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CRYSTALS, INFLAMMATION, AND OSTEOARTHRITIS

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Abstract

Purpose—Calcium pyrophosphate dihydrate (CPPD) and basic calcium phosphate (BCP) crystals are common components of osteoarthritic joint fluids and tissues. Why these crystals form and how they contribute to joint damage in osteoarthritis (OA) remains unclear. With renewed interest in inflammation as a key component of OA the role of calcium-containing crystals in this common disease warrants re-examination.

Recent Findings—There is ample evidence supporting a pathogenic role for inflammation in OA, and the innate immune system likely participates in this inflammatory process. Recent work reinforces the almost universal existence of calcium-containing crystals in tissues from patients with end-stage OA. Calcium-containing crystals may contribute to inflammation in OA tissues through their direct interactions with components of the innate immune system, as well as by inducing or amplifying other inflammatory signals.

Summary—There is increasing evidence that calcium-containing crystals contribute to OA and their inflammatory properties may mediate detrimental effects through innate immunity signals. Calcium-containing crystals may thus represent important therapeutic targets in OA.

Keywords

calcium crystals; osteoarthritis; inflammation

Introduction

OA is a complicated disease which is best explained as an accumulation of large and small injuries to the joint followed by ineffective cartilage repair. It is likely that numerous etiologic factors participate in the pathogenesis and heredity, injury, and advanced age are well-accepted risk factors. By contrast, the role of inflammation in OA is controversial. Some synovial inflammation is frequently observed in OA joints (1) and may be crucial to OA pathogenesis (2). Why inflammation occurs and how it affects key clinical variables in OA, such as pain and progression, remain to be determined. Burgeoning knowledge about innate immunity and its role in diseases that are not traditionally considered inflammatory has reinvigorated interest in inflammation in OA (3).

Articular calcium crystal deposition, comprising of calcium pyrophosphate dihydrate (CPPD) and basic calcium phosphate (BCP) crystals, is seen in a majority of joints affected by severe OA. Calcium-containing crystals possess inflammatory potential similar to the monosodium urate (MSU) crystals that cause gout (4)(5), and can interact directly with synovial cells and chondrocytes to produce pro-inflammatory substances (6). However, the

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relationships between calcium crystals, inflammation and OA are complex. I will review here evidence supporting an important role for calcium crystals in contributing to the inflammation seen in OA.

Calcium-containing crystals in OA

Synovial fluid studies demonstrate calcium crystals in 30–60% of unselected OA patients (7)(8). Fuerst et al. recently showed that 100% of knee and hip cartilages removed at the time of joint replacement for clinical OA contained BCP crystals and 20% contained CPPD crystals (9). These crystals are often found together in a single joint. CPPD and BCP crystals have some distinct properties and are associated with several unique musculoskeletal syndromes. CPPD and BCP crystals develop in the midzone of articular cartilage, and the smallest and earliest crystals show a pericellular distribution. Unlike MSU crystals, which form in supersaturated solutions, the formation of calcium crystals is facilitated by a solid matrix (10) and almost certainly occurs within cartilage and other connective tissues. The precise mechanisms of CPPD and BCP crystal deposition are not known but their formation is affected by altered levels of ATP, inorganic pyrophosphate (PPi) and Pi as well as by changes in the cartilage matrix (11). Extracellular chondrocyte-derived organelles known as articular cartilage vesicles (ACVs) serve as foci of crystal formation in the pericellular matrix (12). It is presumed that crystals are released from cartilage into the synovial space by injury or mechanical wear.

Inflammation in OA

Historically, OA has been considered a non-inflammatory process, as reflected by the longstanding and largely unsuccessful campaign to change its name to “osteoarthrosis”. However, inflammatory synovitis is a common and well-accepted pattern of OA histopathology. Cooke showed that 60% of joints affected by OA had evidence of synovial inflammation at the time of joint replacement (13), and synovial lymphocytic infiltrates correlate with IL-1β levels in synovial fluid and MMP-1 expression in some studies (14). Proteomic studies demonstrate numerous components of the complement cascade and other inflammatory mediators in synovial fluid from OA patients (15). Inflammatory components are also found in OA cartilage (16)(17) and we recently noted the presence of complement and amyloid proteins typically associated with inflammation in articular cartilage vesicles isolated from OA cartilage (18).

