflammation associated with OA. Elevated mast cell counts in the synovial fluid of patients with OA have been reported, with higher levels of histamine, tryptase, and nitrite detected in OA synovial fluid.[60,61] Furthermore, a role for TNF-α in the induction of mast cell chemotaxis in OA has been elucidated.[62] Studies have suggested that the observed increase of mast cells in OA may be due to the expansion of a tryptase but not chymase containing population, with an increase in this population reported in the synovial tissue of OA patients compared with control.[63] The serine protease tryptase is released from mast cells upon degranulation along with other products including heparin, histamine, and many proteases.[64] Tryptase can induce IL-8 release and expression of the adhesion molecule intercellular adhesion molecule 1 (ICAM-1) by epithelial cells and upregulate IL-8 and IL-1β gene expression in endothelial cells, suggesting a potential role of tryptase in the propagation of inflammatory responses through cellular recruitment.[65,66]

The complement system

The complement system is an essential component of the innate immune system, functioning to eliminate macromolecules and foreign bodies by opsonization or cell lysis.[67] A role of complement activation in the pathogenesis of OA has been elucidated, with increased gene expression of complement factors in OA synovium reported.[68] This group pursued studies utilizing a medial meniscectomy model of OA with mice genetically deficient in complement components C5, C6, and CD59a, to reveal that the membrane attack complex of the complement pathway was involved in the development of the disease. Proteomic studies identified differential expression of complement proteins in human OA synovial fluid compared to control, further suggesting a potential role of these proteins in catabolic processes associated with OA.[69]

T cells

Infiltrating $CD3^+$ T cells have been detected in the synovial perivascular area of patients with early stage disease.[70] Furthermore, T cells expressing antigens of early, intermediate, and late activation have been detected in the synovium of patients with advanced OA.[71] $CD4^+$ effector T cells may be classified according to several subsets, with varying immunological functions associated with their characteristic cytokine expression profile. Cells of the T helper 1 (Th1) subset are associated with production of IL-2 and interferon (IFN)-γ, and can establish cell-mediated inflammatory responses such as macrophage and cytotoxic T-cell activation, which functions to clear intracellular pathogens.[72,73] Conversely, cells of the T helper 2 (Th2) subset express IL-4, IL-5, and IL-10, resulting in increased antibody production, and elimination of parasitic infections.[73–75] Detection of $CD4^+$ effector T cells and CD8 + cytotoxic T cells, predominantly in the sublining layer of synovium from patients with OA has been reported.[76] Furthermore, increased levels of IFN-γ and IL-2 were detected compared with IL-4, suggesting a prevalence of Th1 cells.[71,76] In additional studies, infiltration of $CD4^+$ effector T cells and macrophages were found in association with increased expression levels of VEGF and abundant ICAM-1 expression in early OA synovial tissue.[40] VEGF is expressed by macrophages and known to induce endothelial cell chemotaxis and mediating vascular permeability, an important process in inflammation.[77–79] Interestingly, intra-articular injection of VEGF in mice has increased calcification of AC, cartilage degradation, and subchondral bone sclerosis, implicating a role of VEGF in OA.[80] ICAM-1 is upregulated by osteoarthritic synoviocytes in response to pro-inflammatory stimuli and plays an important role in transendothelial migration of T cells and may therefore mediate the migration and infiltration of lymphocytes in the synovium.[81,82]

Using $CD4^+$ T cell depletion in a murine anterior cruciate ligament-transection (ACLT) model for induction of OA, Shen *et al.* elegantly identified a role for $CD4^+$ T cells in the pathogenesis of the disease.[83] Increased levels of infiltrating $CD4^+$ T cells were evident in the synovium and localized throughout the membrane 30 days after ACLT alone. Increased synovial expression of the chemokine macrophage inflammatory protein (MIP)-1γ was detected in the ACTL group compared with the $CD4^+$ T cell depleted ACLT model. Furthermore, T-cell depletion was found to correlate with increased levels of IL-4 and tissue inhibitor of metalloproteinase-1. T cells may further play a role in OA disease progression and contribute to inflammation through the recognition of cartilage matrix molecules, with aggrecan epitopes previously identified as targets of self-reactive T cells in patients with OA.[84]

Current Pharmacological Agents Targeting Inflammation in OA

Current anti-inflammatory therapies recommended for the management of knee and hip OA include COX-2 selective and

nonselective nonsteroidal anti-inflammatory drugs (NSAIDs).[85] Although these pharmacological agents have been reported to provide analgesia and reduce inflammation following short-term treatment,[86] long-term use is associated with adverse side effects, including gastrointestinal toxicity and increased risk of cardiovascular events.[87,88] Furthermore, Reijman *et al.* have reported that NSAIDs use may increase progression of OA,[89] and certain NSAIDs have been found to negatively impact collagen metabolism and GAG synthesis.[90,91] Although administration of NSAIDs and COX-2 inhibitors provide symptomatic relief, the ability of these agents to limit disease progression remains to be seen.

