[Frontiers In Bioscience, Landmark, 23, 318-347, January 1, 2018]

Strategies for reducing inflammation and promoting bone repair in arthritis

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1. ABSTRACT

Chronic forms of arthritis encompass many joint inflammatory disorders, including rheumatoid arthritis (RA), an autoimmune inflammatory disease, and osteoarthritis (OA), typically a 'wear and tear' condition that is now known to also have an inflammatory etiology. The impact of inflammation in the disease prognosis and joint degradation due to impaired repair mechanisms has long been recognized for RA, and now also for OA. Both forms of arthritis are prevalent chronic health conditions, and despite recent advances, their treatment still represents an unmet medical need because of safety and efficacy concerns with currently prescribed drugs. There is an urgent need to develop and test new drugs that selectively target inflamed joints and to control articular inflammation while preventing healthy tissue damage. The therapeutics developed for RA might be useful for OA, since studies in humans and animal models demonstrate a key role for chronic, low-grade

inflammation in the pathogenesis of OA. In this review, we discuss current and emerging new therapies for management of inflammation and promotion of cartilage and/or bone repair in RA and OA.

2. INTRODUCTION

Chronic, non-infectious arthritis encompasses many inflammatory disorders affecting the joints. The most common forms of chronic, non-infectious arthritis are rheumatoid arthritis (RA), an autoimmune inflammatory disease, and osteoarthritis (OA), typically a 'wear and tear' form of arthritis but that now is known to also have inflammatory etiology. Both forms of arthritis are prevalent chronic health conditions, and despite recent advances, their treatment still represents an unmet medical need because of safety and efficacy concerns with currently prescribed drugs. Therefore, there is an urgent need to develop and test new drugs for RA and OA that selectively target inflamed joints thereby mitigating damage to healthy tissues and to control articular inflammation while circumventing collateral damage to healthy tissues. Synovitis, indicated by synovial hyperplasia and low-grade inflammatory infiltrates within the synovial lining, is frequently observed in OA (reviewed in (1)). The inflammation in OA is chronic, low-grade, and differs in its clinical presentations and mechanisms from the 'high-grade' inflammation in RA. Instead, inflammation in OA involves the interplay of the innate immune system and inflammatory mediators, offering opportunities for developing disease-modifying OA drugs like the ones that exist for RA. The discoveries and therapeutics developed for RA might to some extent be useful for OA, since studies in humans and animal models demonstrate a key role for chronic, low-grade inflammation in the pathogenesis of OA. Also, innate immune pathways, such as the complement and pattern-recognition receptor pathways, are pivotal to the inflammation in OA (reviewed in (1)). But clinical trials will be necessary to ultimately determine whether anti-inflammatory therapeutics can prevent or slow disease progression in OA.

The impact of inflammation in the disease prognosis and joint degradation due to impaired repair mechanisms by the cartilage and bone has long been recognized for RA, and more recently also for OA. There appears to be a necessity for halting inflammation in order to fully promote joint repair, as the inflammatory process inhibits proper repair by bone, stromal and/or cartilage cells. Types of joint destruction differ between RA and OA. Bone loss has been recognized as a complication of RA and presents in two different forms: as juxta-articular osteoporosis around inflamed joints, which is a radiological characteristic of early RA, and generalized osteoporosis, which is one of the most common extra-articular manifestations of the disease (2). Marginal erosions secondary to the loss of cartilage and subchondral osteolysis may develop later in the course of the disease and are included in the classification criteria for RA. We will discuss the current interventions for treating inflammation and/or promoting joint tissue repair, and will also present some innovative emerging strategies for future clinical application in the treatment of inflammation and arthritis.

3. INTERVENTIONS FOR TREATING INFLAMMATION AND PROMOTING OR FACILITATING JOINT TISSUE REPAIR.

3.1. Pathology of RA and major current interventions

A major clinical manifestation of RA is the progressive destruction of bone and cartilage in the joints of patients, leading to bone erosion and joint spaces narrowing that are visible by radiography. Typical pathology in the synovium of RA patients includes a thickened hyperplastic synovial layer, neoangiogenesis, and ongoing migration of macrophages and autoreactive lymphocytes into joints resulting from the action of chemokines and proinflammatory cytokines (Figure 1). These inflammatory processes are known to activate bone and cartilage destruction through pathways that include tumor necrosis factor (TNF) and receptor activator of NF-κB ligand (RANKL)-mediated signaling. RANKL and TNF pathways act to enhance bone resorption by stimulating osteoclastogenesis and also by activating synovial fibroblasts. In the case of RA, the osteoblast-osteoclast axis is severely disrupted due to ongoing inflammation, which results in enhanced osteoclast function. Key macrophage and T cell derived cytokines such as TNF, interleukin (IL)-1β, IL-6, and IL-17 act to induce expression of RANKL by synovial fibroblasts, osteoblasts and bone marrow stromal cells, leading to enhanced osteoclast differentiation (3). Additionally, several factors expressed in the RA joint lead to functional inhibition of osteoblasts, and joint repair is compromised, even in the presence of clinical remission of inflammation (4).

Therapeutic interventions in RA have targeted various aspects of the pathways in Figure 1a, including corticosteroids, methotrexate (MTX), anti-TNF agents, IL1b and IL-6 pathway blockage, B-cell depletion via targeting cluster of differentiation (CD) 20, and blockade of lymphocyte co-stimulation via cytotoxic T lymphocyte antigen 4 (CTLA4) (reviewed in (3)). Biphosphonates have also been used, but not all patients respond with respect to bone erosion repair. Therefore, in addition to targeting synovitis, it is important to find strategies that can stop and ultimately reverse bone erosion in RA. The rebalancing of bone cells requires restoring proper communication and function to osteoblasts, and reducing osteoclast

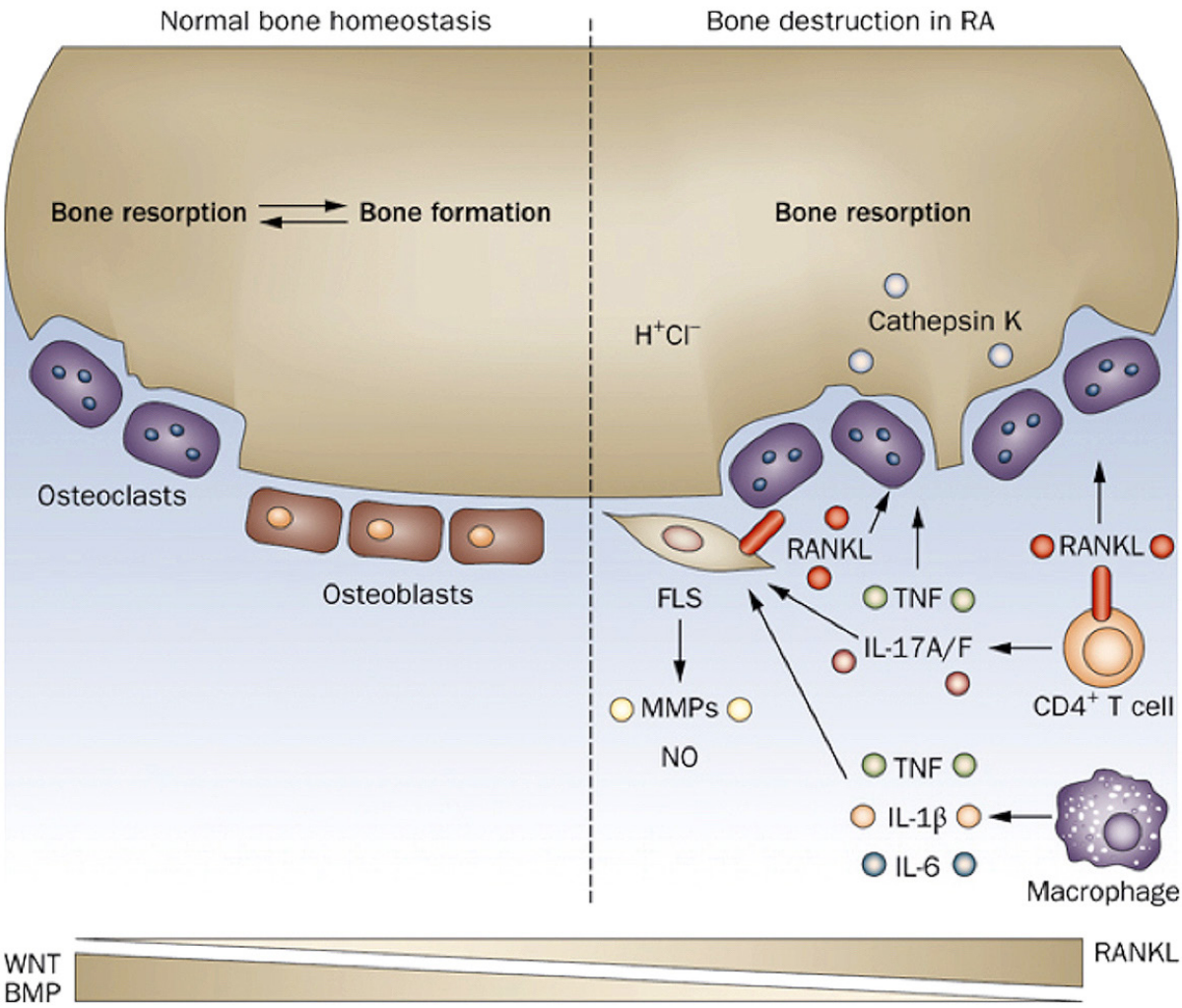

Figure 1. Signaling and cells involved in the pathology in rheumatoid arthritis joints. In normal joints, bone formation and bone resorption are maintained by the balanced or homeostatic function of osteoblasts and osteoclasts. The molecular basis is controlled in part by the opposing actions of Wnt and BMP pathways on osteoblasts and the RANKL pathway on osteoclasts. Under the inflammatory conditions of rheumatoid arthritis (RA), activity of infiltrating macrophages and $CD4^+$ T cells results in expression of proinflammatory cytokines, such as TNF, that drive osteoclast formation via induction of RANKL in the synovium. In addition, RANKL is expressed on synovial fibroblasts and infiltrating T cells. The resulting osteoclasts, and associated local production of H^+ ions and cathepsin K, lead to increased bone resorption and joint destruction. Abbreviations: BMP, bone morphogenetic protein; FLS, fibroblast-like synoviocyte; IL, interleukin; MMPs, matrix metalloproteinases; NO, nitric oxide; RA, rheumatoid arthritis; RANKL, receptor activator of nuclear factor kB ligand; TNF, tumor necrosis factor. Reproduced with permission from (3).

differentiation. Examples of endogenous inhibitors that impair osteoblast function are dickkopf1 (DKK1), an inhibitor of WNT signaling in osteoblasts, and sclerostin (SOST), which inhibits both wingless-type MMTV integration site family (WNT) and bone morphogenic protein (BMP) pathways in osteoblasts. It appears that therapeutics in development would have a greater chance of impacting joint repair if they can inhibit the RANKL axis and reduce SOST and/or DKK1 signaling in the RA joint.

Several therapeutic agents aim to disrupt the communication between the inflammatory cells and bone cells within the RA joint. The osteoimmunology of RA includes the presence of CD4 T cells, which are responsible for initiating and perpetuating RA (Figure 1b) (2). CD4 T cells inappropriately recognize autoantigens on the surface of antigen-presenting cells. This process can be inhibited by abatacept, which interrupts the CD28–CD80/86 co-stimulatory pathway, a mechanism shown to reverse osteoclastogenesis via cytotoxic T-lymphocyte associated protein 4 (CTLA4). Activated T cells release cytokines that directly activate synovial macrophages and B cells. The former produce large amounts of proinflammatory cytokines (TNF, IL-6, IL-1β), which stimulate the expression of RANKL from osteoblasts and synovial fibroblasts and mediate osteoclast differentiation through RANKL-dependent

and independent pathways. Blockade of these pathways reduces the degree of immune response within the joint with direct and indirect beneficial effects on bone metabolism. Depletion of activated B cells with rituximab compromises antigen presentation, production of autoantibodies and formation of immune complexes. Another agent, denosumab, can bind to RANKL and blocks the maturation of pre-fusion osteoclasts to multinucleated osteoclasts. This effectively inhibits the activation, development and fusion of mature cells in the bone microenvironment.

Corticosteroids and non-biologic disease-modifying anti-rheumatic drugs (DMARDs) low-dose use has been associated with increased bone loss and fracture risk in RA, but on the other hand their use effectively dampens inflammation. There are beneficial effects in early RA (2). Low-dose steroids also can have a synergistic effect with the TNF-alpha antagonist, adalimumab, accounting for 18.5.% of the improvement observed in femoral neck bone density in 50 patients with active RA (5). A recent study showed that steroids are associated with a lower risk of vertebral fractures in RA, further supporting the view that appropriate control of disease may protect against bone fragility (6). High-dose MTX has been linked with bone loss in patients with malignant diseases but it does not seem to impair bone formation in RA patients treated with low-dose MTX (~10 mg/week) (7) but contradictory results have been reported (8).

Novel anti-cytokines can suppress systemic inflammation and also somewhat halt systemic bone loss (reviewed in (2)). TNF blockers have shown beneficial effects on reducing inflammation and joint degradation by achieving high rates of sustained clinical remission. In the collagen-induced arthritis (CIA) model, TNF blockade reduces bone resorption and prevents bone mineral density reduction in parallel with improvement in disease activity scores (9). Several studies have assessed the effect of TNF blockade on bone metabolism but there is not sufficient data available on bone yet. Recent studies suggest that the risk for nonvertebral fractures is the same amongst patients with RA treated with TNF inhibitors, MTX or other non-biologic DMARDs (10). For example, a study with 102 patients with a 12 month assessment showed that clinical remission following treatment with infliximab was associated with an arrest in generalized bone loss, which however was not accompanied by improvement of localized hand bone loss (11). The impact of TNF inhibition on altering biochemical markers of bone turnover also has provided conflicting results. One study has shown a transient (not sustained after 12 months) decrease in bone turnover indices in the first 6 weeks (12), whereas other studies reported a persistent improvement both in markers of bone resorption and formation (reviewed in (2)). In summary, TNF inhibition appears to have at least a short-term positive effect on bone remodeling in RA.

