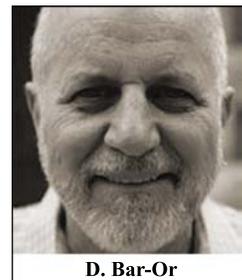


# Inflammatory Pathways in Knee Osteoarthritis: Potential Targets for Treatment

David Bar-Or<sup>\*1-4</sup>, Leonard T. Rael<sup>1,4</sup>, Gregory W. Thomas<sup>1,4</sup> and Edward N. Brody<sup>5</sup>

<sup>1</sup>Swedish Medical Center, Trauma Research Department, 501 E. Hampden Ave., Englewood, CO 80113, USA; <sup>2</sup>St. Anthony Hospital, Trauma Research Department, 11600 W. 2<sup>nd</sup> Pl., Lakewood, CO 80228, USA; <sup>3</sup>Medical Center of Plano, Trauma Research Department, 3901 W. 15<sup>th</sup> St., Plano, TX 75075, USA; <sup>4</sup>Ampio Pharmaceuticals Inc., 373 Inverness Parkway, Suite 200, Englewood, CO 80112, USA; <sup>5</sup>Somalogic Inc., 2945 Wilderness Pl., Boulder, CO 80301, USA



D. Bar-Or

**Abstract:** Osteoarthritis (OA) of the knee is a wide-spread, debilitating disease that is prominent in Western countries. It is associated with old age, obesity, and mechanical stress on the knee joint. By examining the recent literature on the effect of the anti-inflammatory prostaglandins 15d-PGJ<sub>2</sub> and Δ12-PGJ<sub>2</sub>, we propose that new therapeutic agents for this disease could facilitate the transition from the COX-2-dependent pro-inflammatory synthesis of the prostaglandin PGE<sub>2</sub> (catalyzed by mPGES-1), to the equally COX-2-dependent synthesis of the aforementioned anti-inflammatory prostaglandins. This transition could be instrumental in halting the breakdown of cartilage via matrix metalloproteinases (MMPs) and aggrecanases, as well as promoting the matrix regeneration and synthesis of cartilage by chondrocytes. Another desirable property of new OA therapeutics could involve the recruitment of mesenchymal stem cells to the damaged cartilage and bone, possibly resulting in the generation of chondrocytes, synoviocytes, and, in the case of bone, osteoblasts. Moreover, we propose that research promoting this transition from pro-inflammatory to anti-inflammatory prostaglandins could aid in the identification of new OA therapeutics.

**Keywords:** Cartilage, cyclo-oxygenase, inflammation, knee osteoarthritis, prostaglandins, synovial fluid.

## KNEE JOINT STRUCTURE AND COMPOSITION

The knee joint is composed of a few layers of cartilage on both the distal femoral surface and the proximal tibial surface interposed by cartilaginous menisci separating the ends of these bones. Synovial membranes line the cartilage which is at the ends of these bones. The purpose of these features (and other structures such as ligaments, muscles, etc.) is to lubricate the knee joint and to act as shock absorbers for the pressure which arises as a result of walking upright (and even more so when upright walkers are overweight). Upon closer examination at the cellular level, articular cartilage is divided into zones with the most distal being the articular surface followed inwardly by the middle zone and deep zone (Fig. 1). Even deeper is the calcified cartilage, which is separated from the cartilage above by the tidal membrane. Deeper still is the cement line under which is sub-chondral bone.

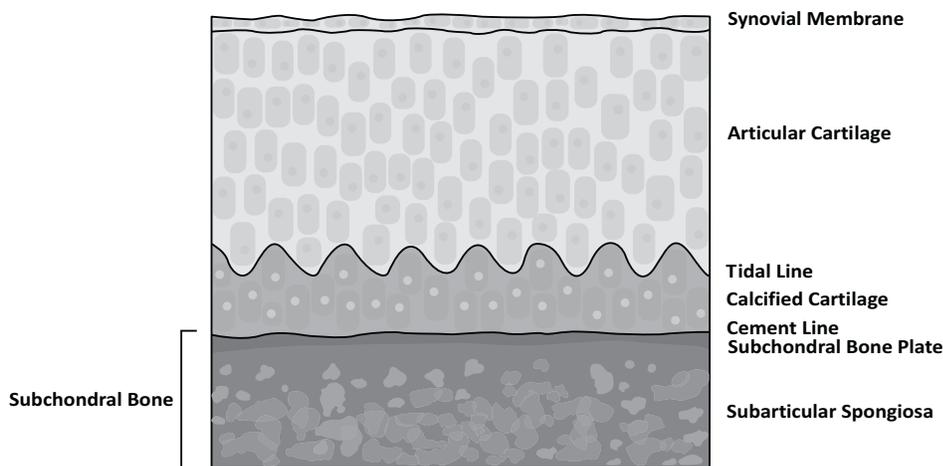
Although simplification of these layers is helpful when discussing the biochemistry of cartilage and bone, it is important to realize that the cellular and biochemical make-up and sensitivity to mechanical stress is somewhat different in all these layers. The articular surface has densely packed collagen fibrils, arranged parallel to the articular surface