Novel pharmacological agents have been developed to target inflammatory mediators associated with OA pathology. A TNF-α-binding antibody, Adalimumab, has been reported to decrease the progression of structural damage in inflamed joints of patients with erosive hand OA, however, an effect on inflammation was not observed.[92] Other groups have found this TNF-α antagonist to provide symptomatic relief for patients with knee OA, including an improvement in pain and swelling.[93] However, the ability of this therapy to slow down the development of structural damage in the OA knee requires further investigation. Additional studies performed to target pro-inflammatory cytokines associated with OA pathogenesis have generated mixed results. Symptomatic efficacy of the IL-1 inhibitor Diacerein for treatment of knee and hip OA has been reported.[94,95] However, no significant improvement in symptoms was observed following intra-articular injection of a recombinant form of IL-1-receptor antagonist (IL-1RA) to patients with knee OA.[96] It appears that the potential of current anti-inflammatory therapies to improve structural damage is limited, and the development of novel immune modulation strategies is required to alter the progression of OA.

The Effect of Inflammation on Tissue Engineered Cartilage

As previously discussed, TERM-based strategies may represent the key to improved cartilage regeneration both in isolated defects and treatment of OA. However, it is evident that inflammation plays a significant role in OA pathogenesis and the immune response is increasingly recognized as a key factor influencing tissue regeneration. For example, disorders such as diabetes mellitus that are associated with an inflammatory state are also characterized by impaired tissue regeneration.[97] TERM strategies for cartilage regeneration may be compromised as a result of dysregulated inflammatory processes and pro-inflammatory mediators in the joint. IL-1β has been reported to reduce collagen type 2 expression and GAG content of human nasal and articular chondrocytes cultured on a type 1 collagen scaffold,[98] and both IL-1β and TNF-α have been shown to impact the integration of engineered cartilage with native tissue.[99] Additionally, these pro-inflammatory cytokines have been shown to inhibit the migratory potential of chondrogenic progenitor cells in osteoarthritic cartilage,[100] which may limit the success of strategies utilizing cell-free scaffolds for recruitment of endogenous cells and *in situ* cartilage regeneration.[24]

In addition to directly impacting engineered cartilage and endogenous cellular repair, inflammation may also hinder stem cell-based repair strategies. Previous studies have reported a detrimental effect of IL-1β and TNF-α on chondrogenic differentiation of MSCs in aggregate culture.[101] Furthermore, IL-1α treatment of chondrogenically differentiating MSCs seeded on a 3D woven poly(ε-caprolactone) scaffold, has been shown to decrease accumulation of ECM components and reduce the mechanical properties of the construct.[102] In addition to these findings, OA synovium-conditioned medium and synovial fluid have been shown to inhibit MSC chondrogenesis.[103,104] It is evident that modulation of the inflammatory environment in an OA joint is vital to maintain the integrity of engineered cartilage, or achieve efficient cell-based repair.

Tissue Engineering Strategies for Immunomodulation in OA

Given that the inflammatory milieu may determine the success of tissue regeneration strategies, regulation of inflammatory processes in the OA joint is required to achieve successful cartilage repair. TERM-based strategies such as the use of biomaterials with immunomodulatory capacity, the delivery of cells and anti-inflammatory proteins, or gene therapy approaches may serve as favorable strategies for gaining control over the pro-inflammatory environment present in OA and subsequently interfere with the disease process.

Biomaterials

The use of biomaterials as a scaffold for the delivery of cells or proteins with immunomodulatory capacity may serve as an efficient strategy to attenuate disease progression in OA and facilitate successful tissue regeneration. However, implantation of biomaterials may induce local tissue injury and inflammation.[105] Studies performed by Grotenhuis *et al.*, have assessed the effect of different biomaterials on macrophage responses *in vitro*, and found that biomaterials alone have the ability to influence macrophage inflammatory phenotype.[106] Additionally, nanocrystalline hydroxyapatites have been reported to increase pro-inflammatory cytokine production by macrophages, indicating activation of the innate immune system.[107] In light of these findings, it is apparent that the interaction of biomaterials with immune cells, such as macrophages, needs to be carefully considered to avoid exacerbating existing inflammation. Anti-inflammatory α-melanocyte-stimulating hormone coated poly (D, lactic-co-glycolic) acid microspheres have been reported to modulate biomaterial-induced inflammation following subcutaneous implantation in rats.[108] However, whether this approach would be beneficial in the context of a chronic inflammatory environment requires further investigation.