IL-6 is a proinflammatory cytokine capable of eliciting a broad spectrum of inflammatory events involved in the onset and progression of RA, such as stimulation of synoviocyte proliferation, recruitment of inflammatory cells, and promotion of joint bone and cartilage erosion. Tocilizumab is a humanized anti IL-6 receptor monoclonal antibody which has been effective in reducing clinical signs and systemic inflammation in RA, in particular decreasing joint swelling and controlling the acute phase response (13). Tocilizumab can reduce structural joint damage (14), suggesting that inhibiting IL-6 blocks osteoclastogenesis independent of its anti-inflammatory properties. Additionally, a rapid and sustained improvement in bone metabolism has been shown following the initiation of tocilizumab and MTX combination treatment in 416 patients with active RA (15). The authors concluded that pharmacological blockade of IL-6 attenuates local and systemic bone loss predominantly (but not exclusively) in clinical responders, dissociating the tight link between disease activity and bone turnover in RA. Tocilizumab may also improve coupling of bone formation and resorption by regulating the wingless signaling pathway. Histological changes of bone marrow in response to tocilizumab in 10 RA patients who underwent total knee replacement have shown increased OPG expression in the bone marrow of patients treated with tocilizumab (16). IL-6 blockade also promotes a decrease of circulating osteoblast inhibitor DKK1 and normalizes osteoprotegerin (OPG)/RANKL ratio just after 2 monthly infusions (17), suggesting a positive influence of tocilizumab on bone formation through modulation of the WNT pathway.

Despite the success of biologics in inhibiting proinflammatory cytokines, a significant part of RA patients remain non-responders and/or cannot tolerate such treatment due to severe side effects (18). Therefore, there has been attention to developing other approaches targeting lymphocytes, such as rituximab, a chimeric anti CD20 antibody that eliminates B-lymphocytes and induces their apoptosis. In the context of RA, the number of Ig-producing cells is drastically reduced, diminishing the synthesis of rheumatoid factors or anti-citrullinated protein antibodies and leading to the inhibition of T to B cell activation process. Rituximab decreases the number of synovial osteoclast precursors and increases OPG/RANKL ratio in the serum of RA patients. Improvement in bone density following rituximab is more pronounced in clinical responders but the findings should be interpreted cautiously due to the small number of patients, the relatively short period of follow-up and the absence of appropriate controls (19).

T cell-mediated processes are central to the initiation and perpetuation of RA. Abatacept (CTLA4-Ig), is a recombinant soluble fusion protein that blocks the co-stimulation of T cells by inhibiting the CD28–CD80/

CD86 pathway between T cells and antigen presenting cells. Abatacept is currently licensed as an alternative treatment in patients failing to respond to other biologic agents. Recent insights have revealed that the binding of Cytotoxic T-Lymphocyte Associated Protein 4 (CTLA-4) to the CD80/CD86 molecule on the surface of osteoclast precursors restrains their maturation and development, suggesting an antiresorptive effect of abatacept in RA (20). In preclinical studies, abatacept prevented parathyroid hormone (PTH)-induced bone loss (21) but the outcome of T cell targeting therapy on bone mineral density has not been investigated yet.

Finally, desonumab, a humanized antibody that specifically binds RANKL, inhibits the radiographic progression of erosive bone damage and increases the bone mineral density in the hands after 12 months of treatment (22). Desonumab also prevents metacarpal cortical bone loss in patients with erosive RA treated with MTX (23). Denosumab increases bone mineral density in the lumbar spine and the hip and reduced markers of bone turnover after 6 months, regardless of the concomitant administration of steroids and/or biphosphonates. The therapeutic role of denosumab is promising, since TNF inhibition has failed to arrest metacarpal bone loss. Whether co-administration of denosumab with other biologic regimens such as TNF inhibitors will have a better outcome in reversing the impact of systemic inflammation on bone hemostasis and structure in RA patients remains to be determined.

3.2. Pathology of OA and major current interventions

Because of the heterogeneity of OA and the large number of molecules involved in its pathogenesis, effective treatment requires a combination of approaches. Only recently it has been appreciated that the low-grade inflammation in OA has an important role in the pathogenesis of this condition. Several inflammatory pathways and mechanisms contribute to the pathogenesis of OA (Figure 2a). In the OA joint, acute, subacute, or chronic injuries and joint tissue breakdown can trigger cycles of progressive tissue damage, failed repair, and inflammation, resulting in cartilage loss and progressive joint degeneration over time (reviewed in (1)). During joint degradation, the innate immune system recognizes features in tissue damage products via pattern recognition receptors such as damage associated molecular patterns (DAMPs). DAMPs are endogenous molecules that signal to innate immune cells (for example, macrophages and mast cells) to trigger a protective response. Once activated, the innate immune system produces an array of inflammatory mediators that normally would initiate immune responses leading to tissue repair. However, prolonged activation of inflammation can be destructive, and promotes the chronic inflammation seen with OA. When inducing inflammation, one of the main classes of pattern-recognition receptors bound by DAMPs are the Tolllike receptors (TLRs). Stimulation of TLRs ultimately leads to activation of inflammatory transcriptional programs via transcription factors such as interferon regulatory factors, nuclear factor B (NF B), and activator protein 1 (AP1) (24). TLR activation has been implicated in the development of synovitis, degeneration of cartilage, and susceptibility to OA. TLR1–7 and TLR9 have been detected in the synovium of individuals with OA or RA (25).

The complement system is another innate immune mechanism by which the body recognizes pathogens, and its activation leads to chemotaxis, exudation of plasma proteins at inflammatory sites, and opsonization of infectious agents and damaged cells. Levels of antibodies and immune complexes also are abnormally high in OA joints, and the immune complexes likely follow antibody targeting of neoepitopes exposed during cartilage degeneration processes. Antibody complexes might activate innate immune responses and worsen inflammatory responses but further investigation is needed to better define the role of antibodies and of the adaptive immune response in OA. Many other factors are upregulated in the OA joint, including cytokines and chemokines, growth factors, adipokines, prostaglandins and leukotrienes, nitric oxide (NO), and neuropeptides (Figure 2a).

Now that low-grade inflammation is appreciated as critical in the pathogenesis of OA, novel anti-inflammatory therapeutics may be useful for treatment. Although lifestyle changes involving exercise and/or weight loss are simple and sometimes effective ways of slowing the progression of OA, such regimens are difficult to implement in elderly individuals or those with limited mobility. Currently, only symptom modifying agents, such as analgesics, nonsteroidal anti-inflammatory drugs (NSAIDs), steroids, and hyaluronic acid, have been approved as therapeutic interventions for OA. The ultimate treatment appears to be joint replacement (26). There are no approved treatments able to restore the original structure and function of the damaged joint, nor can they slow the progression of OA. Moreover, these medications frequently have deleterious adverse effects. Thus a substantial need remains for safe and effective disease modifying OA drugs (DMOADs), and several other biologicals might soon be in the horizon for OA.

Inhibition of the low grade inflammation in OA could form the basis of DMOADs. Because inflammation is present early in the course of OA, before structural changes have occurred, therapeutic strategies targeting the low grade inflammatory processes might be able to halt disease progression and to prevent the onset of radiographic OA. Several antiinflammatory therapeutics have been tested

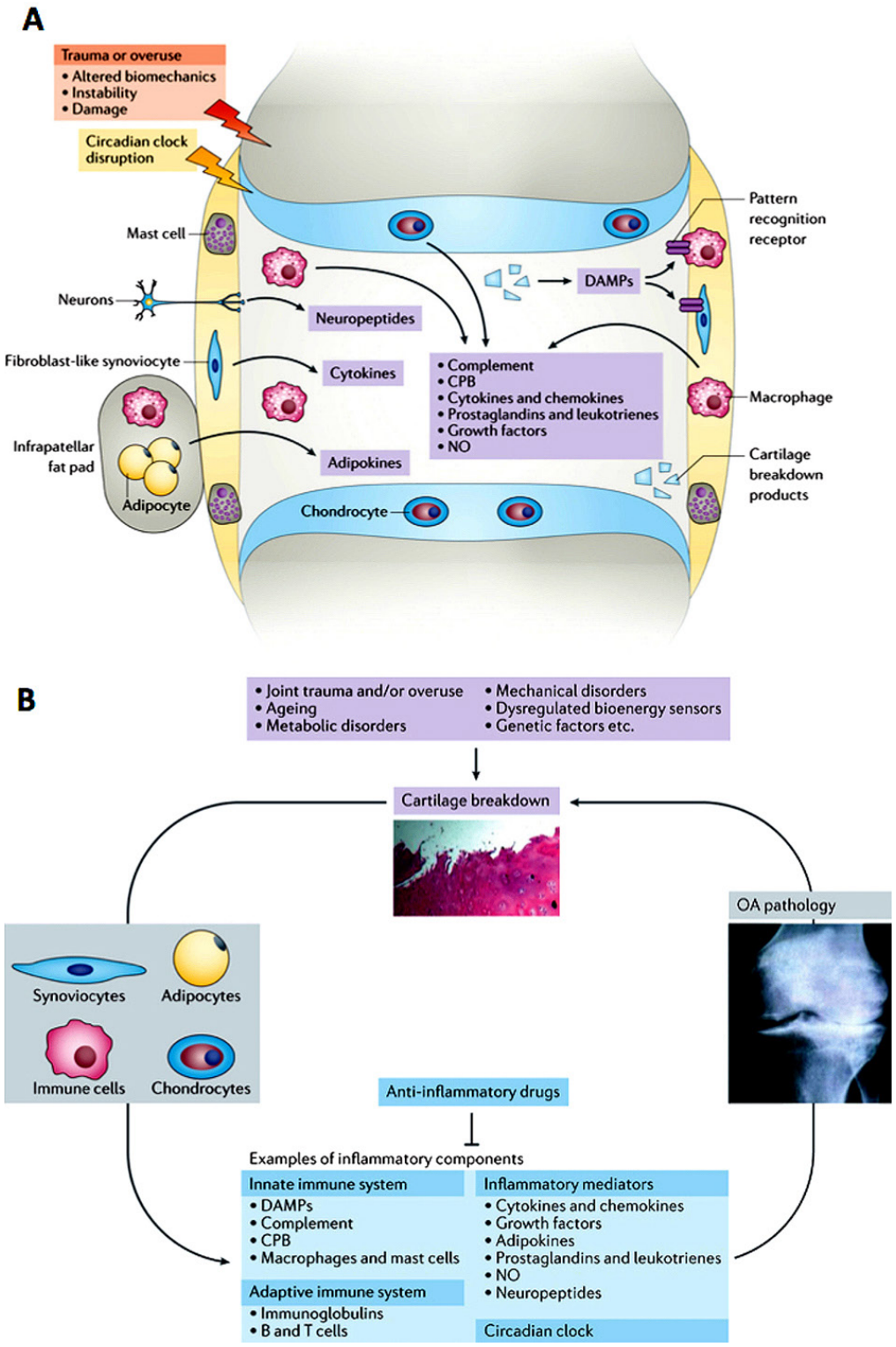

Figure 2. Signaling and cells involved in the inflammatory pathology in osteoarthritic joints. (A) Several inflammatory pathways and mechanisms are likely to contribute to the pathogenesis of OA. In this paradigm, injury or overuse, often in the context of other risk factors, triggers a vicious cycle of local tissue damage, failed tissue repair, and low-grade inflammation involving a number of molecular components and mechanisms in the joint. This low-grade inflammation contributes to or mediates progressive cartilage loss, pain, and joint dysfunction. CPB, carboxypeptidase B; DAMPs, disease-associated molecular patterns; NO, nitric oxide.(B) Targeting low-grade inflammation to break the feed-forward cycle of joint damage and breakdown leading to inflammation that promotes the pathogenesis of OA. Examples of risk factors for OA, cell types involved in its pathogenesis, and molecular components in the inflammatory pathways that are potential therapeutic targets for preventing or treating OA are shown. CPB, carboxypeptidase B; DAMPs, disease-associated molecular patterns; NO, nitric oxide; OA, osteoarthritis. Reproduced with permission from (1).

in human OA, but the results have thus far been disappointing (27). Findings suggest that strontium ranelate, a drug for treating osteoporosis, has disease-modifying effects in individuals with knee OA (28, 29), possibly through antiinflammatory mechanisms involving inhibition of IL1β and matrix metalloprotease (MMP) production. It is unknown whether strontium ranelate can actually prevent or slow the progression of OA in the long term (i.e.,≥3 years) (30). Several promising findings showing disease modifying effects of inhibitors of lowgrade inflammation in animal models of OA warrant follow up in human clinical trials, and these include IL-1β blockade, cyclooxygenase-1 and 2 (COX1–2) inhibitors, nitrous oxide (NOS) inhibitors, MMP inhibitor, and growth factors such as fibroblast growth factor (FGF)18 (reviewed in (1)). Other recent studies have stressed the importance of the cartilage–bone interface in OA by demonstrating that cartilage and subchondral bone act as a single functional unit, in health and in disease (31). Vascular pathology and the loss of mineral density in subchondral bone are important in the initiation and/or progression of OA. Changes in subchondral bone may accelerate progression of pre-existing disease. Therefore subchondral bone is an attractive target for developing DMOADs and biological therapy.