where the collagen is primarily type II. The chondrocytes in this layer secrete proteins that have a lubricating function, especially superficial zone protein (SZP) and lubricin. Both of these proteins are derived from megakaryocyte stimulating factor and are synthesized both in chondrocytes and in synoviocytes from the synovial membrane [1]. This layer has high water content and is more deformable than the other layers, presumably to help absorb mechanical pressure which is exerted downward toward the joint space. The middle zone contains less water and a less organized arrangement of collagen fibers. The deep zone has the least amount of water and a high concentration of proteoglycans, especially aggrecan [2]. Along with type II collagen, aggrecan forms a major structural component of cartilage, especially articular cartilage. Additionally, aggrecan is a structural proteoglycan because it provides a hydrated gel structure (via its interaction with hyaluronan) giving load-bearing properties to cartilage. Finally, aggrecan mediates chondrocyte-chondrocyte and chondrocyte-matrix interactions [3]. Deeper still is calcified cartilage which contains chondrocytes and calcium salts. The total joint cartilage lies on top of the sub-chondral bone which is also affected in severe OA as well as in mild and moderate OA.

## KNEE OSTEOARTHRITIS

Osteoarthritis (OA) is a disease of aging, mechanical stress on joints, and inflammation. It affects almost 27 million people in the U.S. alone, and the percentage of people

\*Address correspondence to this author at the Swedish Medical Center/Trauma Research Department, 501 E. Hampden Ave., Room 4-454, Englewood, CO 80113, USA; Tel: +1.303.788.4089; Fax: +1.303.788.4064; E-mail: [dbaror@ampio-pharma.com](mailto:dbaror@ampio-pharma.com)



**Fig. (1).** Zonal arrangement of joint cartilage. This schematic diagram shows the top superficial layers and the deep bottom layers. Underneath the synovial membrane, the articular cartilage is actually composed of three main zones: the superficial, the middle, and the deep layers, with the deep layer abutting the tidal line. The three layers of articular cartilage have different physical and biochemical properties (discussed within the text). Underneath the cement line is subchondral bone, which is implicated in the progression of OA and is thought to be the source of mesenchymal stem cells (MSCs). MSCs can migrate into the cartilage layers in the presence of certain stimuli.

with this disease is similar in Western Europe. It is the leading cause of chronic disability in the US [4]. In OA, joints become painful, tender, stiff, and sometimes swollen. The focus here is on OA of the knee joint because it affects more than 12% of adults (or roughly 4.3 million) over 60 [5]. Additionally, OA is a significant burden on the U.S. health care system and budget, meaning that finding new therapies would constitute a major impact on life styles and health care economics [6]. Finally, knee OA frequency and severity are directly related to another almost epidemic problem in the U.S. population: obesity [7].

It is useful to separate changes in the knee joint affected by OA into: (1) changes seen in experimental conditions; (2) clinically observed changes in this disease; and (3) biochemical changes in different cell types. A unifying theme in these descriptions is that ordinary wear and tear on the knee always leads to some joint damage. However, the regenerative capacity of cartilage (and bone) can reverse most of these damaging changes, until the combination of mechanical stress, injury, and inflammation overwhelms the regenerative capacity leading to progressive disease. It is in the context of this possible reversibility and healing that identifying new pharmacological approaches to OA could prove clinically useful.

### EXPERIMENTAL CHANGES IN OA

Under normal loading conditions, knee joints experience downward pressure as high as ten times body weight equating to stresses of 5-10 MegaPascals (MPa, where 1 MPa equals 145.03 psi) [8, 9]. Although it may take significantly more stress than this to fracture cartilage, there is evidence that with lower levels of stress (under 25 MPa), surface cartilage as well as underlying bone can be damaged [10, 11]. A study on explanted cartilage showed that a critical threshold which caused chondrocyte death and rupture of the collagen II matrix occurred at ~15-20 MPa. Moreover, proteoglycan synthesis diminished and water content increased at stresses as low as 10 MPa [12]. A single impact study on bovine ar-

ticular cartilage explants showed significant damage to the superficial zone at low impacts and increasing injury to the middle and deep cartilage zones at higher impacts. The chondrocytes in the superficial zone died within 3 minutes of impact as evidence by decreases in cell volume and viability with a corresponding radiation of cell death after 20 minutes post-impact.

