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Review

Inflammation in joint injury and post-traumatic osteoarthritis

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SUMMARY

Inflammation is a variable feature of osteoarthritis (OA), associated with joint symptoms and progression of disease. Signs of inflammation can be observed in joint fluids and tissues from patients with joint injuries at risk for development of post-traumatic osteoarthritis (PTOA). Furthermore, inflammatory mechanisms are hypothesized to contribute to the risk of OA development and progression after injury. Animal models of PTOA have been instrumental in understanding factors and mechanisms involved in chronic progressive cartilage degradation observed after a predisposing injury. Specific aspects of inflammation observed in humans, including cytokine and chemokine production, synovial reaction, cellular infiltration and inflammatory pathway activation, are also observed in models of PTOA. Many of these models are now being utilized to understand the impact of post-injury inflammatory response on PTOA development and progression, including risk of progressive cartilage degeneration and development of chronic symptoms post-injury. As evidenced from these models, a vigorous inflammatory response occurs very early after joint injury but is then sustained at a lower level at the later phases. This early inflammatory response contributes to the development of PTOA features including cartilage erosion and is potentially modifiable, but specific mediators may also play a role in tissue repair. Although the optimal approach and timing of anti-inflammatory interventions after joint injury are yet to be determined, this body of work should provide hope for the future of disease modification tin PTOA.

Introduction

Joint injury is a well-established risk factor for development of OA[1]; approximately 12% of all OA cases may be due to prior joint trauma[2]. After a joint injury, surgery to re-stabilize the joint is often indicated for functional recovery and pain relief. Altered biomechanics from injury put the patient at risk for progressive joint degeneration[3], but surgical re-stabilization does not reduce this risk[4]. Many investigators have speculated that molecular and cellular alterations to joint tissues resulting from injury are not readily reversible by joint stabilization. It is therefore important to understand the biological mechanisms triggered by joint injury, and how they change over time, to develop more successful intervention strategies for prevention and treatment of post-traumatic OA (PTOA).

Low-grade cellular infiltration, cytokine production, and inflammatory activation of articular chondrocytes, synoviocytes and other joint tissue cells are common in OA. Human studies and animal models have revealed details about the course and nature of inflammatory mediator production, inflammatory pathway activation and synovial cellular infiltration after joint injury. The importance of inflammatory cytokines in promoting enzymatic mediators of cartilage catabolism (i.e., MMPs, ADAMTS enzymes) makes early inflammation a suitable target for prevention of PTOA. In this review, we will focus on recent literature regarding time-dependent production of specific inflammatory mediators in both clinical studies as well as animal models of PTOA and the multiple sources of inflammation in OA both local and systemic. Further, we discuss current understanding of the activation of inflammatory pathways as well as pharmacologic targeting of specific mediators of inflammation using disease models of PTOA.

Methods

A PUBMED search limited to the past 10 years was pursued for this narrative review using the following strategies: (1)

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"Inflammation AND osteoarthritis AND post-traumatic", (2) "Immunity AND osteoarthritis NOT Rheumatoid", and (3) "Inflammation AND osteoarthritis NOT Rheumatoid" which together yielded 1358 references. We removed review articles, redundant citations and irrelevant references. We limited references that were more than five years old to those that provide important contributions and context. A small number of references not revealed in the search but known to the authors were added.

Inflammatory mediator production after joint injury

Common injuries associated with PTOA development include disruption of the anterior cruciate ligament[5], meniscus[6,7] and intra-articular fracture[8]. These injuries result in hemarthrosis, chondrocyte death and bruising of underlying bone, and in the release of inflammatory mediators in the acute post-injury period. A recent study reported synovial fluid (SF) levels of inflammatory cytokines from 111 patients presenting with traumatic ACL tears, and categorized levels according to time since injury[9]. Fluid was aspirated at presentation, which ranged from the day of injury to 23 days later. Levels of IL-1β, IL-6, IL-8 and TNFα were highest in fluids aspirated on day 0–1 post-injury, but then remained elevated compared to uninjured controls. Levels of cytokines peaked one day earlier than markers of cartilage proteoglycan breakdown, and IL-8 and TNFα were higher in those with concomitant osteochondral fracture[10]. Similar trends were seen in a study of patients aspirated within 48 h of ACL injury[11].