Antibody therapy for treatment of chronic forms of OA is becoming a reality. OA is associated with increased expression and activity of several secreted proinflammatory cytokines, which activate catabolic pathways and promote the production of matrix-degrading enzymes. Besides IL-1β and TNF, several other cytokines and chemokines, including IL-6, IL-8, and IL-17, are implicated in OA. These proinflammatory cytokines bind to their respective cell-surface receptors and activate inflammatory signaling pathways culminating in activation of NF-κB, a transcription factor that can be induced by stress-related stimuli, including excessive mechanical stress and extracellular matrix (ECM) degradation products. Once activated, NF-κB regulates the expression of many cytokines, chemokines, adhesion molecules, inflammatory mediators, and several matrix-degrading enzymes. Therefore, proinflammatory cytokines, their cell-surface receptors, and NF-κB and associated signaling pathways are excellent therapeutic targets in OA. For inhibiting TNF in OA, published preclinical studies suggest that monoclonal antibodies and single-chain Fv antibody against TNF can potently inhibit inflammation and prevent cartilage damage initiated by this cytokine (32). In contrast with full-length IgG, ESBA105, a topically applied TNF inhibitor, also penetrates into cartilage and can be expected to reverse the catabolism of articular cartilage in arthritis. These studies recognized the value of anti-TNF therapy as a treatment option for severe OA and proposed that larger controlled trials should be established to investigate this possibility. Additionally, infliximab and etanercept are anti-TNF therapies approved for treatment of RA (33), and these may be useful for the severe OA cases where there is a strong inflammatory component.

In OA, proinflammatory cytokines are the crucial biochemical signals that stimulate chondrocytes to release cartilage-degrading proteases. The rationale for use of anticytokine therapy in OA is based on evidence from *in vitro* and *in vivo* studies that demonstrated specific effects of the proinflammatory cytokines IL-1β and TNF in the initiation and progression of articular cartilage destruction (34). Further evidence suggests that, in addition to IL-1β and TNF, other pro-inflammatory cytokines, including IL-6, members of the IL-6 protein superfamily, IL-7, IL-17, and IL-18, are also capable of promoting cartilage degradation (35). Other cytokines released during the inflammatory process in the OA joint may be regulatory (IL-6, IL-8) or inhibitory (IL-4, IL-10, IL-13, and interferon-γ (IFN-γ)). It has been suggested that therapeutic intervention with the purpose of blocking or reversing structural damage is likely to be more effective when there is a possibility of preserving normal homeostasis (34), attempting to restore physiological functions in the joint and to block catabolic pathways activated by inflammatory mediators. Therapeutic strategies that concurrently use growth factors, for example transforming growth factor beta (TGFβ), insulin-like growth factor 1 (IGF1), FGF2, platelet-derived growth factor (PDGF), and connective tissue growth factor (CTGF), may be required in advanced cases of OA in which the repair responses of the cartilage may be severely compromised (35).

Other interesting approaches for treating OA include targeting angiogenesis and neurogenesis. By reducing blood vessel and nerve formation from the subchondral bone into articular cartilage, one may be able to prevent or reduce joint pathology and pain symptoms in OA (36). Rodent models have shown that vascularization changes occur early during OA development, and articular cartilage loses its resistance to vascularization over time, sustaining microcracks and damage (37). Inflammation also drives synovial angiogenesis by activating macrophages (37). Interestingly, angiogenesis and nerve growth are linked by pathways that involve the release of proangiogenic factors, for example vascular endothelial growth factor (VEGF), nerve growth factor (NGF), and neuropeptides (37). Studies of humans have shown that increased vascular penetration and nerve growth expression in the meniscus is a potential source of pain and also enhanced inflammation and tissue damage, driving disease progression in knee OA (38). Angiogenesis might be stimulated by similar inflammatory mechanisms in the synovium, contributing to joint effusion through impaired synovial fluid drainage (39). Novel antiangiogenic therapeutics and inhibitors of proangiogenic and neurogenic factors

might be promising therapies for inflammatory joint disease in both RA and OA.

3.3. Current modalities for introducing therapeutics in the joint

Of all the modalities to introducing specific therapeutics in the joint for treating OA or RA, intra-articular injection appears to be favored. This is because local administration offers several advantages over systemic delivery, including increased bioavailability, reduced systemic exposure, and fewer off-target effects and/or adverse events. OA, which affects individual joints, and polyarticular inflammatory pathologies, including RA and gout, have high incidence and long-term therapeutic need. Thus, there has been great interest in achieving successful localization of therapeutics at the pathological site, to maximize efficacy and reduce drug cost. The joint is highly vascularized and a subsynovial capillary network delivers systemic soluble molecules to the joint space (Figure 3a). Small molecules also leave the joint space via the vasculature, whereas larger substances such as proteins exit via the lymphatic system (reviewed in (40)). Macromolecules enter the joint from the circulation via the synovial capillaries and are first sieved by the fenestrated endothelium of the capillaries (Figure 3a). Small molecules also enter via the capillaries, but encounter resistance from the extracellular matrix of the synovial interstitium. Intra-articular injection bypasses both of these constraints to allow molecule entry into the joint space. One major challenge to sustaining therapeutic effect, however, is that both large and small molecules rapidly exit the joint, via the lymphatic system and small blood vessels, respectively. It is also difficult for drugs to reach chondrocytes from a systemic delivery method since they must first reach the synovial fluid and then diffuse through the extracellular matrix of cartilage. Unless it is already microcracked or damaged, the cartilage matrix is highly anionic and increasingly impermeable to molecules much greater than the size of albumin (~67 kDa), depending upon their charge and conformation (41). Intra-articular therapy improves drug delivery to cartilage and can therefore increase therapeutic efficacy. In an inflamed joint, capillary permeability increases, thereby enhancing the entry of macromolecules into the joint space.

Although macromolecule entry into joints is constrained, their removal from joints occurs via the lymphatic system independent of size (Figure 3a). For example, the rate of macromolecule removal from the joint is increased in patients with RA, reflecting enhanced drainage from the joint space due to a greater synovial lymph flow (42). Unfortunately, lymphatic drainage is efficient, rendering the intra-articular time of proteins in joints just a few hours or less, making it difficult to sustain therapeutic effects in chronic joint disorders. For small molecules, which rapidly diffuse from the joint via the synovial capillaries, it has been proposed that the best strategy is targeting to enhance accumulation at the joint following delivery. For example, various molecules ranging in size still experience quick clearance from joints. For example, the values reported range from 0.2. h for acridine orange (M_W 370 Da), 1.2.–13 h for albumin, and 26 h for hyaluronic acid (M_W 3 × 10^6 Da). Half-lives of NSAIDs and soluble steroids cluster at around 1 to 4 h in joints (43). Overall, these values illustrate the challenges facing intra-articular delivery for treating chronic conditions such as arthritis.

And although intra-articular injection can circumvent the entry restrictions imposed by synovial sieving, it cannot avoid rapid lymphatic clearance of a therapeutic agent. Therefore, gene delivery is a strategy that has been proposed as a promising approach to promote sustained and localized protein production to enable an increased intra-articular dwell time (44). Gene delivery to the joint has been successful in several animal and clinical models. In animal models, injections of both the knee and ankle/paw joints are feasible in rabbits, rats, and mice. For example, both viral and nonviral vectors, if administered systemically, generally only achieve transient expression. Local delivery to the joint facilitates the accumulation of anti-arthritic proteins in and around articular tissues, where they are needed the most. Local delivery also reduces exposure of extra-articular tissues and organs, reducing potential side effects. Gene transfer to synovium and intra-articular expression of the IL-1Ra gene via viral vectors, has been shown to be efficient in reducing inflammation and cartilage loss in several animals models. Interestingly, direct transfer of an adenoviral IL-10-expressing vector (Ad-vIL-10) to the mouse paws (periarticularly) prevented the development of CIA (45). Local delivery resulted in a strong and persistent CIA suppression, without evidence of adverse effects. Also, when only one or two paws were injected with the adenoviral vectors, all four paws were protected from developing disease. Another study highlighted distant protection of joints following periarticular administration of adenovirus in the knee, ankle, and paw. Injection of Ad-vIL-10 into the knee appeared to delay the onset of CIA in the distal paws and reduced the severity of disease (46). The mechanism of protection appeared to be production of vIL-10 in the knee, followed by circulating vIL-10 protein being the major mediator of distal protection. In another study, a peptide was given in the knee joint of a mouse CIA model. Although the IKK inhibitor peptide (NEMO-binding domain peptide) was given locally, beneficial immune effects were systemic, ameliorating bone scores and inflammation in the paws (47). Therefore, it appears that localized injection of vectors or therapies in larger joints can have a localized and also a systemic effect, improving disease severity

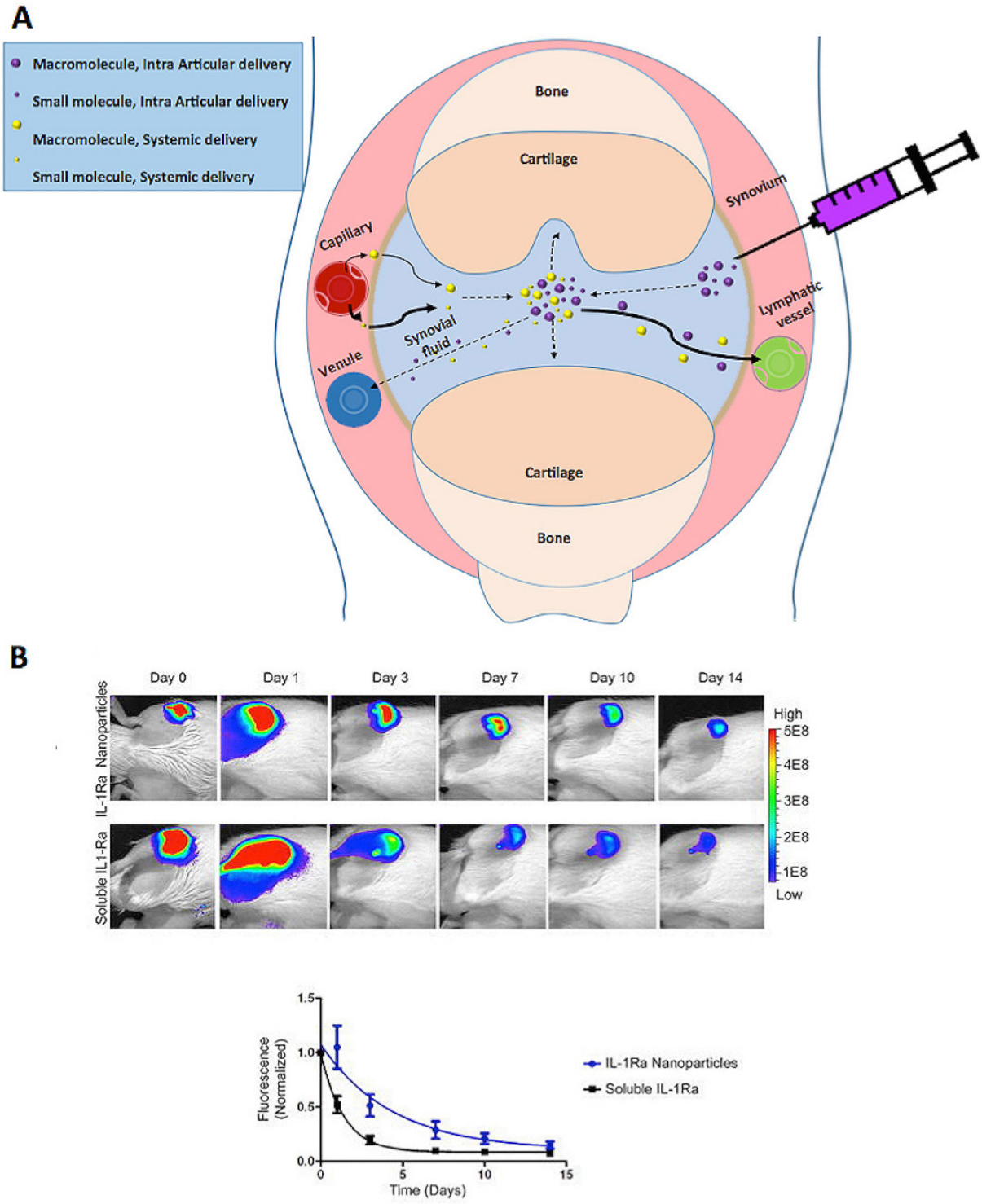

Figure 3. Intraarticular injections are an effective way to deliver therapeutics to the joint. (A) Schematic of challenges associated with retaining certain molecules in the joint due to effusion and lymphatic flow. Macromolecules enter the joint via the synovial capillaries from the circulation and are sieved by the fenestrated capillary endothelium. Small molecules also enter, but the major resistance to their entry is provided by the extracellular matrix of the synovial interstitium. Intra-articular injection bypasses both of these constraints to entry. However, both large and small molecules rapidly exit the joint, via the lymphatic system and small blood vessels, respectively. (B) An example of successful slower egress of particles when targeting is employed to retain molecules in joints *in vivo*. Here, IL-1Ra-tethered nanoparticles are retained longer than soluble IL-1Ra in the intra-articular joint space. Top panel, images of IL-1Ra tagged with a near-infrared (IR) dye (AF750-maleimide) that was then tethered to particles. IL-1Ra-tethered particles or soluble IL-1Ra were injected into the right stifle joint of 8–10 wk old rats. Left stifle joints were injected with saline. Total IR photon counts (relative fluorescence units) were measured by IVIS imaging over 14 days. Lower panel, IL1-Ra-particles show sustained signal over time as compared to soluble IL1-Ra as assessed by measuring photon counts. The half-life was estimated at 3.0.1 days for IL-1Ra-particles (n = 6) vs. 0.9.6 days for soluble IL-1Ra (n = 5). Reproduced with permission from (102).

in smaller, distal joints. We will next discuss small molecules, gene therapies, cell therapies, and then emerging approaches to targeting these molecules to enhance intraarticular therapy of arthritis in the following sections.