Interestingly, incubation in hypertonic saline protected chondrocytes, whereas incubation in hypotonic saline led to more severe cellular injury and chondrocyte swelling, a phenomenon seen in OA [13, 14]. More recently, porcine patellae were isolated, subjected to mechanical impact, and then cultured for up to 14 days. RNA was then extracted from the cartilage, and mRNA levels were measured by RT-PCR. Collagen type I was up regulated in these extracts, which suggests that the normal cartilage type II collagen is being replaced by the fibroblast like type I collagen. Transcription for metalloproteinases, especially MMP 1 and MMP 13, was also up regulated. These proteinases (and others) are well-known inducers of matrix degradation in OA [15]. Therefore, an effort to repair some of the matrix damage exists, but this leads to a weaker matrix compared to pre-injury.

### CLINICAL CHANGES IN OA

Examination of clinical specimens from patients suffering from OA show a similar progression of cellular and tissue changes with a strong correlation to symptoms such as pain, stiffness, and immobility in the affected knee. This disease is characterized by a general progressive loss of articular cartilage, weakening of the cartilage matrix, remodeling of sub-chondral bone, and often growth of bony protuberances within the joint space (osteophytes seen in radiographic analysis of patients). One of the first pathological changes is the loss and disorganization of the type II collagen fibrils within the superficial layer of articular cartilage especially around chondrocytes [16, 17]. This degradation is ascribed primarily to collagenase-3 (MMP 13), but other metalloproteinases play a role, particularly MMP 3 (strome-

lysin 1), MMP 2 (gelatinase A), MMP 9 (gelatinase B), MMP 1 (collagenase-1), MMP 8 (collagenase-2), and MMP 14 (membrane type 1 MMP) [18]. The proteoglycan aggrecan (as well as others in this category) is also degraded by aggrecanases [19].

The natural inhibitors of the MMPs, tissue inhibitors of metalloproteinases (TIMPs), are deficient in OA, although the ratio of MMPs to TIMPs is lower than that seen in rheumatoid arthritis [20]. As collagen type II and proteoglycans are progressively degraded in the superficial layer, they are up-regulated in the deeper, middle zone and deep zone of the cartilage [21]. As the disease progresses, the re-synthesis of the degraded molecules becomes less and less efficient with gradual erosion of the articular cartilage complete to the sub-chondral bone layer. Also involved is the synovial space with some thickening of the synovial membrane sometimes seen in early stages of OA. More importantly, the synovial membrane and presumably the synoviocytes in the membrane produce pro-inflammatory cytokines IL-1 $\alpha$ , IL-1 $\beta$ , and TNF- $\alpha$  in the early stages of OA [22].

### BIOCHEMICAL CHANGES IN OA

As OA progresses, erosion of all layers can occur, and defects can extend into the sub-chondral bone and finally to the sub-articular spongy bone. In fact, MRI analysis shows bone changes even when erosion is superficial [23, 24]. Osteochondral defects can also be repaired, depending on their sizes with small defects more likely to be repaired than large ones. The defect is first filled with a blood clot allowing bone marrow to communicate directly with the joint tissue. Mesenchymal stem cells from bone marrow are activated under these conditions and differentiate into chondrocytes and osteoblasts in a complex series of transformations leading to joint repair [25]. The details of the biochemistry of these differentiation steps will not be discussed here, but are well studied. For this review, primary emphasis is placed on the “struggle” between mechanical stress/inflammation and the regenerative processes that repair cartilage and underlying bone.

Using existing knowledge on OA pathophysiology, the ideal therapeutic for OA would provide the following benefits: halt inflammatory responses in the knee joint, protect chondrocytes and perhaps synoviocytes from apoptosis due to mechanical load stresses, repair defective matrix perhaps by increasing aggrecan and other proteoglycan synthesis, and stimulate mesenchymal stem cells to migrate into the defective regions to repopulate the bone and cartilage with the appropriate cells. These effects should reduce pain and increase mobility in the joint.