Anti-inflammatory molecules are also observed post-injury. Following ACL injury, SF levels of IL-10 were elevated in the first two weeks post-injury, but were lower in fluids aspirated later[11,12]. *In vitro*, exposure to IL-10 along with IL-4 protects cartilage from inflammatory effects of blood products, suggesting a potential chondroprotective role in response to the hemarthrosis that often accompanies ACL injury[13]. A study of human subjects using microdialysis catheters showed an increase in IL-10 in the joint capsule following exercise[14], suggesting IL-10 may also contribute to the beneficial effects of exercise. IL-1 receptor antagonist (IL-1Ra), the natural antagonist of IL-1α and β, is detectable in SF from uninjured joints[15]. Levels of IL-1Ra are reported to be elevated in SF aspirated in the acute phase after ACL injury, but then lower than normal in fluids aspirated later (three to > six weeks after injury)[16]. A decrease in IL-1Ra levels could leave the activity of IL-1 unopposed in the injured joint. However, there is some disagreement as to whether SF IL-1α or β are consistently elevated following injury[16,17]. SF handling and method of detection are variables that might explain differences between studies, but whether SF levels of a specific molecule necessarily reflect local tissue concentration and importance in pathogenesis is unclear.

After the acute phase, cytokine concentrations generally decrease but some are reported elevated months to years after joint injury. In a study of chronic ACL tears (aspirated over 6 months since injury) there was a modest difference in the concentration of SF IL-1β between patients and healthy controls, and levels correlated with the degree of chondral damage[18]. SF IL-1Ra levels were also higher compared to controls. Other authors have reported that SF IL-1 levels drop to normal in patients aspirated up to three months after injury, while levels of IL-6 and TNFα remained elevated 6 months post-injury[19]. None of these studies employ longitudinal sampling of SF from the same patients. A recent review of cross-sectional studies from ACL-injured patients[20] concluded that in the first week post-injury[20], levels of IL-6, IL-8, and IL-1Ra were consistently elevated, while there was more variability in reported levels of IL-1α and β. In the subacute period (1 week–2 months), IL-6 levels remain elevated across studies, and other cytokines and chemokines such as MCP-1, MIP-1β, and IFNγ have been reported. In the subacute to chronic phase (2 months to >1yr), while IL-6 and IL-8 remain elevated, IL-1Ra levels were often reported lower than controls. TNFα elevations in the subacute period persist into the chronic phase (>1yr post-injury). There are clearly different phases of inflammation following injury[21]. The initial injury results in acute tissue damage, prompting a robust release of pro- and anti-inflammatory mediators. We speculate that the pro-inflammatory response in certain patients lacks adequate control post-injury, thereby perpetuating chronic inflammation and tissue damage leading to PTOA (Fig. 1).

Soluble inflammatory mediators in animal models

A variety of animal models that involve injury by surgical, traumatic or chemical means[22] are used to understand biologic and mechanical mechanisms in PTOA. Commonly used surgical models include meniscectomy or ACL transection in rodents, rabbits, and sheep. Loading injury models in rodents, including noninvasive tibial plateau fracture, have also been developed[23,24]. By altering the mechanism or extent of injury, the cadence of OA progression can be altered[25]. With appreciation of the post-injury inflammatory response seen in patients, these models are being used to investigate the role of inflammation in PTOA.