4. NEWLY DEVELOPING MODALITIES FOR ANTI-INFLAMMATION OR BONE REPAIR

4.1. Drugs or small molecules

Dexamethasone provided in a cross-linked hyaluronic acid hydrogel formulation (cHA gel) has been used to treat knee OA in clinical practice owing to their chondroprotective and anti-inflammatory effects. In a rat model, a strong staining of type II collagen was found in both the cHA-Dex gel groups compared with saline group or cHA alone group. Similar result was found for the mRNA level of aggrecan and opposite result for type X collagen. Hematoxylin and eosin staining in the synovial membrane showed less synovial lining cell layers and reduced inflammatory cell infiltration in cHA-Dex gel-treated animals compared with saline or cHA only groups. Altogether, cHA-Dex gel has better chondroprotective and anti-inflammatory effects in rat surgery-induced osteoarthritis than cHA alone (48).

Salubrinal is novel promising agent for OA and other arthritis therapy. Salubrinal is a synthetic agent that elevates phosphorylation of eukaryotic translation initiation factor 2 alpha (eIF2α) and alleviates stress to the endoplasmic reticulum. A recent study demonstrated, in a mouse model of OA, that daily administration of Salubrinal attenuated the degradation of the tibial and femoral articular cartilage and the inflammation of synovium by downregulating NFκB signaling (49). Salubrinal's suppressive effects on the proteolytic activity of MMP13, as well as its elevation of the phosphorylation of NFκB p65 in the mouse model of OA, were consistent with *in vitro* data. The OA model was generated by inducing damage in the medial collateral ligament and the medial meniscus. As post-traumatic OA may cause injuries in those regions, the results from this study support the idea that Salubrinal could be applied to treat post-traumatic OA. Salubrinal significantly suppressed cartilage degradation at 3 weeks (femur) and 6 weeks (tibia) following the induction of OA in this model. Significant suppression of OA symptoms was detected only in the tibia samples, although the actual mean histological score was close between the femur and the tibia. This model indicated that intra-articular injection of salubrinal delayed the progression of OA, and attenuated articular cartilage degradation and synovial inflammation, but without causing a pathological thickening of subchondral bone (Figure 4a). Future application of Salubrinal in human OA might necessitate an extended period of chondroprotection beyond three to six weeks and this could be achieved by selecting proper dosages and higher administration frequencies. Also, daily intra-articular injection may not provide a convenient option in clinical situations, and sustained delivery of Salubrinal potentially could be examined using implantable scaffolds or targeted nanoparticles. With the exception of Salubrinal's effects on chondroprotection, interesting future studies could examine the effects on chondroregeneration including expression of matrix components such as type II collagen and aggrecan or effects on mesenchymal stem cells within the joint.

Salubrinal also has significant promise for RA therapy. A recent preclinical study has shown salubrinal downregulated the expression of dual specificity protein phosphatase 2 (DUSP2) and inflammatory genes in immune cells *in vitro*, and its administration in a collagen-antibody induced arthritis (CAIA) mouse model significantly reduced inflammatory responses *in vivo* (50). In the *in vitro* assays, genome-wide microarray analysis revealed that DUSP2 might mediate salubrinal's partial attenuation of the inflammatory effects of lipopolysaccharide (LPS) and *phorbol* 12-myristate 13-acetate (PMA)/ionomycin in RAW264.7. and Jurkat cell cultures. In the *in vivo* analysis with CAIA mice, inflammation was induced by the administration of a cocktail of antibodies that recognize auto-antigenic epitopes in type II collagen, which offers a rapid disease onset and synchronicity over the classic collagen-induced arthritis (CIA) model. The clinical and histological scores as well as thickness measurements of the paws in CAIA mice support the efficacy of salubrinal in suppression of inflammation. On day 12, for instance, the administration of salubrinal improved the clinical and the histological scores (Figure 4b). Salubrinal's advantage is that it attenuates bone destruction by elongation factor 2 alpha (eIF2α)-mediated downregulation of nuclear factor of activated T-cells 1 (NFATc1), a master transcription factor for osteoclastogenesis (51). Furthermore, salubrinal can downregulate MMP13, a collagenase that digests not only aggrecan in cartilage but also collagen in bone. It is important to discover whether salubrinal can be administered with other agents, such as DMARDs or biologics, to induce synergistic therapeutic effects.

Kartogenin is a novel small molecule identified by image-based high-throughput screening. Kartogenin has been shown to have chondroprotective effects *in vitro*, and is efficacious in OA animal models of OA (52). Kartogenin can therefore replenish cartilage from endogenous stem cells by inducing the selective differentiation of multipotent mesenchymal stem cells (MSCs) into chondrocytes (53). Kartogenin binds filamin A, disrupts its interaction with the transcription factor core-binding factor β subunit (CBFβ), and induces chondrogenesis by regulating the CBFβ and runt-related transcription factor-1

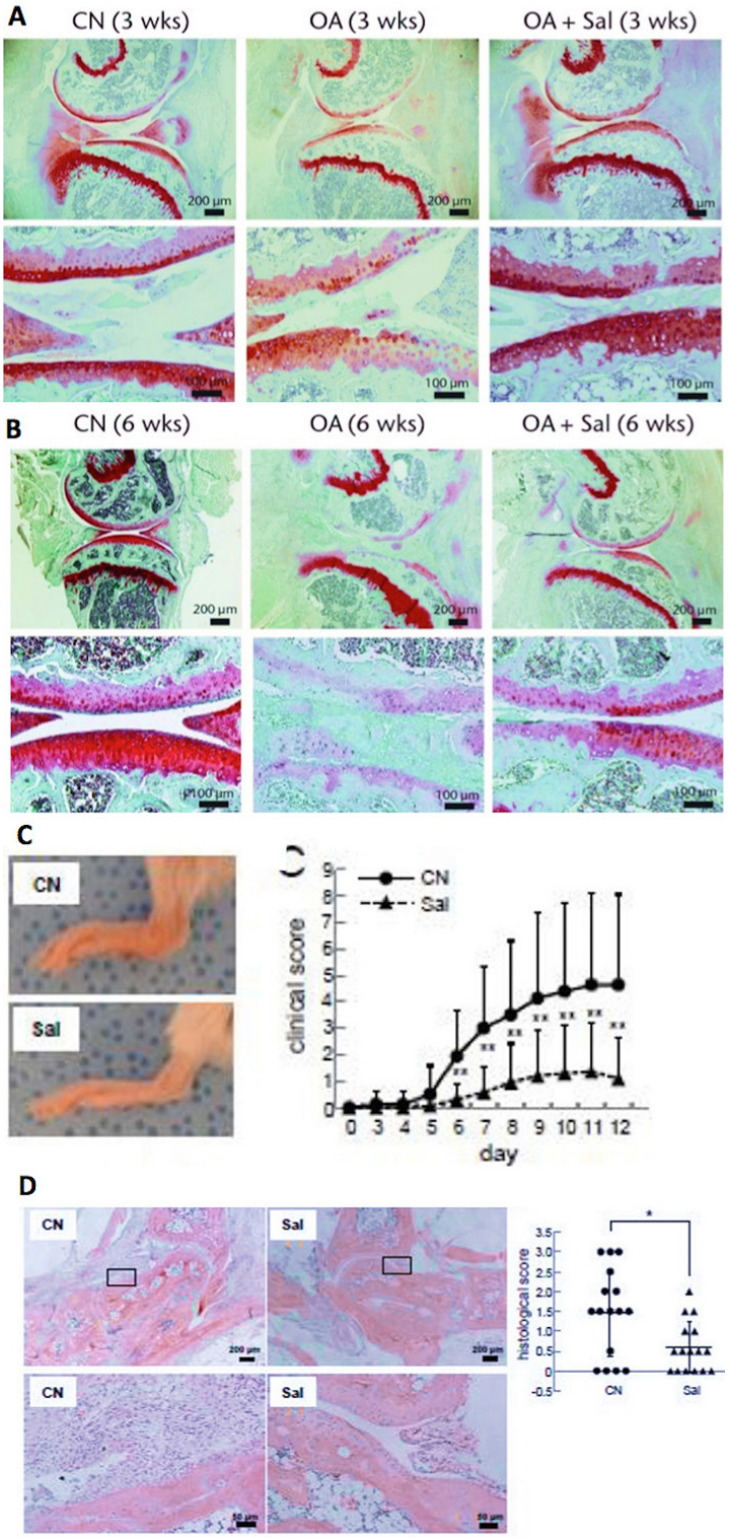

Figure 4. An example of a small molecule drug that improves inflammation and bone erosion scores in osteoarthritis and rheumatoid arthritis. Images of safranin O staining of the sagittal knee joint section, with (A) samples 3 weeks after induction of osteoarthritis (OA, placebo sample; OA + Sal, Salubrinal-treated OA sample; CN, sham control sample) and (B) samples six weeks after induction of OA. The three panels in the top row show an entire knee section (bar = 200 μm), while the three panels in the bottom row show high magnification images (bar = 100 μm). (C) Suppression of the progression of collagen-antibody induced arthritis (CAIA) by salubrinal. Hind paws are shown 12 days after induction of CAIA, with clinical scores. (D) H&E staining of the hind paws. The scale bars are 200 μm (upper) and 50 μm (lower). Right plot, histological scores. The single and double asterisks indicate p<0.0.5 and p<0.0.1, respectively. CN=placebo, Sal=salubrinal. Reproduced with permission from (50).

(RUNX1) transcriptional program. This work has generated excitement about harnessing the potential of stem cells for cartilage repair. Another pilot study in rat showed promise for kartogenin in preventing joint deterioration in post-traumatic OA. OA was induced in rats by anterior cruciate ligament transection surgery in the right knee joint. Sham surgery was performed on the right knee joint of control group rats. All rats underwent *in vivo* magnetic resonance imaging (MRI) at 3, 6, and 12 weeks after surgery, and quantitative MR relaxation measures (T1ρ and T2) were determined to evaluate articular cartilage changes. Animals were sacrificed at week 12 and the knee joints were removed for micro-computed tomography (micro-CT) and histology. Kartogenin treatment significantly lowered the T1ρ and T2 relaxation times, indicating decreased cartilage degradation; also, treatment significantly decreased cartilage and bone turnover markers. Kartogenin also prevented subchondral bone changes, showing promise as a drug to potentially prevent joint deterioration in post-traumatic OA. This work invigorates research into OA small-molecule therapy and regenerative medicine (54) and provides new insights into the control of chondrogenesis that may ultimately lead to a stem cell-based therapy. Kartogenin and other structurally related small molecules that can promote selective differentiation of MSCs into chondrocytes may prove to be extremely useful for improving the outcome of cell-based therapy by stimulating endogenous mechanisms for repair of damaged cartilage, thus enhancing the joint's intrinsic capacity for cartilage repair.

4.2. Protein-based biological agents in development

Biological therapy is a thriving area of research and development, and although it has been well established for chronic forms of RA, the transfer of information to OA therapy has been slow. OA involves three main tissues in the synovial joint: articular cartilage, bone, and synovium. Biological therapy may have benefits for all of these tissues. The presence of systemic inflammation in some patients may provide a rationale for biological therapy, even though it is virtually impossible to reverse cartilage damage at late stages of the disease. Biological therapy is unlikely to be suitable for less severe OA, which can be treated with conventional and complementary treatment. Therefore, understanding when biological therapy for OA is adequate or not will be an important priority of future studies. Future research must be directed toward defining the risk-to-benefit ratio for biological therapy, especially if the purpose of the therapy is to target mediators of low-grade inflammation, especially for obese patients with insulin resistance and diabetes. This will be extremely challenging, because mediators of low-grade inflammation are likely to be important physiological effectors on other organ systems. Among biologics in development are gene therapies, cell therapies, and targeted therapies or delivery systems.

Challenges to the development of biologics may include an increased number of reports suggesting that biological disease modifying antirheumatic drugs (DMARDs) induced or exacerbated interstitial lung disease in RA patients treated with biologics targeting TNF, CD20, IL-1β and IL-6 receptors (55). The possibility of biologic-induced lung disease makes it more difficult to choose an optimal biologic regimen for RA treatment, as the relationship between biological therapy safety and the induction or exacerbation of interstitial lung disease has not been established. A framework to assess baseline disease severity, particularly standardizing the evaluation of the pulmonary condition stage and monitoring the outcome during the biological therapy treatment is highly needed and may help guide treatment decisions and predict treatment benefits.

4.3. Peptide delivery

Delivery of peptides and proteins to the joint has been accomplished via direct delivery of recombinant molecules or via nanoparticle administration (56). Nanoparticles can serve to target therapeutics to the joint, and peptides have been used also to target nanoparticles to collagen II in joints. Rothenfluh *et al* have recently developed a nanoparticle that can bind to collagen II alpha 1 to target articular cartilage up to 72-fold more than nanoparticles displaying a scrambled control peptide sequence following intra-articular injection in the mouse (57). These functionalized nanoparticles were sufficiently small to enter the cartilage matrix and use it as a reservoir, offering the advantage of improved bioavailability within the matrix itself. Targeting the cartilage matrix is a well-suited approach for treating early events of cartilage degradation and could be a critical approach for the treatment of the initial stages of several forms of arthritis. Another group targeted synovial vasculature (58) with success using peptides that attenuated autoimmune arthritis. Peptides were screened for enrichment of their ability to home to the inflamed joint in Lewis rats. Interestingly, these peptides (NQR) could distinguish between the vasculature of inflamed joints specifically and could bind to endothelial cells in the joint but not in the liver. Also, the peptides suppressed autoimmune adjuvant induced arthritis, as well as inhibited the migration of CD3 T cells into the joints. Another peptide was used to target delivery of a cytokine (IL-4) to joints of rheumatoid tissue (59), although the receptor the peptide bound to unidentified. This group showed that IL-4 could be delivered with MMP-cleavable sequences and that the cytokine was functional, augmenting production of IL-1R antagonist (IL1-Ra) by fibroblast like synoviocytes. *In vivo* imaging showed that the IL4 peptide fusion

was retained in synovial but not in skin tissue grafts and could induce signal transducer and activator of transcription 6 (STAT6) phosphorylation. These therapeutics are exciting in that they could enable one to develop therapies for other autoimmune diseases besides arthritis. Targeting of cytokines such as NGR peptide conjugation to TNF has been successful in the treatment of cancer in preclinical models and in clinical trials. Tissue targeting has the potential to augment the therapeutic window of the drug, enabling it to be safer than existing therapeutics.