Inflammation may be clinically important in OA and may predict pain. (19). Some of the clinical heterogeneity, the pattern of joint involvement and the prevalence of osteophytes may be explained by variability in the amount of articular inflammation. For example, macrophages may play a key role in osteophyte formation in murine models (20). Cooke et al. suggested that synovial inflammation may be more common in polyarticular than in mono-or oligoarticular joint disease (13). Recent studies have correlated elevated circulating levels of C-reactive protein with increased incidence and severity of knee OA (21)(22).

While the mechanisms of inflammation in OA are uncertain, the prevailing theory is that inflammatory mediators are released from cartilage into the synovial space, where they activate synovial cells to produce chemoattractants and initiate an inflammatory response. Typically, cartilage-generated cytokines or fragments of cartilage matrix proteins, such as collagens, fibronectin and hyaluronan are thought to begin this process. Based on their work and the work of others, Scanzello et al. recently summarized evidence supporting a role for the innate immune system in OA(23). Innate immunity is an ancient host-defense system initiated by exposure to molecular patterns common to many pathogens (PAMPs) and danger signals released by damaged or dying cells or tissues (DAMPs). Central to this system are toll-like receptors (TLRs), a family of membrane receptors that recognize

bacterial lipopeptides and lipopolysaccharides as well as extracellular matrix fragments. TLR activation leads to elevated levels of nuclear factor kappa B (NFκ B) which activates the pro-inflammatory cytokine interleukin 1β (IL-1β). It is postulated that cartilage matrix degradation products initiate an innate immune response through TLRs. Nucleotide-binding, leucine-rich repeat containing proteins (NLRs, formally known as Nod-like receptors) are also important receptors in innate immunity (24)(25). They include several proteins, known as NODs, that participate in the formation of the large signaling complexes known as inflammasomes. These, in turn, activate inflammatory caspases and IL-1β.

Many lines of evidence support a role for innate immunity in OA (23). For example, IL-1β is clearly central in mediating many of the cartilage changes seen in OA (26). In addition, TLRs may directly or indirectly activate matrix metalloproteinases (MMPs) such as a MMP1 and MMP13. These enzymes directly contribute to cartilage degradation, and may amplify the inflammatory response by generating cartilage matrix fragments that bind to TLRs (27). Levels and types of proteoglycans are also altered in OA cartilage matrix. Increased levels of certain proteoglycans in OA cartilage, such as osteoadherin, may contribute to inflammation in OA. Osteoadherin binds C1q and is a potent activator of complement (28).

Inflammatory actions of calcium-containing crystals

The phlogistic properties of calcium-containing crystals have been recognized since their initial clinical descriptions (4)(5). Few reviews on the subject of inflammation in OA acknowledge a potential role for calcium crystals in the synovial inflammation (2)(23). CPPD crystals are more inflammatory than BCP crystals, and under certain conditions, CPPD crystals can produce a vigorous inflammatory response, as during attacks of pseudogout. Synovial fluid BCP crystals are much less commonly associated with evidence of inflammation. However, soft tissue deposition of BCP crystals, including such syndromes as pseudopodagra, can also be associated with clinical inflammation. It is postulated that proteins and other substances bound to crystals affects their ability to initiate inflammation. Recent work on kidney stones and crystal effects on vascular smooth muscle cells reinforces older findings showing that synthetic crystals are far more injurious than native crystals (29) and that the substances bound to crystals can dramatically affect cell-crystal interactions (30). There is much about the factors that modulate the inflammatory actions of calcium crystals that is unknown..

While it seems obvious that calcium-containing crystals should participate in inflammation in OA, few clinical studies actually support this hypothesis. Schumacher et al. failed to demonstrate an association between the presence of calcium crystals and cell counts in non-inflammatory OA synovial fluids (31). In contrast, Gordon et al. were able to correlate x-ray evidence of calcification, and synovial proliferation or inflammation in OA knees at the time of autopsy (32). Further work in this area is necessary and the recognition that synovial inflammation can be patchy and correlates poorly with synovial fluid cell counts should refine methodology in future studies. There are currently few reliable and accurate methods to identify BCP crystals in synovial fluid and such identification methods need to be designed and tested before further studies can be performed.