Data from multiple species demonstrate production of inflammatory mediators post-injury, including cytokines and chemokines observed in human patients[26–30]. Recent studies address how inflammatory gene expression varies with time after injury in common rodent models. Wei and colleagues[31] compared cartilage transcriptomic profiles in rats undergoing meniscal transection to sham-operated controls at days 3, 7 and 21 post-injury. 337 differentially expressed genes were observed at day 3, with smaller numbers at day 7 (79) and day 21 (112). CCL2 (MCP-1), a chemokine involved in macrophage recruitment, was upregulated at all three time-points although highest at day 3. Pathway analysis revealed that genes involved in extracellular matrix (ECM) turnover and the inflammatory response were observed. mRNA expression analysis in the murine destabilization of medial meniscus (DMM) model also provided insight on time-dependent transcriptional regulation after injury[32–34]. In this slowly progressive model, global upregulation of transcription was seen early (2–4 weeks), followed by down-regulation at 8 weeks and more modest up-regulation again at 16 weeks post-DMM indicating distinct phases of transcriptional activity after injury. Consistent with other investigators, genes and pathways related to ECM remodeling were activated most strongly indicating an attempted reparative response. But genes involved in inflammatory processes were also identified, notably the chemokine CCL21 which was upregulated at all post-DMM time points examined (2, 4, 8 and 16 weeks post-surgery). The significance to disease of this particular chemokine, known for controlling normal trafficking of lymphocytes and dendritic cells, has yet to be determined. Taken together, these studies demonstrate that activation of inflammatory genes occurs most robustly early after injury, but is sustained at lower levels, in agreement with human observational data.

As surgical intervention does not reduce the risk for PTOA, an important question is whether acute post-injury inflammation is independent of joint instability. Two recent approaches have addressed this question. In an ovine model of ACL tear followed by immediate reconstruction to re-establish stability[35], cartilage changes were observed 2 weeks after, and persisted but did not progress significantly by 20 weeks post-surgery[36]. Similarly, IL-1β, IL-6 and IL-8 were increased in synovium at 2 weeks, but normalized by 20 weeks suggesting resolution of acute inflammation in the absence of chronic instability. The authors concluded that the immediate post-injury inflammatory response contributes