4.4. Gene therapies

The biology of joints makes them very difficult targets for specific and selective drug delivery. This is especially true for current biologics, which are typically protein-based. Given the challenges with protein-based biologics egress from joints, gene transfer might be an important strategy that can solve the delivery problem in a clinically reasonable fashion. Vast pre-clinical data confirms that genes can be efficiently transferred to tissues within joints by intra-articular injection using a variety of different vectors in conjunction with *ex vivo* and *in vivo* strategies (60). Using the appropriate gene transfer technologies, long-term, intra-articular expression of transgenes at therapeutic concentrations can be achieved for treating RA and OA preclinically. However, despite this promise, a limited number of clinical trials have been completed, which confirm safety and feasibility but only three protocols have reached Phase II; as yet, there is no unambiguous evidence of efficacy in human disease. Only two clinical trials are presently underway, both Phase II studies using allogeneic chondrocytes expressing TGF-β1 for the treatment of OA. Phase I studies using adeno-associated virus to deliver IL-1Ra in OA and interferon beta (IFN-β) in RA are going through the regulatory process.

4.4.1. Current use of AAV in arthritis clinical trials

Eight current or recent clinical studies were found for adeno-associated vector (AAV) use in arthritis therapy. These include AAV-IFNβ for RA using a single intra-articular injection in the metacarpophalangeal, proximal interphalangeal, or distal interphalangeal joints (NCT02727764) (61) Another Phase I/II trial is currently recruiting and determines the maximum tolerated doses following single intra-articular injection. The study will permit subjects who are concurrently on anti-TNF antagonists. For subjects on DMARDs, a stable regimen for inflammatory arthritis for the previous three months, with no changes in doses in the four weeks prior to screening will be required. The primary objectives were to evaluate the safety of intra-articular administration of tgAAC94, a recombinant AAV containing the TNFR:Fc fusion gene in subjects either taking or not taking TNF antagonists currently (NCT00126724) (61). Unfortunately, one patient had a fatal adverse event from contracting disseminated histoplasmosis (62). The patient's receipt of concurrent anti-TNF therapy and other immunosuppressive therapy while she was living in an area where histoplasmosis was endemic was thought to be the most likely explanation for the infection. The fatal infection appeared unrelated to exposure to the AAV administered in the trial. This case reinforces the importance of considering infectious complications in patients receiving treatment with a TNF antagonist. Finally, a current Phase I trial using AAV is recruiting moderate knee OA patients to examine the local and systemic safety of three intra-articular doses of the recombinant self-complementary AAV2 vector expressing IL-1Ra (AAV.IL-1Ra) (NCT02790723) (61). The primary outcome measures will include collecting information on the number of subjects experiencing severe adverse events for up to 53 weeks. The study expects to enroll at least 9 patients and the study design will compare AAV-IL1Ra to IL-1Ra and Anakinra and Kinaret at low (10^{11}), medium (10^{12}) and high AAV viral particle doses (10^{13}). Regarding other vectors, there are no current clinical trials examining herpes or adenovirus as delivery vectors in arthritis.

4.4.2. Newly developing viral vector gene therapy strategies

4.4.2.1. Herpes

There seem to be no recent preclinical efforts in herpes simplex virus (HSV) for arthritis therapy. Back in 1999, a report suggested promise for HSV-based vectors for arthritis gene therapy using a first-generation, replication defective herpes simplex virus (HSV) vector (S/0-) deficient in the immediate early gene ICP4, and a second-generation HSV vector derivative (T/0-) deficient for the immediate-early genes ICP4, 22 and 27, each carrying a soluble TNF receptor or IL-1Ra transgene. Following a single intra-articular injection of the vectors into rabbit knee joints, only the second-generation, HSV T/0- vector expressed detectable levels of soluble TNFR in synovial fluid. When tested in an experimental model of arthritis generated by intra-articular overexpression of IL1b using retrovirus transduced synovial cells, the HSV T/0- vector expressing IL1Ra was found to inhibit leukocytosis and synovitis significantly. The improved levels and duration of intra-articular transgene expression achieved via HSV-mediated gene delivery suggest that an HSV vector system could be used for therapeutic applications in patients with RA (63).

4.4.2.2. Adenovirus

An interesting highlight from the literature was a study using adenovirus (Ad) that examined the effects of delivering basic fibroblast growth factor

(bFGF), bFGF combined with IL-Ra and/or IGF-1 both in human OA chondrocytes and a rabbit OA model induced by anterior cruciate ligament transection (ACLT) in knees. Human OA chondrocytes received Ad vectors expressing bFGF, IL-Ra, or IGF-1 vectors. Chondrocyte proliferation, glycosaminoglycan (GAG) content, expression of type II collagen, disintegrin and metalloproteinase with thrombospondin motifs 5 (ADAMTS-5), MMP-13, MMP-3 and TIMP metallopeptidase inhibitor 1 (TIMP-1) were determined. In the rabbit OA model, Ad vectors were injected intra articularly into the knee following ACLT. *In vitro*, Ad-bFGF significantly promoted chondrocyte proliferation, and increased GAG and type II collagen synthesis. When Ad vectors were used to transduce cells in different combinations, there was significant enhancement on the GAG content, type II collagen synthesis, and TIMP-1 levels, while ADAMTS-5, MMP-13, and MMP-3 levels were reduced. *In vivo*, the transfected genes were expressed in synovial fluid and intra-articular delivery of Ad-bFGF enhanced the expression of type II collagen in cartilage and decreased cartilage Mankin score compared with the OA control group. Multiple-gene transfection in different combinations further enhanced the effects of bFGF alone, suggesting that bFGF can be effective in treating experimental OA and may synergize with other gene therapy vectors to augment repair (64).

4.4.2.3. Adeno-associated and lentiviral vectors

Several new developments are being described for AAV use in arthritis. In a large animal model (6 horses), equine chondrocytes and synovial cells were treated with Ad, serotype 2 AAV (rAAV2), or self-complementary (sc) AAV2 vectors carrying green fluorescent protein (GFP) following bilateral metacarpophalangeal joint injections. The localization of GFP expression within the joints, as well as the presence of *in vivo* joint inflammation and neutralizing antibody titers in serum and joint fluid were assessed. *In vitro*, greater transduction efficiency and sustained gene expression were achieved by scAAV2 compared to rAAV2 in equine chondrocytes and synovial cells. *In vivo*, AAV2 demonstrated less joint inflammation than Ad, but similar neutralizing antibody titer. The scAAV2 vectors induced superior gene transduction than rAAV2 in articular cells, and both rAAV2 and scAAV2 vectors seemed safer for intra-articular compared to Ad vectors (65). Another interesting study showed that the combination of rAAV2/5-TRAIL (tumor necrosis factor superfamily member 10) gene therapy and epirubicin chemotherapy provided augmented antiarthritic effects in a mouse model of CIA. Epirubicin treatment up-regulated death receptor -4 and -5 expression and down-regulated c-FLIP (CASP8 and FADD Like Apoptosis Regulator) expression, thereby enhancing the activation of procaspase 3, procaspase 8, and procaspase 9 in fibroblast like synoviocytes (FLS). The intraarticular injection of rAAV2/5-TRAIL combined with epirubicin treatment significantly reduced the severity and incidence of CIA and joint swelling in the animals. Histologic evaluations revealed that inflammatory cell infiltration, cartilage destruction, and bone erosion were significantly reduced in the joints of the mice receiving the synthetic treatment (66). An interesting recent AAV application has been in cartilage repair in porcine joints. AAV can be useful for cellular modification to help promote MSC differentiation for cartilage repair applications. It is well known that cellular differentiation in cartilage repair tissue is challenged by incomplete chondrogenic differentiation, leading to the formation of fibrocartilage. Also, ingrowing osteochondral progenitor cells have the tendency to undergo terminal chondrogenic differentiation, resulting in endochondral ossification. AAV expressing chondromodulin 1 (AAV-Chm1) has been used with success in porcine joints and showed strong anti-angiogenic effects, as wells as inhibition of chondrocyte hypertrophy and ossification (Figure 6) (67). AAV-Chm1-infected cells maintained a chondrocyte like phenotype, forming a hyaline-like cartilage matrix that was superior to that formed by uninfected or control infected (AAV-GFP) cells.

Lentiviral (Lv) vectors also have been used in preclinical studies for RA. Lv-EGFP (enhanced green fluorescent protein) or Lv-angiostatin were injected into joints of mice. The EGFP vector revealed that the lentivirus can infect a variety of cells in the pannus and synovial tissue. The Lv-angiostatin vector inhibited progression of CIA and improved the radiographic changes, reducing bone erosion by 50% in the ankle joints (68). Fibroblasts genetically modified to overexpress IL1b generate a model of rat knee swelling which can be reversed with intra-articular delivery of Lv-IL1Ra (69). An Lv-GFP also could transduce a vast number of cells within the hypertrophic synovium. The mechanism is though to involve a dramatic increase in IL1Ra expression following the onset of inflammation in the synovium, which appears to arise from the proliferation of joint cells (type B synoviocytes) stably transduced by the Lv-IL1Ra vector. Despite the potential utility, clinical applications of the lentiviral vectors have not been initiated for arthritis. AAV vectors are also capable of gene transfer into non-dividing cells *in vivo* and are less immunogenic and cytotoxic. However, the lack of efficient packaging systems has limited the translation of AAV to the clinic. Now, there are some emerging clinical studies (4) employing AAV, as we discussed above, and this holds great promise for AAV becoming a staple vector for the gene therapy of arthritis.

4.4.3. Newly developing non-viral vector gene therapy: sonoporation

Sonoporation consists of a gene delivery technique in which ultrasound (US) is utilized to

increase cell membrane permeability, thus allowing the delivery of compounds or nucleic acids into cells. Over the past few years, sonoporation has been examined in several tissues. Sonoporation has been found to be enhanced by US contrast agents, commonly referred to as microbubbles, which should be used alongside ultrasound in order to keep gene transfer high while avoiding tissue damage from high intensity US. US stimuli promotes the burst of microbubbles, inducing acoustic cavitation, which augments cell membrane porosity and promotes high transfection efficiency of gene constructs (70, 71). US has been considered largely non-toxic at low intensity and it is considered a safe technique for gene delivery. It is well known that continued ultrasonic waves lead to increased temperatures, and are utilized clinically mainly to treat patients with pain, and such waves are not recommended for gene delivery (72). Pulsed waves, on the other hand, are expected to only increase cell membrane porosity, making these the least harmful and safest types of ultrasonic wave to use for gene transfection/transduction.

Sonoporation – with the assistance of US contrast agents – has proven efficient in delivering genes/compounds to various tissues, such as the myocardium, kidneys, peritoneal cavity, pancreas, lungs, corneas, vertebrae, tumors (72), skeletal muscle (73), and also to synovia (72, 74). In 2007, reports showed that plasmid DNA encoding luciferase was transduced into normal synovial tissue using sonoporation, confirming that the efficacy of transduction is significantly higher with a combination of sonoporation and microbubbles than with sonoporation alone (70). In this study, male rats were used and localization of GFP reporter gene expression was detected in the synovium around the patella, femur, and tibia one day post-transduction. It appears that the cell types transduced were patellar and other ligament fibroblasts and a large proportion of synovial (surface) fibroblast-like synoviocytes. In the mouse knee, immunostaining for luciferase expression post-sonoporation in another study showed that the joint synovium fibroblasts expressed the reporter gene. In both studies, the articular cartilage did not appear to express the reporter genes, suggesting the fibroblastic tissues in and around the joint are the most amenable to gene delivery by sonoporation. In an antigen-induced arthritis rabbit model, GFP was expressed the highest the fifth day post-sonoporation and gradually declined until day 40 (74), and reporter gene expression was detected in the synovial lining and pannus. Longer-expressing vectors might be very useful in the joint to sustain therapeutic effect and these might include mini-circles or episomes (75).

Sonoporation also has been shown to promote the uptake of drugs such as MTX, increasing its anti-inflammatory effects following intraarticular injection in a rabbit rheumatoid arthritis model, when compared to its effects while administered systemically (76). Sonoporation can also be used to transduce siRNA into cells *in vivo*; there are reports of its utilization to transduce siRNA targeting TNF (siTNF) into the synovium of a rat arthritis model, successfully inhibiting bone destruction by suppressing osteoclast differentiation and locally inhibiting arthritis (72). Fluorescently-labeled siRNA transfected the synovial tissue, as well as the synovium around the patella, femur, and tibia, with no fluorescence seen in the surface layer of the articular cartilage. Sonoporation was found to be a safe method for gene transduction, with no adverse effects locally (in joint tissues) or systemically.