### INFLAMMATION IN OA

Inflammation was disregarded as a major contributor to OA for decades primarily due to the observation of fewer inflammatory white blood cells in synovial fluid from OA patients than from rheumatoid arthritis patients. Although inflammatory changes are most easily seen in the synovial membrane, it is likely that this is a secondary response to the initial damage and inflammatory response in cartilage from OA patients.

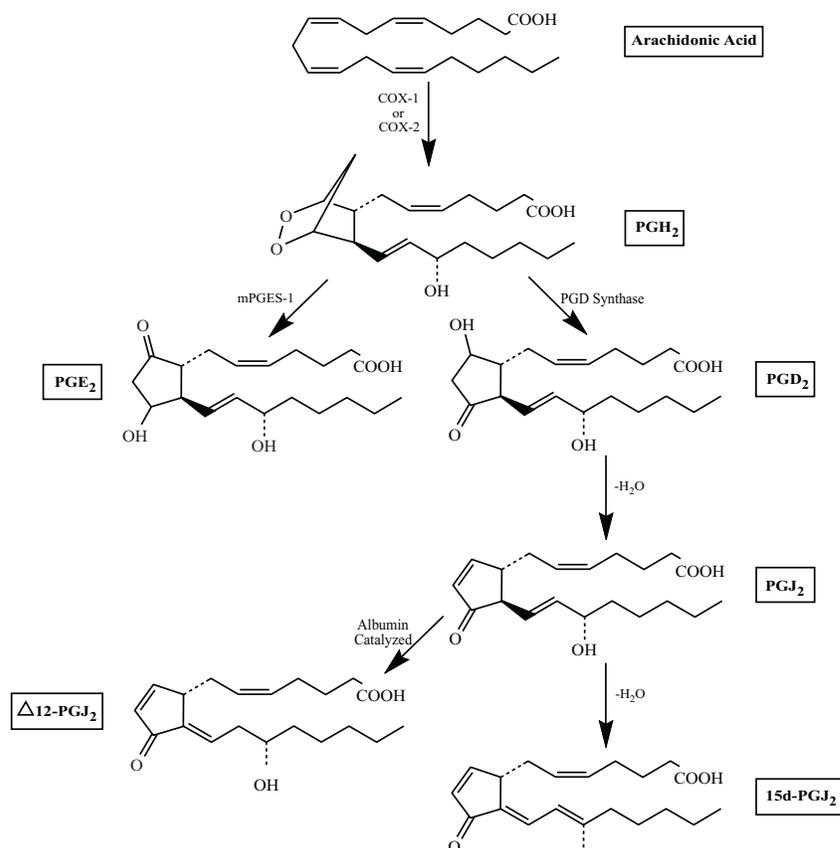
If age, obesity, and mechanical stress contribute to cartilage erosion, how does this translate into an inflammatory response? The main candidate seems to be the innate immune system. The innate immune system is primarily a rapid response system for pathogens via receptors known as pathogen-associated molecular patterns (PAMPs). However, it is also characterized by a different set of receptors known as damage-associated molecular patterns (DAMPs) or alarmins [26]. The alarmins react to danger signals generated endogenously in animals after cellular damage, abnormal proteins, leaky vasculature, and fragments of cartilage matrix. This could explain the activation of inflammatory cells, such as dendritic cells, in the early stages of OA [27]. The alarmins SA100A8 and SA100A9 seem to be expressed on activated macrophages in a mouse model of collagen-induced OA. Increased levels of the pro-inflammatory cytokine IL-1 $\beta$  were also found in the synovium of these diseased mice. This seems to be the mechanism in humans as well since increased alarmin mRNA was found in synovial membranes from early OA patients compared to controls. Moreover, a positive correlation was demonstrated between progression of the disease in humans and blood levels of the two alarmin proteins.

Contribution to the response attributed to alarmin comes from plasma protein leakage into synovial fluid due to changes in endothelial cells during OA progression. These are interpreted as danger signals by the immune system and contribute to the inflammatory response via TLR-4 signaling [28]. It has also been shown that chondrocytes and synoviocytes up-regulate TLR-2 and TLR-4 at cartilage lesions sites causing the release of inflammatory cytokines directly into the arthritic cartilage [29]. IL-1 $\beta$  is not the only inflammatory cytokine induced by the innate immune response to OA. A variety of cytokines, especially IL-6, IL-8, and TNF $\alpha$  are present in early OA lesions and synovial fluid. These cytokines are well-known inducers of MMPs and aggrecanases thus leading to joint degradation [30].