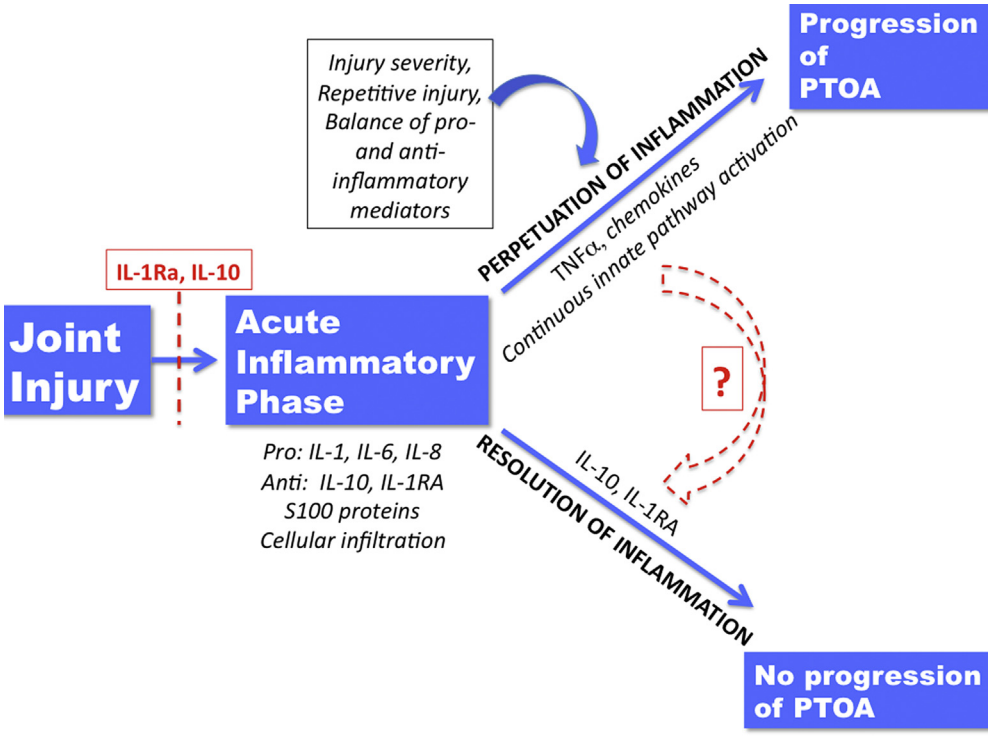

Fig. 1. Perpetuation of inflammation as a mechanism leading to PTOA: Joint injury sets off a robust acute inflammatory response that includes production of both pro- and anti-inflammatory cytokines, molecular "danger" signals such as the S100A family of proteins, and activation of the complement cascade. In certain individuals the influence of anti-inflammatory factors (i.e., IL-10 and IL-1RA) produced in the early post-injury period may allow for resolution of inflammation and reduce the risk of progressing to PTOA. However, perpetuation of inflammation via continued activation of innate inflammatory pathways may promote progression to PTOA in the injured joint. A number of factors may lead to perpetuation of inflammation after joint injury. These include the severity of the initial injury, the balance of pro- and anti-inflammatory factors produced in the acute and subacute post-injury periods, and repeated macro- or micro-injury which can promote continuous cycles of inflammatory activation involving innate pathways such as the complement cascade and danger-signal mediated pathways. Anti-inflammatory approaches (i.e., intraarticular IL-1Ra) to interfere with the acute phase (dashed red line) are already being tested in animal models and hold promise for prevention of PTOA. Whether resolution of chronic inflammation (dashed red arrow) can be accomplished to slow progression of structural change and chronic disability remains to be determined.

to the early cartilage degeneration observed. In another recent model, an injury was created in New-Zealand white rabbits by drilling two holes into the subchondral bone in a non-load bearing area of the inter-condylar notch[37]. Synovial expression levels of IL-1β, IL-6, and IL-8 as well as IL-1Ra were increased 2–3 fold within 72 h of injury, but then decreased to baseline by 3 weeks. Dexamethasone injected intra-articularly reduced expression of inflammatory markers and decreased cartilage degeneration in this model[38]. These two recent studies suggest that an early biologic inflammatory response to joint tissue injury can occur in the absence of gross instability, and that the early inflammatory response contributes to PTOA development. How much of this response is related to surgically induced bleeding into the joint remains unclear, but may be relevant to patients with joint injuries associated with hemarthrosis.

Post-injury inflammation reflects activity of multiple joint tissues

It is likely that multiple joint tissues contribute to inflammation after joint injury. Synovial macrophages, fibroblasts and chondrocytes are sources of cytokines and chemokines[39], and a wide variety of other inflammatory molecules. Infrapatellar fat pads from patients with knee OA have increased numbers of macrophages, granulocytes and lymphocytes[40], and this tissue can contribute to production of inflammatory cytokines in knee OA patients[41,42]. Fibrocartilaginous structures such as the meniscus in the knee[43] and the labrum in the hip[44] respond to and produce inflammatory mediators. Understanding which tissues and cell types contribute to inflammation has important implications for therapeutic targeting. Several studies have focused on the cell/tissue source of soluble pro-inflammatory products and enzymatic mediators of progressive cartilage loss (proteases of the MMP and ADAMTS families). Haslauer and colleagues[45] recently studied mRNA expression of proteases in joint tissues from mini-pigs after ACL transection. Synovium, ligament and scaffold tissue were predominant sources of MMP-13 and ADAMTS-4 in the first two weeks after ACL tear. MMP-13 increased up to 350-fold in synovium and 1000-fold in ligament by 14 days post-injury. In cartilage, ADAMTS-4 levels did not change while MMP-13 was down-regulated. These results are consistent with ACL-transection models in rabbits and rats[46–48], rodent meniscectomy[30,49] models, and observations in canine cranial cruciate ligament tears[27]. Thus, non-cartilaginous soft tissues may be important sources of inflammatory molecules and proteases that contribute to cartilage matrix catabolism early after injury. Whether anti-inflammatory targeting of specific extra-cartilaginous cells or tissues will improve interventions is yet to be determined.