Moreover, gene delivery to muscle can also be very useful in promoting the sustained gene expression needed for treating chronic conditions such as arthritis. Aside from the traditional need for treating muscle diseases and immunization, successful gene delivery to skeletal muscle is critical for delivering genes in systemic conditions such as bone metastatic cancer, for example. In an attempt to promote sustained gene expression, our group examined delivery of an episomal vector (pEPIto) by sonoporation of skeletal muscle. It was shown that long-term (~10 months) gene expression could be achieved *in vivo* with a minimal toxicity profile, assessed by inflammatory gene expression and muscle histopathology evaluations (73). And although no current clinical studies are ongoing for sonoporation in arthritis, there is one study utilizing sonoporation to enhance chemotherapeutic (platinum and gemcitabine) delivery to malignant neoplasms of the digestive system at the Beijing Cancer Hospital (NCT02233205). This gene delivery strategy holds great promise in the future for translation in chronic arthritis conditions, especially if long-term expression utilizing certain vector types (episome, for example) can be achieved.

4.5. Cell therapies

4.5.1. Clinical use of cell therapy in arthritis

Autologous chondrocyte implantation/transplantation (ACI) is widely used in clinical practice and more than 15,000 patients have received this treatment worldwide (77). ACI shows good to excellent results 2 years post-transplantation, but only ~29% of patients retain a satisfactory outcome in cartilage repair at a 3-year time point. The commercial product Carticel (autologous cultured chondrocytes) is mostly used in localized cartilage defects; therefore cartilage damage with generalized OA has typically been an exclusion criterion for treatment. More recently, ACI has been attempted in clinical trials for OA with a mean age of 37 and an average defect size of ~5 cm^2 (78). About 8% of the patients experienced treatment failures, but in the

92% that experienced some functional improvements, had a delayed need for arthroplasty. In another study with patients >45 and a mean defect size of ~12 cm^2, 72% of patients experienced food or excellent clinical improvements (79). Despite benefits, there are still some limitations with ACI including the limited cells available, multiple surgical procedures, and *in vitro* chondrocyte dedifferentiation and donor site morbidity caused by cartilage harvest.

An interesting recent use of chondroprogenitor cells for cartilage repair has been reported. A promising cell source, cartilage stem/progenitor cells, has attracted recent attention. Because their origin and identity are still unclear, their application potential is under active investigation. There appears to be a group of stem/progenitor cells derived from adult human chondrocytes, highlighted by dynamic changes in expression of the mature chondrocyte marker, collagen type II (COL2), and MSC marker CD146. These cells are termed chondrocyte-derived progenitor cells (CDPCs). The stem cell-like potency and differentiation status of CDPCs were determined by physical and biochemical cues during culture. A low-density, low-glucose two-dimensional culture condition was shown to be critical for the emergence and proliferation enhancement of CDPCs (80). CDPCs showed similar phenotype as bone marrow mesenchymal stromal/stem cells but exhibited greater chondrogenic potential. Moreover, the two-dimensional cultured CDPCs proved efficient in cartilage formation and in repairing large cartilage defects (6–13 cm^2) in knees of 15 patients (Figure 5) (80). These findings suggest a phenotype conversion between chondrocytes and CDPCs and provide conditions that promote the conversion. These insights expand our understanding of cartilage biology and may enhance the success of chondrocyte-based therapies.

MSC also can be an alternative to ACI or CDPC strategies since they are more easily collected from various tissues including bone marrow and adipose tissues and have a high proliferation rate, an ability to differentiate into chondrocytes, and have immune suppressive activities. These regenerative characteristics have supported the use of MSC in several trials. In 2011, one report with 4 patients with moderate to severe knee OA showed that autologous BM-MSC (8–9x10^6 per knee) reduced pain with walking in 75% of patients and other improved physical parameters (81). In another study, MSC therapy was applied to 12 patients with severe knee OA. Patients received 40x10^6 MSC and cartilage quality was significantly improved in 11 out of 12 patients as assessed by T2 MRI (82). ASC from the infrapatellar fat pad have been used to treat OA prepared with platelet rich plasma (PRP)(83, 84). Patients (twenty-five) received these cells intra-articularly and had significant improvements in cartilage by MRI at the 2-year follow-up (84). Although promising, the effect of ASC+PRP versus PRP was unclear, since PRP appeared to give a similar benefit. The benefits of MSC therapy likely can be augmented by implementing tissue engineering or gene modification in order to enhance the cellular regenerative ability.

4.5.2. Current use of MSC or similar cells in arthritis clinical trials

For controlling inflammation, a study is aiming to evaluate the safety and efficacy of human umbilical cord mesenchymal stem cells plus DMARDs (NSAIDs or MTX) for RA in China (NCT02643823) (61). The primary outcome measure will be severity of adverse events and RA serology for Rheumatoid factor and C-reactive protein and Disease Activity Score (DAS 28) index, with a 12-month patient follow-up. For promoting tissue repair, there a trial recruiting patients to evaluate the efficacy and safety of a single intraarticular injection of autologous adipose derived MSC (10^8 cells in 3ml) following high tibial osteotomy in OA patients (NCT03000712) (61). MRI will be performed to follow up responses, and several grading and healing outcome scores will be used to compare results to the saline treatment. The intra-articular injection of MSCs is expected to stimulate the regeneration and growth of cartilage, and to improve pain and joint function with cartilage regeneration compared to high tibial osteotomy alone. Another study recruiting from the University of Navarra is a Phase I/II aiming to determine the safety, feasibility and effectiveness (clinical and radiological) of intra-articular administration of autologous mesenchymal stem cells (MSCs, also 10^8) with platelet rich plasma in patients with knee OA (NCT02365142) (61). A very interesting study at the University of Leeds (NCT02696876) (61) is recruiting patients to examine whether synovium brushing can help increase the reparative capacity of the knee. The discovery of a resident population of MSCs within synovial fluid with ready access to cartilage and other joint tissues offers a novel strategy for joint repair. Current arthroscopic procedures result in the removal of all synovial fluid MSCs due to continuous irrigation throughout the procedure. This study might benefit the patient by increasing the reparative capacity of the joint by bolstering MSC numbers and retaining those cells within the joint after surgery. By accessing MSCs from the synovium it is anticipated that these cells would be entrapped/migrate into the marrow clot formed by microfracture of the subchondral bone. These MSCs would supplement those from the marrow and may result in faster, better quality repair. MSCs will likely be emerging as important therapeutics for RA as well, given that MSC-derived trophic factors (secretome, exosomes) can promote chondrocyte proliferation, reduce chondrocyte apoptosis and fibrosis, and act in an anti-hypertrophy fashion (85). Also, MSC can reduce inflammation and in certain contexts inhibit osteophyte

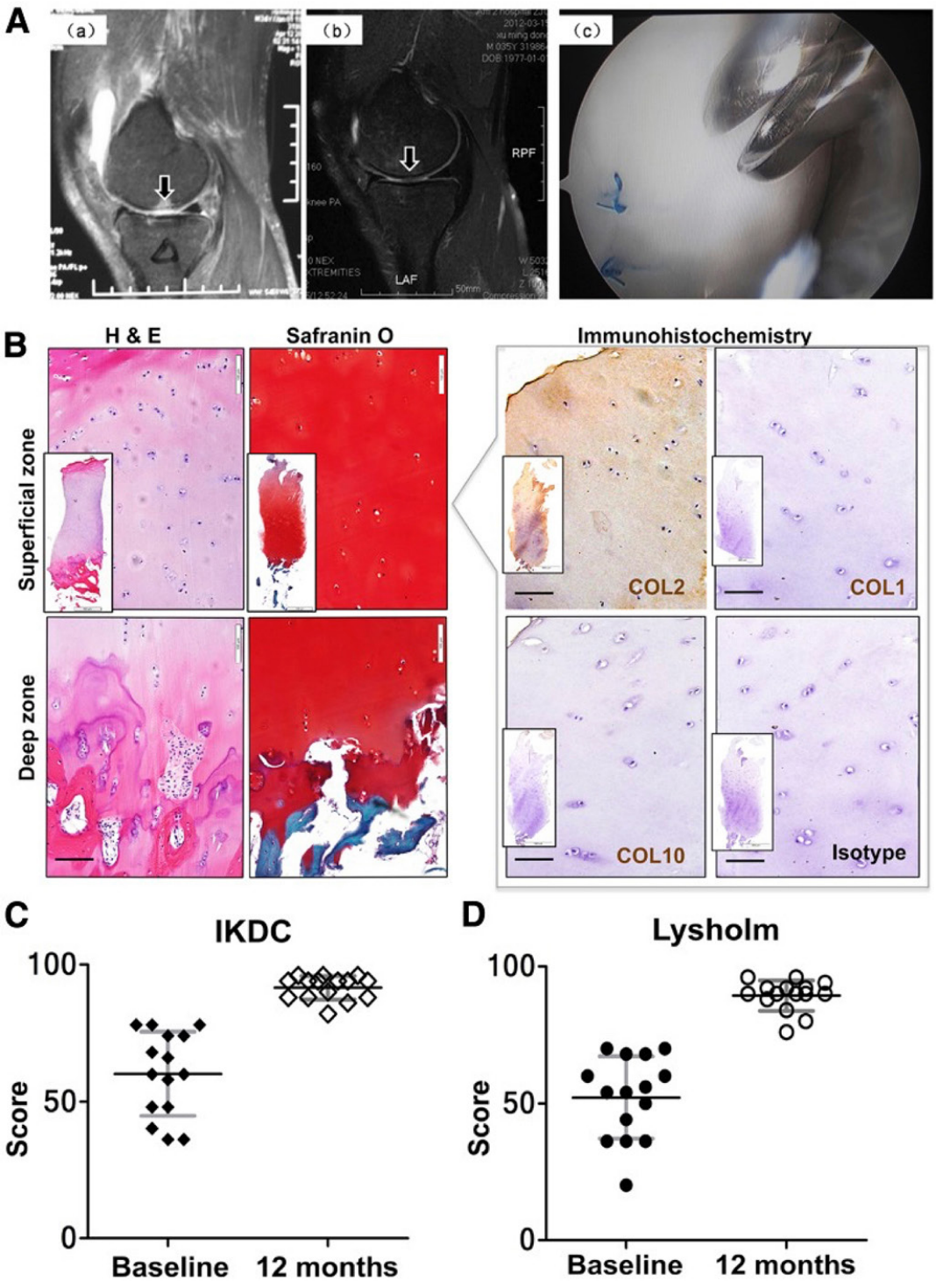

Figure 5. Example of a novel cell therapy for cartilage repair using chondrocyte-derived progenitor cells (CDPCs) transplanted in patients with large size (>6 cm^2) cartilage defects. (A): Representative magnetic resonance imaging (MRI) and arthroscopy images to assess cartilage repair. (a) Preoperative MRI/sagittal oblique imaging of femoral condyle with cartilage defect; (b): MRI of repaired cartilage 12 months after CDPC transplantation. Complete attachment and filling of the defect are shown (arrows: transplanted areas); (c): Second arthroscopy 12 months after CDPC transplantation. (B): Histological analysis of repaired tissue, 12 months after CDPC transplantation. Insets show low-magnification structural overview of cylinder harvested with bio-punch (bar, 500 μm). High-magnification micrographs show the superficial zone and deep zone of repaired cartilage in the repair tissue. (B, left): H&E staining (bar, 100 μm); (Safranin O staining (bar, 100 μm). (B, right): Immunohistochemistry of COL2, COL1, and COL10 for repaired cartilage. Tissue immunostaining was detected with diaminobenzidine (reddish brown), with hematoxylin nuclear counterstain (bar, 100 μm). (C): Clinical scoring evaluations of IKDC score. Each data point represents one patient, compared with preoperative situation (n = 15; p = 1.4.3 × 10−6). (D): Clinical scoring evaluations of Lysholm score. Each data point represents one patient, compared with preoperative situation (n = 15; p = 1.0.4 × 10−7). Abbreviations: H&E, hematoxylin and eosin; IKDC, International Knee Documentation Committee. Reproduced with permission from (80).