Another contributing factor in the pathogenesis of knee OA is the role of free radicals. Abnormal chondrocyte metabolism results in the production of enough free radicals that exceeds the antioxidant buffering capacity [31]. In addition to damage to proteins, lipids, nucleic acids, and matrix components, free radicals are also important intracellular signaling molecules that amplify the inflammatory response [31]. By acting on the mitochondrial and cell signaling machinery, free radicals can induce structural and functional alteration of the extracellular matrix resulting in matrix stiffness and brittleness in knee OA patients [32].

### PROSTAGLANDIN EFFECTS IN OA

The downstream effects of the pro-inflammatory prostaglandins released in OA should also be taken into account. One of the first effects of pro-inflammatory cytokines is the activation of phospholipase A2 (PLA2) which cleaves cellular membranes thereby liberating arachidonic acid (Fig. 2). PLA2 consists of three broad classes of enzymes, each of which has subclasses that may be regulated during the course of inflammation and recovery [33]. Also induced by pro-inflammatory cytokines is cyclo-oxygenase 2 (COX-2). COX-2 is highly up-regulated during inflammation and catalyzes



**Fig. (2).** Prostaglandin synthesis pathways. Following the activation of PLA2 by pro-inflammatory cytokines, arachidonic acid is liberated from cellular membranes. COX-1 and COX-2 convert arachidonic acid to the unstable prostaglandin PGH<sub>2</sub>. PGH<sub>2</sub> can be converted to the pro-inflammatory prostaglandin PGE<sub>2</sub> by mPGES-1 or converted to PGD<sub>2</sub>, a prostaglandin with well-known anti-inflammatory effects. These effects are mostly due to further non-enzymatic conversions of PGD<sub>2</sub> to PGJ<sub>2</sub> and then 15d-PGJ<sub>2</sub>. Intriguingly, PGJ<sub>2</sub> can also convert to Δ<sup>12</sup>-PGJ<sub>2</sub> in the presence of albumin.

the conversion of arachidonic acid into PGH<sub>2</sub> (Fig. 2). PGH<sub>2</sub> is the precursor to many eicosanoids (such as prostacyclin and thromboxane), but special emphasis will be placed on just two of these, PGE<sub>2</sub> and PGD<sub>2</sub> (Fig. 2). The former is the major pro-inflammatory prostaglandin, and the latter is the precursor to the prostaglandins associated with recovery. PGD<sub>2</sub> halts and reverses the inflammatory response in a multitude of experimental systems.

**INITIAL RELEASE OF PRO-INFLAMMATORY PROSTAGLANDINS IN OA**

Microsomal prostaglandin E synthase-1 (mPGES-1) is the major source of PGE<sub>2</sub> derived from PGH<sub>2</sub> in OA chondrocytes. Levels of both mRNA and protein for mPGES-1 were elevated in OA versus normal cartilage. Treatment of chondrocytes with either IL-1β or TNFα increased expression of mPGES-1 in a dose- and time-related manner [34]. The synthesis of MMPs and aggrecanases and the degradation of collagen (described above) lead to OA-associated changes in cartilage and bone. To a first approximation, this is a direct result of the synthesis of PGE<sub>2</sub> via COX-2 in conjunction with mPGES-1 [35]. This same pathway is induced when mechanical shear is applied to chondrocytes, emphasizing again the interplay between mechanical stress and inflammation of knee joints [36, 37].

**CONTRIBUTION OF ANTI-INFLAMMATORY PROSTAGLANDINS IN OA**

Description of all of the cellular components of inflammation (for example contribution of neutrophils, monocytes, macrophages, etc.) will not be included here, because the desired focus is to emphasize the switch that takes place in cells and tissues after the onset of inflammation. Particularly, the switch of prostaglandin synthesis is very important since this initiates a decrease in inflammation and, to a large degree, the recovery of normal cellular function. Carageenan, a polysaccharide polymer derived from red algae, is often used experimentally to provoke an inflammatory response. In a rat pleurisy model, there is an initial infiltration of inflammatory polymorphonuclear cells in the pleura for the first 12 hours. Then, monocytes migrate to the inflamed region and differentiate into macrophages. The inflammatory response is over at 48 hours post-injection. COX-2 protein and PGE<sub>2</sub> were elevated initially with peak activity observed at 2 hours post-injection. However, at 48 hours post-injection, the COX-2 levels were increased 3.5-fold compared to 2 hours with a sharp diminution of PGE<sub>2</sub> levels [38]. This transition depends on a switch of COX-2 coupling from mPGES-1 to PGD<sub>2</sub> synthase which is important for the resolution of inflammation, but whose control is poorly understood. PGD<sub>2</sub> is unstable in tissue and undergoes two spontaneous dehydration steps to generate 15d-PGJ<sub>2</sub> (Fig. 2). 15d-PGJ<sub>2</sub> is an im-

portant prostaglandin in the resolution of inflammation [39]. This is also true for chondrocytes from OA patients [40-42]. Therefore, the switch in prostaglandin synthesis seems to be a major factor in stopping inflammation and initiating healing in OA cartilage. Indeed, there are many other reactions involving COX-2, both during the inflammatory and anti-inflammatory phases of arthritis and other inflammatory diseases [43].