Calcium-containing crystals have direct effects on synoviocytes and chondrocytes which may increase articular inflammation. BCP crystals induce the production of MMPs, prostaglandins and inflammatory cytokines from resident articular cells (33). CPPD crystals may haves similar effects (34). These effects appear to be related to NFκB and MAPK signals, as well as nitric oxide-dependent pathways, but exactly how crystals interact with cells, and whether this requires crystal internalization or is receptor-mediated remains

uncertain. Much of this work was performed with BCP crystals and much less is known about the effects of CPPD crystals.

Recently, both TLRs and NLRs have been implicated in the inflammatory effects of calcium-containing crystals. Several years ago, Liu-Bryan et al. showed that CPPD crystals induce nitric oxide production in a TLR2-dependent manner from normal bovine chondrocytes (35). In 2006, Martinon et al. implicated the NLRP 3 inflammasome in MSU and CPPD crystal induced inflammation (36). CPPD crystals are now included on lists of DAMPS released by dying or injured cells (3). Extracellular ATP is also a DAMP. Interestingly, high levels of extracellular ATP stimulate CPPD crystal formation, likely by serving as a precursor of pyrophosphate production (37). We recently showed that extracellular ATP is coordinately regulated with production of pyrophosphate, the anionic component of CPPD crystals, in chondrocytes (38). Thus, ATP and CPPD crystals may amplify the effects of one another. DAMPs and PAMPs may also work together to increase IL-1β activation so that low levels of both classes of ligands, when present simultaneously, are able to stimulate an inflammatory response (39). Calcium crystals could increase levels of other stimulants of the innate immune response by augmenting levels of MMPs that break down matrix proteins and generate matrix protein fragments or through mechanical disruption of cartilage matrix.

Calcium-containing crystals also directly affect inflammatory cells. For example, CPPD crystals interfere with the effects of TNF-α on neutrophils (40). MSU and CPPD crystals can inhibit neutrophil apoptosis and prolong the inflammatory response through this mechanism. Higo et al. recently showed that opsonized CPPD crystals regulate Bcl-2 proteins involved in neutrophils apoptosis (41). CPPD crystal exposure activates ERK-dependent pathways in neutrophils, but a direct role for NLRs or TLRs has not been established, and little is known about the effect of BCP crystals on inflammatory cells. MSU crystals can directly interact with integrins on platelets causing them to discharge their inflammatory contents (42) but, whether this also occurs with calcium-containing crystals is not known.

Crystals may also affect other immunomodulatory factors, such as microparticles. Microparticles are small extracellular vesicles released from platelets, endothelial cells, lymphocytes and red cells. While principally characterized in rheumatoid arthritis, they have also been noted in OA synovial fluid, where they act as immunomodulators. Messer et al showed that synovial fluid-derived microparticles stimulate B-cell activating factor (BAFF), as well as other inflammatory and anti-inflammatory factors from synovial cells, and suggested that they may play a key role in induction and amplification of autoimmunity (43). Synovial fluids from patients with crystal arthritis had higher levels of microparticles than crystal-free OA synovial fluids. The small number of fluids examined and the inclusion of both gout and pseudogout, as well as the absence of attempts to identify BCP crystals, makes further work necessary, but suggests that crystal-induced microparticle production may be another mechanism through which crystals contribute to inflammation in OA.

Conclusions

CPPD and BCP crystals are commonly found in OA tissues and are clearly capable of directly and indirectly inducing inflammation. With increasing recognition of the potential importance of inflammation in OA and identification of the innate immune system as a potential mediator of this inflammation, a role for calcium crystals as potential therapeutic targets in OA warrants re-investigation.

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Key points

- Calcium pyrophosphate dihydrate (CPPD) and basic calcium phosphate (BCP) crystals are common components of osteoarthritic joints.
- Calcium-containing crystals contribute to joint damage in osteoarthritis in part through their interactions with components of the innate immune system.
- Further studies of the role of crystals as contributors to the inflammation seen in OA is warranted.