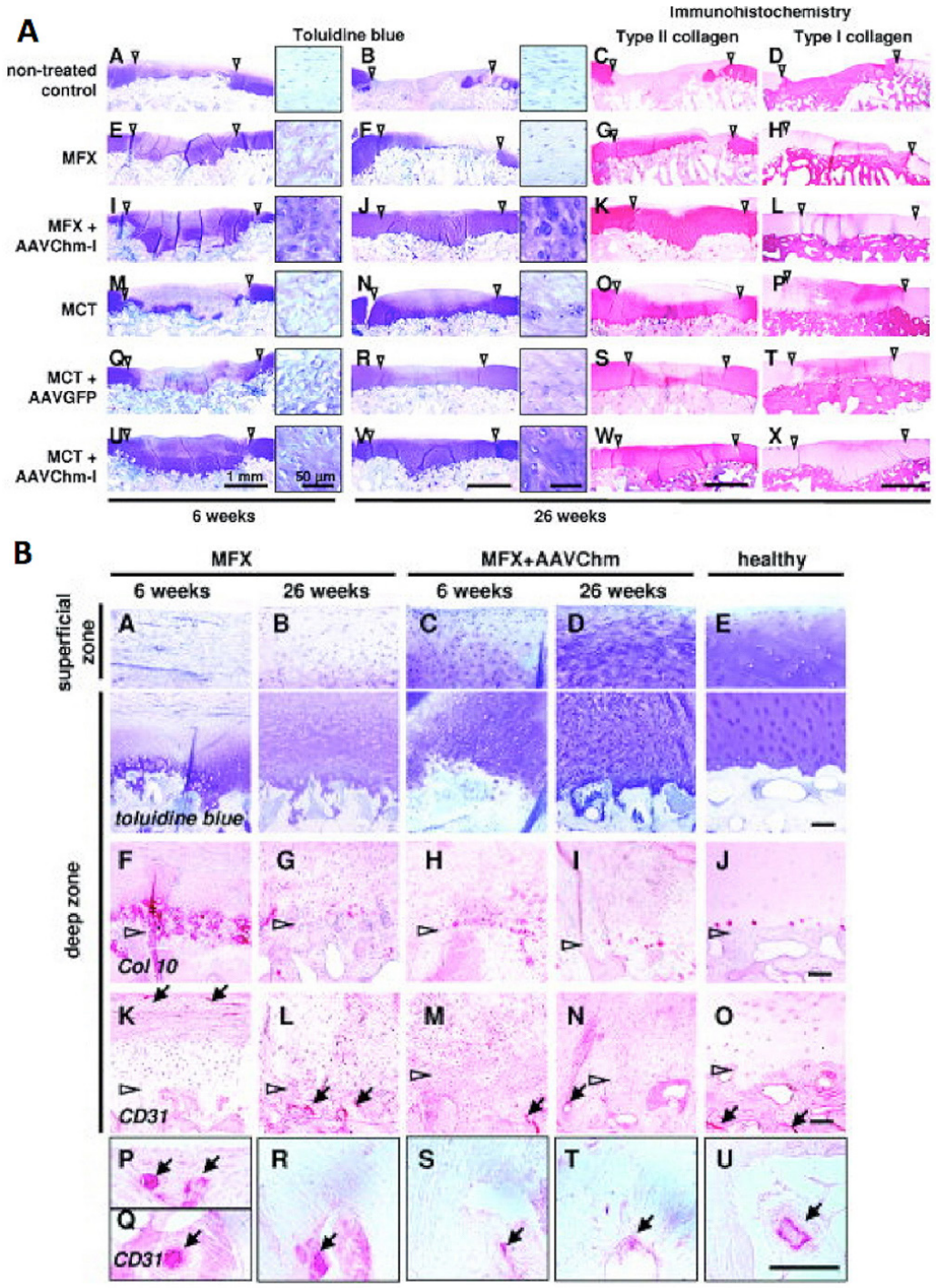

Figure 6. An example of AAV therapy for repairing cartilage in a porcine model. (A) Porcine cartilage lesions 6 and 26 weeks after microfracturing (MFX) or matrix-associated cell transplantation (MCT). A–D, Toluidine blue staining and staining for type II collagen (C) and type I collagen (D) in untreated control cartilage lesions. E–H, Toluidine blue staining and staining for type II collagen (G) and type I collagen (H) in lesions treated by MFX. I–L, Toluidine blue and staining for type II collagen (K) and type I collagen (L) in lesions treated by MFX and with adeno-associated virus vectors expressing chondromodulin 1 (MFX+AAV-Chm-1). M–P, Toluidine blue staining and staining for type II collagen (O) and type I collagen (P) in lesions treated by MCT. Q–T, Toluidine blue and staining for type II collagen (S) and type I collagen (T) in lesions treated with MCT+AAV-GFP. U–X, Toluidine blue and staining for type II collagen (W) and type I collagen (X) in lesions treated with MCT+AAV-Chm-1. Insets, higher-magnification views. Arrowheads, defect borders. Bars = 1 mm, and in insets = 50 µm. (B) Detection of chondrocyte hypertrophy and vascularity in superficial and deep zones of the repair tissue in porcine knee joint lesions 6 and 26 weeks after treatment by microfracturing (MFX) or by MFX and with adeno-associated virus vectors carrying chondromodulin 1 cDNA (MFX+AAV-Chm-1) and in healthy porcine articular cartilage. A–E, Toluidine blue staining. F–J, Type X collagen staining. K–O, Immunostaining for CD31-positive vessel structures (arrows) in superficial fibrous tissue of MFX-treated defects (K) and in subchondral bone marrow spaces (L–O), but not in hypertrophic cartilage or hyaline-like repair cartilage. P–U, Higher-magnification views of K–O. Both P (superficial zone) and Q (subchondral bone space) are higher-magnification views of K. Arrowheads, osteochondral junction. Bars = 50 µm. Reproduced with permission from (67).

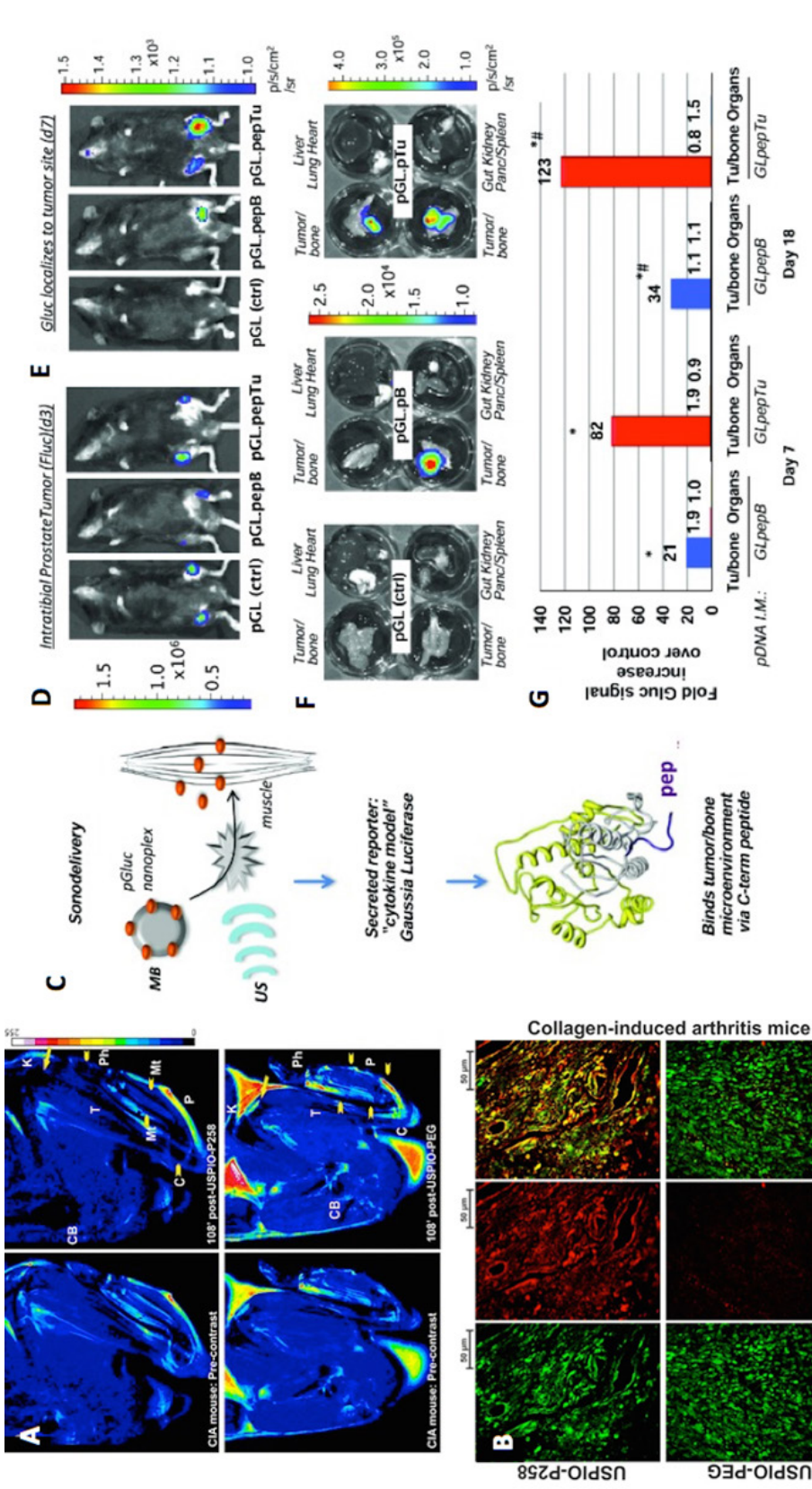

Figure 7. Example of targeted strategies. (A) Example of targeting nanoparticles using an IL-7 receptor alpha (*IL-7Rα*) targeting peptide. *In vivo*, MRI imaging shows detection of targeted nanoparticles accumulating at an area affected by inflammatory arthritis (collagen-induced arthritis, CIA). Color overlay of rapid acquisition with relaxation enhancement (RARE) magnetic resonance images of the hind limbs of mice with *CIA* injected with either ultra-small super paramagnetic particles of iron oxide (*USPIO*)-P258 or USPIO-poly(ethylene glycol) (*USPIO-PEG*) in pre-contrast and ~2 h (108') post contrast. *Arrowheads*, hind paw; *arrow*, knee. *Color overlays* are related to the negative signal enhancement, which is observed by the shift of colors from *blue* to *black*; on the post-USPIO-P258 image *arrowheads* and *arrow* indicate the negatively contrasted constituents of the hind limb. *C*, calcaneus, *CB*, coxal bones, *K*, knee, *P*, paw, *Ph*, phalanx, *Mt*, metatarsal bones, and *T*, tibia. (B) Immunofluorescent co-localization of *USPIO*-P258 (stained *red* by Texas Red) with *IL-7Rα* (stained *green* by fluorescein) on the joints of mice with CIA is evidenced by the *yellow/orange* color (*Merge*). No co-localization was observed for USPIO-PEG. Note that anti-PEG antibody recognizes both USPIO-P258 and USPIO-PEG via their PEG coat. *Reproduced from reference (95) under the terms of the Creative Commons Attribution 4.0. International License.* (C) An example of a secreted reporter protein, Gaussia luciferase (Gluc). Gluc was targeted via modification at the C-terminus with tumor or bone targeting peptides that enable localization to intratibial prostate tumors. Left panel, schematic of the gene delivery method using ultrasound mediated transfection of microbubbles carrying plasmid DNA (GLuc) and a polymer nanoplex. (D) TC2R-*Luc*-marked prostate tumor cells implanted in tibia on day 0 give a detectable signal by day 3. Plasmids expressing Gluc (p*GL*) are delivered intramuscularly (I.M.) via sonodelivery on day 4, and mice imaged for GLuc expression at day 7 (E). (F) *Ex vivo* imaging on day 7 shows that only targeted p*Gluc* show signals in tumor/bone metastases. (G) Quantification of fold GLuc signal over control in tumor/bone versus normal organs on day 7 or 18 postsonoporation. In addition, **p*<0.0.4 compared with normal organs; #*p*<0.0.3 Tu/bone on day 18 compared with Tu/bone signals on day 7. Ctrl refers to delivery of p*GL*, a plasmid expressing *Gluc* with a scrambled nonspecific peptide sequence. Reproduced with permission from (103).

formation. It will be intriguing to assess their role in RA and control of inflammation and promotion of bone and cartilage repair in eroded joints.

4.5.3. Newly developing cellular agents for anti-inflammation or bone repair applications

MSC are an emerging therapeutic for arthritis with many anti-inflammatory and regenerative applications, naïve or transduced with viruses. Adenovirus-transduced MSC overexpressing viral interleukin 10 (vIL-10) have promise in controlling levels of activated T cells in a collagenase model of osteoarthritis (86). Although there was no significant difference in knee OA scores between any of the groups, a trend toward less damage was observed when animals treated with vIL-10 expressing MSCs. There was a significant reduction in the amount of activated CD4 and CD8 T cells in the vIL-10-expressing MSC group. Adenoviral transfer into joints of Sprouty2 (SPRY2), a tumor suppressor gene that blocks both extracellular signal regulated kinase (ERK) and protein kinase B (AKT) signaling cascades, suppressed adjuvant-induced arthritis in rats (87). AdSPRY2 suppressed the production of proinflammatory cytokines and MMPs, and the activation of ERK and AKT signals in adjuvant-induced arthritis (AIA) ankle joints. An interesting use of virus is the use of foamy virus-adenovirus for gene therapy of arthritis. These are hybrid vectors capable of being efficient transducers of cells like adenovirus vectors with the advantage of stable integration into the host cell genome (88).

4.6. New developments in bispecific antibodies and targeting of therapeutic agents

4.6.1. Bispecific antibodies

Biologicals targeting inflammatory cytokines have been applied in the clinic and a new trend exists for double-target and multi-target drugs have significant advantages in therapy. A bispecific antibody against both human IL-1β and IL-17A was tested in collagen-induced arthritis (CIA) mice, with Adalimumab and Dexamethasone as controls. The antibody could bind IL-1β and IL-17A and alleviated the severity of arthritis, decreasing the serum levels of inflammatory cytokines. Several genes were downregulated including IL-1β, IL-2, IL-6, IL-17A, TNF-α, IFN-γ, and MMP-3, and IL-10 was upregulated. Reduced histological damage and inhibition of bone destruction was observed with the treatment. The antibody also inhibited nuclear translocation of the p65 subunit and cytoplasm IκB-α degradation by blocking IL-1β and IL-17A, upstream of the NF-κB pathway. High doses of bispecific antibody had a more beneficial effect on CIA mice than Adalimumab and Dexamethasone, suggesting promise for this bispecific antibody for RA therapy (89). Another bispecific antibody neutralized both TNF and receptor activator of NF-κB ligand (RANKL). This was a humanized 8G12 antibody produced in Chinese hamster ovary cells. The h8G12 antibody reduced TNF-mediated apoptosis of fibroblastic cells (L929) and significantly inhibited leukocyte infiltration in a murine allergic contact inflammation model. Concurrent with the inhibition of apoptosis, the 8G12 antibody significantly reduced the number of osteoclast-like cells in a dose-dependent manner. These results demonstrated that the 8G12 antibody neutralized the activities of TNF and RANKL, suggesting a potential use for treating RA (90).