Synovial thickening and cellular infiltration

Cellular infiltration into synovium also occurs in patients with joint injuries. Macrophage content of the synovium is increased in patients with knee OA and patients with joint injuries[39,50,51]. Molecular markers of macrophage activation in SF and serum were recently associated with progression of joint space narrowing and severity of pain in knee OA[52]. In patients presenting with meniscal

tears, lymphocytic perivascular infiltrates were also present and correlated with knee pain[53], but reports in other injury populations are lacking. Cellular infiltration in the early phases of joint injury in humans, prior to OA onset, needs to be more fully investigated.

Synovial inflammation in animal models

Recent data has suggested that synovial cellular infiltration and lining layer thickening are frequent findings in both large and small animal models of PTOA[23,36,37,54,55]. A synovial response was recently demonstrated post-injury in the DMM model using a comprehensive histological evaluation[54]. Synovitis was most severe in the first two weeks after surgery in both DMM and sham-operated limbs, but dropped to control levels by 8 weeks post-surgery in sham, and sustained out to 12–16 weeks in DMM-operated limbs. At least two noninvasive PTOA models have shown that the severity of the synovial reaction may be related to the severity of injury. Lewis and colleagues used a low or high-energy load applied to the murine knee joint to create a tibial plateau fracture noninvasively[23]. The degree of thickening and cellular density correlated to the severity of fracture. Similar results were seen in a murine model of repetitive load, where synovial thickening was reported 5 days post-injury and related to energy of the load applied[56]. Infiltrating cell types were not examined in these models, but clearly both invasive and noninvasive models of PTOA may be useful to examine the role of synovial pathology and cellular infiltration post-injury. The available data suggests that synovial changes occur early post-injury, are sustained at lower levels, and are related to severity/extent of injury (Table I).

In the DMM model, Jackson and colleagues used flow cytometric analysis of cells from synovial tissue 1-week post-DMM to show that total cell numbers and proportions of inflammatory monocytes and macrophages were increased[54]. Furthermore, mice deficient in protease-activated receptor-2 (PAR-2) were partially protected from OA. PAR-2 deficiency caused less robust macrophage responses to LPS stimulation, but did not alter chondrocyte activity, suggesting that protection was mediated by the reduction in macrophage activation. Synovial macrophage depletion was shown to protect against osteophytosis and MMP-mediated cartilage matrix degradation in the collagenase model of joint instability[57,58], but has not been reported in injury models. Synovitis and cartilage erosion following ACL transection were reduced in CD4 deficient mice[59], and reduced production of the chemokine MIP-1γ was hypothesized to be a potential mechanism. In the same model, cartilage erosion progressed more slowly in CD8 deficient mice[60]. These studies support a role for both infiltrating macrophages and T-lymphocytes post-injury in disease progression. The effects of these cell types at different stages and the mechanisms by which they impact disease need further exploration. Other leukocyte populations such as dendritic cells have been noted in certain models[61], but significance to disease has not been established.