Cell therapy

Stem cells such as MSCs are considered a promising cell type for OA cell therapy and cartilage tissue engineering due to their potential to differentiate and replace chondrocytes, as shown using muscle-derived stem cells modified to express BMP-4 and an inhibitor of angiogenesis.[27,109] However, as previously discussed the presence of an inflammatory environment may impede the use of MSCs in cell replacement strategies. Alternatively, paracrine effects of MSCs may act

to recruit endogenous progenitor cells or be immunomodulatory.[110] Trophic factors released by MSCs in response to pro-inflammatory cytokine stimulation have been reported to reduce the expression of IL-1β, MMP-1, and MMP-13 by OA synovium explants, indicating an anti-inflammatory and anti-catabolic effect of MSCs.[111] Furthermore, MSCs exert a suppressive effect on activated immune cells and studies investigating the suppressive effect of MSCs on T-cell proliferation have reported a major role of the anti-inflammatory cytokine IL-10 in the suppression of T-cell activation.[112,113] An immunosuppressive effect of MSCs on mast cells has been demonstrated following coculture experiments, with a decrease in mast cell degranulation, TNF-α production and chemotaxis observed.[114] Macrophages exhibit a high degree of plasticity, with the potential to change phenotype according to environmental cues. They can be categorized as classically activated (M1), which produce high levels of pro-inflammatory cytokines or alternatively activated (M2) macrophages, which are associated with anti-inflammatory properties and play a role in wound healing and immune regulation.[115] Coculture studies have been performed with MSCs and macrophages to investigate the immunomodulatory capacity of MSCs on macrophage phenotype. MSC-treated macrophages were reported to express the M2 macrophage marker CD206, with increased expression of IL-10, and low expression of the M1-associated cytokines IL-12 and TNF-α.[116,117] Furthermore, transplanted collagen scaffolds seeded with MSCs and polymer complexed-IL-10 plasmids have resulted in macrophage polarization, with an observed increase in $CD63^+$ M2 polarized and a decrease in $CD80^+$ M1 polarized macrophages.[118] Additionally, delivery of MSCs with IL-10 polyplexes reportedly increased the retention rate of MSCs *in vivo*, which was associated with increased IL-10 levels and decreased pro-inflammatory cytokines.

Intra-articular injection of MSCs has been reported to inhibit the development of posttraumatic arthritis in an intra-articular fracture mouse model, with an increase in systemic IL-10 levels observed.[119] Moreover, intra-articular injection of adipose-derived stem cells (ASCs) has been shown to reduce synovial lining thickness and decrease cartilage damage in a collagenase-induced OA mouse model.[120] ASCs were identified in close proximity to synovial macrophages and reduced IL-1β gene expression levels were detected in synovial tissue, indicating immunomodulatory activity of these stem cells within the OA joint. In addition to this study, Desando *et al.* have observed reduced synovial lining thickness and cellular infiltration, and decreased cartilage expression of TNF-α following intra-articular injection of ASCs in a rabbit ACLT model.[121] In light of these findings, the use of MSCs or ASCs may serve as a suitable cell therapeutic strategy to attenuate inflammation in OA via paracrine mechanisms and through the modulation of activated immune cells, which contribute to disease pathogenesis.

The use of induced pluripotent stem cells (iPSCs) may provide an additional cell source for cartilage tissue engineering.[122] iPSCs generated from the reprogramming of mouse fibroblasts have been successfully differentiated toward the chondrogenic linage, and shown to integrate with native cartilage tissue and produce cartilage matrix in an *in vitro* cartilage defect model.[123] Furthermore, chondrogenic differentiation of MSC-like cells derived from human iPSCs has been shown by Guzzo *et al.* to result in a cellular phenotype that more closely resembles articular chondrocytes, compared with undifferentiated iPSCs.[124] iPSC-derived MSCs inhibit lymphocyte proliferation and suppress Th2-associated cytokine production in a similar manner to MSCs, and have been found to elicit a comparable immunomodulatory effect in a mouse model of allergic inflammation.[125,126] Given that MSCs are associated with an age-related reduction in proliferation and differentiation capacity, iPSC-derived MSCs may serve as a useful cell source for large-scale generation of MSCs for cell therapy approaches in OA.[127]