4.6.2. Targeted cytokines

Interleukin-23 (IL-23), a heterodimeric cytokine of covalently bound p19 and p40 proteins, has recently been closely associated with development of several chronic autoimmune diseases such as psoriasis, psoriatic arthritis or inflammatory bowel disease. Released by activated dendritic cells, IL-23 interacts with IL-23 receptor (IL-23R) on Th17 cells, thus promoting intracellular signaling, a pivotal step in Th17-driven pro-inflammatory axis. One study examined the effect of inhibiting the binding of IL-23 cytokine to its cell-surface receptor by novel inhibitory protein binders targeted to the p19 subunit of human IL-23. The inhibitory binders were selected from a combinatorial library derived from a scaffold of albumin-binding domain (ABD) of streptococcal protein G, and ribosome display selection, to yield a collection of ABD-derived p19-targeted variants, called ILP binders. Using *in silico* docking in combination with cell-surface competition binding assay, a group of inhibitory candidates was identified that diminished binding of recombinant p19 to the IL-23R on human THP-1 monocytes. The best p19-blockers also inhibited IL-23-driven expansion of IL-17-producing primary human $CD4^+$ T-cells. These novel binders are unique IL-23-targeted probes useful for producing novel p19/IL-23-targeted anti-inflammatory biologics (91).

Cytokines also can be targeted via peptides or ligand-mediated targeting towards receptors upregulated in cells during the pathogenesis of arthritis. For example, peptides have been described that are able to target nanoparticles or molecules to arthritic joints by binding to collagen II or molecules unique to the arthritic vasculature (endothelial cells). Another interesting targeting peptide has been recently described that can target IL-7Ra. IL-7 and IL-7Ra are over-expressed in the RA synovium. IL-7 plays a crucial role in T cell activation and osteoclastogenesis by upregulating T cell-derived cytokines, including RANKL. In RA synovitis, not only T cells, but also synovial macrophages and fibroblasts over-express IL-7Rα, thereby making IL-7Rα the transcript most differentially expressed between RA and other

inflammatory joint conditions such as osteoarthritis and psoriatic arthritis (92). Synovial fibroblasts also produce a high quantity of soluble IL-7Rα subsequent to their stimulation by cytokines such as TNF, IL-1β and IL-17 (93). The increased expression and high serum concentrations of soluble IL-7Rα is associated with poor response to anti-TNFα therapy in patients with RA (94). After several steps of phage display and peptide screening, two IL-7Rα-specific heptapeptides were selected based on their affinity for the target (extracellular domain of IL-7Rα, which contains a fibronectin type III repeat-like sequence) (95). The linear peptide had the lowest affinity for fibronectin itself, thus it was examined for its ability to be specific *in vivo* in molecular imaging. After grafting to ultra-small super paramagnetic particles of iron oxide, this peptide produced a strong contrast on magnetic resonance imaging (MRI) in mice with CIA, even at 2 hours post injection. The co-localization of the peptide/particles with IL-7Rα-expressing cells in the synovial tissue from CIA mice and its ability to discriminate the level of IL-7R expression and the disease severity confirmed its efficacy as an *in vivo* IL-7Rα imaging agent. Interestingly, a cyclic peptide, which was less adequate for molecular imaging because of higher affinity for fibronectin, had a strong ability to compete with IL-7 for the IL-7Rα binding sites, making it a potential candidate for blocking applications. Accordingly, P725 prevented the signal transducer and activator of transcription 5 (STAT5) activation induced by IL-7 in ADC-stimulated Jurkat cells. The two peptides identified in this work demonstrate that IL-7Rα targeting in RA presents potential applications for *in vivo* molecular imaging and putative blocking and/or targeting purposes (95).

4.6.3. Targeted nanoparticles

Interesting nanotherapies in development include liposomes, nanoparticles and conventional micelles loaded with limited amounts of drugs that may be unstable in the circulation and that can be targeted to arthritic joints. A new drug delivery system of pH-sensitive polymeric micelles has been recently described based on an acid-labile hydrazone bond (96). Amphiphilic conjugates of a polyethylene glycol (PEG)-based derivative and the hydrophobic drug prednisolone (PD) self-assembled into PD micelles with a drug loading of ~20%. When the micelles reached the acidic environment of synovial fluid, the hydrazone bonds hydrolyzed, releasing free PD. Intravenous injection of PD micelles into mice with collagen-induced arthritis led to PD accumulation in affected joint tissues. PD concentrations in plasma and joints of arthritic mice were significantly higher after injection with PD micelles than after injection with free PD. The enhancement effect in joints was 4.6.-fold based on the area under the concentration-time curve. *In vivo* pharmacodynamics showed PD micelles to have better anti-inflammatory and disease-modifying effects than free PD, suggesting that PD micelles may be promising for targeted drug delivery in inflammatory disease.

4.6.4. Imaging and therapeutic strategies

The ability to select patients who will respond to therapy is especially acute for inflammatory diseases, where the cost of therapies is high and progressive joint damage to joints is often irreversible. An imaging strategy based on targeting a radioimaging agent to folate-expressing macrophages in inflammatory sites has been developed. By assessing uptake of a folate receptor-targeted Technetium-99m radioimaging agent (^{99m}Tc-EC20) by a subset of macrophages, the success of therapies can be monitored non-invasively in several murine models of inflammatory pathology. A decreased uptake of the agent can be detected in inflamed lesions upon initiation of successful therapies, but no decrease in uptake is detected upon administration of ineffective therapies. This predictive imaging assay could help reduce costs and minimize morbidities associated with failed inflammatory disease therapies and is under clinical development (97). The folate receptor also can be used to target drugs and nanoparticles to macrophages in areas of inflammation. Recently, folate-anchored carbon nanotubes have been used to target MTX to inflammatory regions in arthritis models. Folate was conjugated to amidated multi-walled carbon nanotubes and MTX was loaded into the pristine and functionalized-nanotubes. Nanotubes could release ~60–66% of drug in phosphate buffered saline (PBS, pH 7.4.) in 24 h. *In vivo,* Folate conjugated nanotubes significantly inhibited arthritis progression, and the biological half-life and volume of distribution of MTX as compared to controls (MTX-loaded naked nanotubes or free MTX). In *in vivo* biodistribution studies, MTX was found to be significantly higher in arthritic joints from folate functionalized nanotubes as compared to controls. This study highlighted that a sustained and targeted drug delivery system can be utilized based on targeting the folate receptor (98); dendrimers and PEGylated forms of nanoparticles have also been used with the folate receptor system (99).

5. CONCLUSIONS AND FUTURE DIRECTIONS.

There is great promise in the new and developing agents for treating chronic forms of non-infectious arthritis such as OA and RA. Drugs that will prove beneficial will likely be multi-functional and inhibit inflammation within the joint while enabling tissue repair to occur. Promising new developments in OA include cellular therapies with chrondroprogenitor or MSCs, since cartilage is very challenging to repair. For RA, promising development include targeted cytokines, which may be able to reduce inflammation

while removing inhibitory signals preventing the repair of bone by osteoblasts. Restoring balance to joints will perhaps require highly specific drugs such as targeted drugs, which may impact several pathways or cells at once within the diseased joint. Gene delivery is experiencing a comeback in several chronic conditions, and this may include high promise for sustained delivery of biologics to joints in the context of arthritis. The dynamics of fluid and molecule kinetics in joints suggests a great need remains for strategies that can promote a sustained accumulation of therapeutics in joints to impact the many cells and signaling that are imbalanced in OA and RA. Finally, the ability to image responses to treatment in real-time with new targeted imaging agents will enable researchers to continue making great progress in adapting current biological or small molecule drugs to arthritis therapy or aiding in the development of new ones. New drugs that are emerging might include epigenetic drugs.

Epigenetic drugs may be able to control inflammation and promote repair of tissue affected by arthritis. RANKL-induced changes in chromatin state appear to be important for osteoclastogenesis, but these epigenetic mechanisms are yet not well understood. A small molecule (I-BET151) has been described that targets bromo and extra-terminal (BET) proteins that 'read' chromatin states by binding to acetylated histones. I-BET151 suppresses pathologic bone loss in TNF-induced inflammatory osteolysis, inflammatory arthritis and post-ovariectomy models by suppressing osteoclastogenesis. The mechanisms appear to be via a c-myc gene (MYC)-NFATc1 axis, which is important for osteoclastogenesis. These interesting findings suggest that targeting epigenetic chromatin regulators may hold promise for treatment of inflammatory and estrogen deficiency-mediated pathologic bone resorption (100). Other new developments are the use of micro RNA (miRNA or miR) to modulate changes in inflammatory gene expression profiles and promote osteogenesis or repair genes. For example, our group has recently shown that cytokine IL-27 promotes osteoblast differentiation, reduces expression of osteoblast inhibitory genes, and reduces osteoclast differentiation, in an apparent coordination with TGFβ/BMP/SMAD (mothers against decapentaplegic, Drosophila homolog) and JAK (Janus kinase)/STAT pathways. Interesting, the effects of this cytokine can be augmented by miRNA co-treatment. miR-29b and miR-21 augmented IL-27 proosteogenic while downregulating osteoclastogenic signals and also worked to reduce inflammatory signaling in activated macrophages, while miR-21 and miR-20b worked with IL-27 to reduce inflammatory gene expression in fibroblasts and T cells. It appears that several miRNAs can be utilized to enhance IL-27's impact on modulating osteogenesis and reducing proinflammatory signaling, and these effects may be extended also to other osteoimmune cytokines (101). Future directions promise to take biological and small molecule targeting to the next level, innovating at the level of delivery vehicles (nanoparticles, nanotubes, etc), to innovations in peptide or ligand-mediated targeting and these approaches might be applicable to several classes of drugs and biologics. Bi-specific targeting is of special promise and the approach might further enhance the specificity of drug or biologic targeting *in vivo*.

6. ACKNOWLEDGEMENTS

We would like to acknowledge support from National Institutes of Health grants R21CA179699 and R01CA196947 (MLF), and support from the College of Veterinary Medicine and the Department of Basic Medical Sciences at Purdue University.

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Abbreviations: RA, rheumatoid arthritis; OA, osteoarthritis; TNF, tumor necrosis factor; RANKL, receptor activator of NF-κB ligand; IL, interleukin; MTX, methotrexate; CD, cluster of differentiation; DKK1, dickkopf 1; SOST, sclerostin; BMP, bone morphogenic protein; DMARDs, disease-modifying anti-rheumatic drugs; CIA, collagen-induced arthritis; OPG, osteoprotegerin; WNT, Wingless-Type MMTV Integration Site Family; CTLA-4, Cytotoxic T-Lymphocyte Associated Protein 4; PTH, parathyroid hormone; DAMPs, damage associated molecular patterns; TLRs, toll-like receptors; NF-κB, nuclear factor kappa-B; AP-1, activator protein 1; NSAIDs, nonsteroidal anti-inflammatory drugs; DMOADs, disease modifying OA drugs; MMP, matrix metalloproteases; COX, NOS, FGF, fibroblast growth factor; ECM, extracellular matrix; IFNγ, interferon gamma; TGFβ, transforming growth factor beta; IGF1, insulin-like growth factor 1, PDGF, platelet-derived growth factor; CTGF, connective tissue growth factor; VEGF, vascular endothelial growth factor; NGF, nerve growth factor; Mw, molecular weight; cHA-Dex, crosslinked hyaluronic acid hydrogel with dexamethasone; eIF2a, Eukaryotic Translation Initiation Factor 2A; DUSP2, dual specificity protein phosphatase 2; CAIA, collagen-antibody induced arthritis; LPS, lipopolysaccharide; PMA, phorbol 12-myristate 13-acetate; NFATc1, nuclear factor of activated T-cells 1; MSCs, mesenchymal stromal/stem cells; CBFβ, transcription factor core-binding factor β subunit; RUNX1, runt-related transcription factor 1; micro-CT, micro computed tomography; IL-1Ra, interleukin 1 receptor antagonist; STAT6, signal transducer and activator of transcription 6; IFNβ, interferon beta; AAV, adeno-associated vector; HSV, herpes simplex virus; ICP4, immediate early gene 4; T/0, second generation HSV vector derivative deficient for the immediate early genes ICP4, 22, and 27; bFGF, basic fibroblast growth factor; ACLT, anterior cruciate ligament transection; GAG, glycosaminoglycan; ADAMTS-5, disintegrin and metalloproteinase with thrombospondin motifs 5; TIMP-1, TIMP metallopeptidase inhibitor 1; rAAV2, serotype 2 AAV; sc, self-complementary; GFP, green fluorescent protein; cFLIP, caspase 8 and FADD like apoptosis regulator; FLS, fibroblast like synoviocytes; TRAIL, tumor necrosis factor superfamily member 10; Chm1, chondromodulin 1; EGFP, enhanced green fluorescent protein; US, ultrasound; siRNA, small interfering RNA; pEPIto, an episomal plasmid; ACI, autologous chondrocyte implantation/transplantation; COL2, collagen type II; CDPC2, chondrocyte-deriver progenitor cells; BM-MSC, bone marrow-derived MSC; PRP, platelet rich plasma; ASC, adipose-derived MSC; vIL-10, viral interleukin-10; SPRY2, sprouty 2; ERK, extracellular signal regulated kinase; AKT, protein kinase B; AIA, adjuvant-induced arthritis; ABD, albumin-binding domain; MRI, magnetic resonance imaging; STAT5, signal transducer and activator of transcription 5; PEG, polyethylene glycol; PD, prednisolone; 99mTc, Technetium-99m; PBS, phosphate buffered saline; BET, bromo and extra-terminal domain; MYC, c-Myc gene; miRNA, micro RNA, SMAD, Mothers Against Decapentaplegic, Drosophila, Homolog Of, JAK, Janus Kinase

Key Words: Arthritis, Therapeutics, Targeted, Biologics, Gene delivery, Intra-articular, Review

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