Systemic influences on inflammation

Just as local inflammatory networks have been shown to play a role in osteoarthritis (OA) pathogenesis, systemic factors have also been implicated, and the strongest influence appears to be obesity. Obesity has long been recognized as a risk factor for OA[62]. While some of the risk may be due to increased joint loading, the association of obesity with the development of OA in non-weight bearing joints suggests that other mechanisms are involved[63]. The systemic effect of obesity on OA development is believed to be mediated, in part, by inflammatory substances (free fatty acids, reactive oxygen species cytokines and adipokines) produced by adipose tissue which can be released into the bloodstream[64]. Specific adipokines such as the molecules leptin and adiponectin have inflammatory and catabolic influences on joint tissues[65]. In addition, obesity is associated with higher levels of IL-6 and TNFalpha[66]. In addition, a number of studies have suggested that weight loss is an important intervention in the management of OA. The results of the IDEA trial indicated that moderate weight loss affects both biomechanical and inflammatory pathways in OA patients, as measured by knee compressive forces and IL-6 levels respectively[67]. A moderate reduction in total fat mass is associated with lower CRP levels and a moderate loss of abdominal fat mass is associated with reduced levels of IL-6[68]. How systemic inflammation from obesity may modulate the post-injury inflammatory response in patients suffering pre-disposing joint trauma has yet to be studied, but it is likely that multiple risk factors contribute to overall risk of OA development and progression in an individual patient. Data from animal models has indicated a complex interplay between obesity, systemic inflammation, and physical activity and joint loading. When fed a high-fat diet, mice are more prone to develop knee OA, and exhibit changes in systemic inflammatory and anti-inflammatory mediators (including adiponectin and leptin)[69]. Moderate wheel-running had a protective effect against the development of OA[70]. But surprisingly the effects of exercise were not mediated by weight loss, but rather through an effect on systemic inflammatory networks. These series of investigations in mice not only support a systemic inflammatory influence of obesity on OA, but also point to a systemic anti-inflammatory effect of exercise.

Anti-cytokine and chemokine interventions

The impact of cytokines in PTOA has recently been reviewed[71]. A few notable cases will be discussed, starting with IL-1. Despite differing reports on IL-1 concentration after joint injury, this cytokine is a potent mediator of cartilage catabolism, and many models have supported its importance in PTOA. Early work demonstrated that intraarticular injection of IL-1Ra after canine ACL transection reduced osteophytosis and cartilage lesions[72]. Adenoviral transfer of IL-1Ra into equine joints has shown[73,74].

Table I
Important features of the inflammatory response after joint injury supported by recent work in patients and animal models of PTOA

Feature	References
Signs of inflammation occur early after joint injury.	9,11,23,54,56
Inflammation is sustained at lower levels.	11,18,19,54
Patterns of inflammation change with time after injury.	20,21,31–33
The extent/severity of the initial injury influences severity of synovial inflammation.	10,23,56
Multiple joint tissues and cell types contribute to inflammation after injury.	27,30,39,43–45,56,59,60
Inflammation can occur even in the absence of joint instability.	35–37
Inflammation contributes to cartilage damage and pain responses after joint injury.	45,54,59,71,75,77,86,87,96
Certain aspects of post-injury inflammation may be protective and important for repair.	13,88,111
Inflammation is a modifiable feature of PTOA.	30,38,54,71,75,77,86,87,96

Knockout of IL-1β protected against structural disease development in the DMM model[75], and IL-1 antagonism reduced PTOA after tibial plateau fracture in mice[76,77]. Zhang *et al.*[78] combined anti-inflammatory approaches, using retroviral transduction to over-express human IL-10 and IL-1Ra in rabbits after excision of the medial collateral ligament and medial meniscectomy. Both IL-10 and IL-1Ra expression attenuated cartilage damage 2 weeks post-injury, and over-expression of both proteins was superior to either alone. Small studies have been carried out in patients. Intraarticular injection of IL-1Ra in 160 patients with knee OA did not improve mean symptom scores compared to placebo[79]. In contrast, a study of 11 patients given intra-articular IL-1Ra within 30 days of ACL injury demonstrated improvement in symptoms compared to placebo at 2 weeks[80]. Although data is limited, it is possible that this strategy may be more efficacious in the early post-injury phase.

The receptor for IL-17 is expressed in OA synovium and cartilage[81]. IL-17 and can promote release of other inflammatory cytokines from chondrocytes and synovial fibroblasts *in vitro*[81,82]. Therefore, Chen *et al.* developed a DNA aptamer that effectively blocked the IL-17 receptor[83]. In the murine DMM model, intra-articular injection reduced synovial thickening and expression of IL-6. Protection from cartilage degeneration post-injury was not observed, however the importance of synovial inflammation in symptom development may support therapeutic targeting of synovial responses post-injury.