Anti-inflammatory protein delivery

Anti-inflammatory IL-10 has the ability to suppress pro-inflammatory mediator production by activated macrophages.[128] Various studies on immune modulation by MSCs have implicated IL-10 as an important mediator.[113] Interestingly, synergistic activity of IL-4 with IL-10 has been previously reported.[129] Recent studies have demonstrated a protective effect of IL-4 in combination with IL-10 on blood-induced cartilage damage compared to IL-10 treatment alone.[130] The use of these factors may be beneficial to limit cartilage damage resulting from joint bleeding during surgical procedures for treatment of OA-related defects. A study utilizing a rat model of instability-induced experimental OA has reported that IL-4 may have a chondroprotective effect in mechanical stress-induced OA through suppressing nitric oxide production by chondrocytes and preventing cartilage destruction following intra-articular injection.[131] The use of biomaterial scaffolds for localized delivery of anti-inflammatory proteins may be a beneficial strategy to antagonize pro-inflammatory mediator production in an osteoarthritic joint, and subsequently counteract the negative influence of pro-inflammatory mediators on cartilage regeneration.

Gene delivery

Gene therapy approaches for the treatment of cartilage defects in OA have been previously examined. For example, the potential of overexpression of the cartilage transcription factor SOX9 by MSCs for AC repair has been evaluated, utilizing a rabbit full-thickness cartilage defect model.[132] Improved integration of newly formed tissue with native cartilage and positive staining for type II collagen was reported following implantation of transduced MSCs seeded on a polyglutamic acid scaffold. The therapeutic efficacy of inflammatory mediator overexpression in an OA environment has also been evaluated. A decrease in gross pathologic abnormalities was observed following adenoviral vector-mediated IL-1RA gene delivery, in an equine experimental OA model.[133] The effects of retrovirally delivered human IL-1RA and IL-10 have also been assessed following injection in to the knee joint in a rabbit model of OA.[134] Intra-articular expression of these genes was found to have a chondroprotective effect with an evident reduction in cartilage degradation. Administration of both IL-1RA and IL-10 was reported to have a greater effect, compared with administration of either gene alone. The delivery or overexpression of anti-inflammatory mediators via gene therapy approaches may therefore offer a novel therapeutic strategy for the treatment of OA, through attenuating the destructive effect of inflammation on cartilage (Table 2).

TABLE 2. PRECLINICAL STUDIES UTILIZING GENE DELIVERY WITH THERAPEUTIC POTENTIAL FOR TREATMENT OF OSTEOARTHRITIS

Gene	*Vector*	*Delivery method*	*Disease model*	*Species*	*Effect*	*Reference*
SOX9	Adenovirus	Intra-articular delivery of transduced rabbit bone marrow MSCs	Full-thickness cartilage defect	Rabbit	Induction of MSC chondrogenesis *in vitro* Newly formed cartilage tissue *in vivo*	Cao *et al.*[132]
IL-1RA	Adenovirus	Direct vector intra-articular injection	Osteochondral fragment exercise model	Horse	Decrease in gross pathologic abnormalities	Frisbie *et al.*[133]
IL-1RA	Plasmid DNA vector	Direct intra-articular injection of plasmid	Partial medial meniscectomy model	Rabbit	Reduced progression of OA	Fernandes *et al.*[135]
IL-1RA	Retrovirus	Intra-articular injection of *ex vivo* transduced synoviocytes	Anterior cruciate ligament resection model	Dog	Reduction of cartilage lesions and reduced progression of OA	Pelletier *et al.*[136]
IL-1RA & IL-10	Retrovirus	Intra-articular delivery of transduced rabbit synoviocytes	Excision of the medial collateral ligament plus medial meniscectomy	Rabbit	Chondroprotective effect Reduced cartilage degradation	Zhang *et al.*[134]
TGF-β1	Retrovirus	Intra-articular injection of transduced human chondrocytes	Full-thickness cartilage defect model	Goat	Increased chondrocyte proliferation and deposition of collagen type II	Noh *et al.*[137]

IL-1RA, IL-1-receptor antagonist; MSCs, mesenchymal stem cells.

Conclusion

Modifying the intra-articular environment in OA through the attenuation of destructive processes affecting cartilage and synovium would offer great therapeutic benefit. Synovial inflammation is a key player in the pathogenesis of OA and is highly associated with cartilage destruction and disease progression. Furthermore, infiltrating inflammatory cell populations play a key role in propagating inflammatory responses. Modulation of synovial inflammation has been shown to impact clinical symptoms and prevent structural damage. The immune response is increasingly recognized as a key factor influencing tissue regeneration, and gaining control of the pro-inflammatory environment associated with OA is vital to achieve efficient tissue repair with TERM-based strategies and halt disease progression. Combining cell-based therapy using MSCs with anti-inflammatory genes to deliver immunomodulatory factors *in vivo*, may serve as a favorable strategy to attenuate destructive inflammatory processes and promote regeneration of cartilage defects in an OA joint.

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