A number of chemokines have been explored as potential targets. MCP-1 is associated with knee pain in patients with knee injuries[84,85], and mice deficient in the high affinity MCP-1 receptor (CCR2) are protected from developing pain-related behaviors post-DMM[86]. CCR2 deficiency had no effect on cartilage degeneration. In contrast, deficiency of CCR5 was shown to partially protect against cartilage erosion in this same model[87]. Interestingly, loss of CCR5 did not impact synovitis or bone remodeling, suggesting a direct effect on cartilage. CXCR2 may protect cartilage by promoting phenotypic homeostasis[88]. Genetic loss of CXCR2 in mice resulted in more severe OA after DMM surgery. Chemokines clearly influence PTOA development in both positive and negative ways, and can influence development of structural disease and symptoms. Whether targeting specific chemokines or their receptors will be effective clinically remains to be tested; systemic effects need to be carefully considered.

PTOA progression risk is likely related to an inflammatory profile rather than the effect of a single mediator. The available data suggests that there is a crucial balance between protective and deleterious effects of the initial inflammatory response to injury that needs to be better understood. This balance likely reflects the role of inflammation in tissue repair responses. As individual inflammatory mediators can have cell-specific and time-dependent effects, expression in various joint tissues and times post-injury needs further investigation. Alternatively, targeting specific pathways leading to chronic inflammation, rather than specific mediators, may provide promise. Recent studies have uncovered a few molecular pathways that are important in triggering and perpetuating inflammation after joint injury.

Inflammatory pathway activation in animal models

The complement cascade

The complement proteolytic cascade is an essential innate inflammatory mechanism for clearance of pathogens and damaged cells [reviewed in[89]]. Complement deposition in synovium from patients with meniscal tears was reported in the past[90]. More recently, it was reported that chondrocytes and synovial macrophages actively produce complement components and inhibitors, and that production is increased in OA[91,92]. There are several molecular components of cartilage matrix that can activate or inhibit the complement cascade[93–95]. Mice with impaired ability to generate the complement membrane attack complex are partially protected from development of OA after surgically induced meniscal injury, and therapeutic targeting of complement was also effective[96]. The optimal timing, risks and benefits of such a strategy for use in patients needs further evaluation.

Molecular "danger signal" and pattern-recognition receptor pathways

Pattern-recognition receptor systems including the Toll-like receptors (TLRs) NOD-like receptors (NLRs) and the receptor for advanced glycation end-products (RAGE), are utilized by the innate immune response to tissue injury[97]. In the setting of non-infectious tissue damage, these receptors respond to a variety of damage-associated molecular patterns (DAMPS), also termed "danger signals" or "alarmins", released during cellular stress and ECM damage. Activation of these receptors promotes inflammatory signaling, cytokine and chemokine production. "Danger signals" such as HMGB1[98], S100A8 and S100A9[99], and tenascin-C[100] can be produced by cells subjected to oxidative and metabolic stress, or elaborated from damaged ECM[101]. The role of selected DAMPs and the receptors that bind them have been studied in PTOA.

S100A proteins are produced by multiple cell types (macrophages, neutrophils, *etc*) and found in serum and synovium of OA patients[102,103]. Human chondrocytes produce these molecules, and exposure of cartilage explants to S100A8 and S100A9 promoted catabolic (MMP-1, MMP-3, MMP-13, IL-6, IL-1β) and suppressed anabolic gene expression (aggrecan and Collagen type II) in a TLR-4 dependent manner[104]. Moreover, S100A9 deficient mice exhibited reduced disease in the collagenase model, but not the DMM model of OA[104,105], and disease was inhibited by pharmacologically reducing S100A9 expression[106]. The differential effects in these two models were attributed to more robust synovial activation in the collagenase model, which is induced by intra-articular injection of bacterial collagenase leading to instability of connective tissues. Whether results are applicable to injury-induced PTOA models that exhibit more extensive synovial reaction than DMM remains to be tested.

Elevated levels of Tenascin-C (TnC), a danger signal which binds to TLR4, are observed in the joint after injury[107,108]. TnC induces GAG release and aggrecanase activity in cartilage explants[109] and chondrocyte cultures[110], and expression correlates with aggrecanase activity in a rat ACL-tear model[110]. But *in vivo* studies in deficient mice (TnC−/−) have revealed a predominant role for TnC in supporting cartilage repair. TnC−/− mice develop cartilage erosion more rapidly after ACL and MCL transection, and cartilage repair is delayed in a focal cartilage defect model[111]. It is possible that the effect of TnC is modified by other factors present *in vivo,* but additional work is necessary to clarify.

An interesting recent study showed that a 32-mer peptide of aggrecan, generated by sequential enzymatic cleavage in joints of patients with OA, can promote catabolism and suppress anabolic gene expression in murine and human chondrocytes, synovial fibroblasts and macrophages[101]. MyD88 and TLR-2 were shown to mediate these effects. Whether this peptide plays an important role in modulating disease progression after joint injury *in vivo* remains to be tested. Enzymatic aggrecan degradation plays an important and early role in development of progressive cartilage damage in multiple PTOA models, thus highlighting its potential for future therapeutic manipulation.

The pro-inflammatory effects of molecular danger signals are mediated through a variety of pattern recognition receptors. Of these receptors, the TLRs have been most studied in PTOA. Catabolic

responses of cartilage explants and cultured chondrocytes in response to many of the danger signals mentioned previously are mediated through TLR-2 or TLR-4. However, mice deficient in expression of TLR-1, -2, -4, or -6, as well as the key TLR signaling adaptor molecule MyD88 were not protected from development of cartilage erosion 8 weeks after partial meniscectomy[112]. Other timepoints, and pain-related outcomes particularly relevant to inflammation have not been examined. The data regarding S100 proteins[105] points to the importance of investigating multiple models, as the mechanism of disease induction may be an important variable impacting results. Therefore, whether observations from this study extend to other models and human PTOA has yet to be determined.

Conclusion

Activation of inflammatory mechanisms is critical to development of PTOA, and part of the joint response to injury. Aspects of inflammation observed after injury in humans include inflammatory mediator production (i.e., cytokine and chemokines, DAMPs), cellular infiltration and innate inflammatory pathway activation involving multiple tissues. These aspects are often recapitulated in animal models of PTOA in a time-dependent manner. Multiple models have demonstrated that inflammation after injury occurs early, but is sustained and modifiable (Table I). However, we still have a limited understanding of the specific changes that correspond to inflammatory responses over time after different injuries. Inflammatory response to joint injury may be altered by aging and systemic factors, providing a molecular context for the interaction of multiple OA risk factors (i.e., joint injury, age, obesity). Weight loss and physical activity can be accompanied by anti-inflammatory effects in both patients and animal models, providing hope that the systemic effects of obesity on OA could be mitigated by non-pharmacologic approaches. Models have also revealed specific targets that show potential to slow cartilage erosion, synovitis, and/or pain-related outcomes. However, results to date have illustrated the complexity of the inflammatory response post-injury, which exerts both deleterious and potentially protective roles. This complexity may relate to multiple cellular sources and targets in the joint, and the role of inflammation in tissue repair. Therefore, multiple models exhibiting different phenotypes need to be examined in order for a comprehensive picture of the impact of inflammation in PTOA to emerge. The strong association of inflammation with pain and joint dysfunction in patients necessitates examination of symptomatic measures in PTOA models when possible. It is likely that the nature and impact of future anti-inflammatory intervention will be dependent on time post-injury, and tissue or cell type targeted. And although early intervention is likely important for prevention of cartilage deterioration, there may be more than one optimal “window of opportunity” for targeted anti-inflammatory approaches to impact progression or development of chronic disability and pain (Fig. 1).

Author contributions

Conception and design: JL, NS, CRS; acquisition of literature: JL, CRS, interpretation of data: JL, NS, CRS; drafting the article: JL, CRS; revising critically for important intellectual content: NS, CRS; final approval of the submitted version: JL, NS, CRS.

Competing interest statement

The authors have no conflicts of interest to disclose related to this work.

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