### ROLE OF PGJ<sub>2</sub>-DERIVED PRODUCTS IN OA HEALING

There is another product derived from PGJ<sub>2</sub> which may be important to healing called  $\Delta$ 12-PGJ<sub>2</sub>. However, there is much less evidence for this compared to 15d-PGJ<sub>2</sub>. Like 15d-PGJ<sub>2</sub>,  $\Delta$ 12-PGJ<sub>2</sub> has two electrophilic reactive carbon atoms as opposed to only one reactive carbon in the parent compound PGJ<sub>2</sub> (Fig. 2). Injection of monkeys with a single dose (1mg/kg) of the precursor PGD<sub>2</sub> led to a 20 to 180-fold increase in the urinary output of  $\Delta$ 12-PGJ<sub>2</sub>. This increase was reduced by 50% if the monkeys were treated with the non-selective COX inhibitor indomethacin. Thus,  $\Delta$ 12-PGJ<sub>2</sub> seems to be a natural metabolite of PGD<sub>2</sub> [44]. Although this metabolite has been studied for its cytotoxic effects, only one published study suggests that  $\Delta$ 12-PGJ<sub>2</sub> participates in the repair process of OA cartilage and bone.

Siddhivarn *et al.* subjected osteoblastic cell lines to mechanical stretching and assessed biochemical and cellular parameters. It was found that 1% elongation for up to 24 hours was optimal for maximizing bone nodule formation with maintenance of >90% cell viability [45]. First of all, this mechanical stretching induced bone nodule formation, whereas none was seen in non-stretched controls. More intriguingly, stretching stimulated COX-1 and COX-2 synthesis with COX-1 increasing about 50% at 4 hours post-stretching, but COX-2 increased between 5 minutes and 24 hours post-stretching from 400% to 700%, respectively. PGE<sub>2</sub> and PGD<sub>2</sub>, as well as the PGD<sub>2</sub> derivative  $\Delta$ 12-PGJ<sub>2</sub>, were not detected in non-stretched cells, but were abundant in the stretched cells. Similarly, the stretching induced the synthesis of PGD synthase which catalyzes the switch from inflammatory to healing reactions. Remarkably, no 15d-PGJ<sub>2</sub> was found after induction of this healing/bone nodule response. Furthermore, abundant evidence suggests that the healing response in OA cartilage is partly transmitted in 15d-PGJ<sub>2</sub>-induced healing by the binding and activation of the nuclear receptor PPAR $\gamma$ . After interaction with 15d-PGJ<sub>2</sub>, PPAR $\gamma$  migrates to the nucleus and activates the transcription of a variety of effector molecules for resolving inflammation. Remarkably, stretching in these experiments also stimulated transcription of the PPAR $\gamma$ -1 gene even though its usual ligand (15d-PGJ<sub>2</sub>) was unavailable. This leads one to suspect that  $\Delta$ 12-PGJ<sub>2</sub> may be an important factor in shutting down inflammation and promoting healing in bone and possibly cartilage. This hypothesis remains to be proved.

Why is this hypothetical contribution of  $\Delta$ 12-PGJ<sub>2</sub> in OA healing intriguing? Although it was first thought that the conversion of PGD<sub>2</sub> to 15d-PGJ<sub>2</sub> or  $\Delta$ 12-PGJ<sub>2</sub> were both dependent on the presence of serum albumin, it was later found that the well studied 15d-PGJ<sub>2</sub> was derived from PGD<sub>2</sub> independently of albumin [46]. However, the synthesis of the

other PGD<sub>2</sub> metabolite,  $\Delta$ 12-PGJ<sub>2</sub>, does depend on interaction with albumin. Coincidentally, one of the more promising therapeutics for OA is derived from the low molecular weight fraction of commercially available human serum albumin [47].

### IMPORTANCE OF PROSTAGLANDIN SWITCH IN OA HEALING

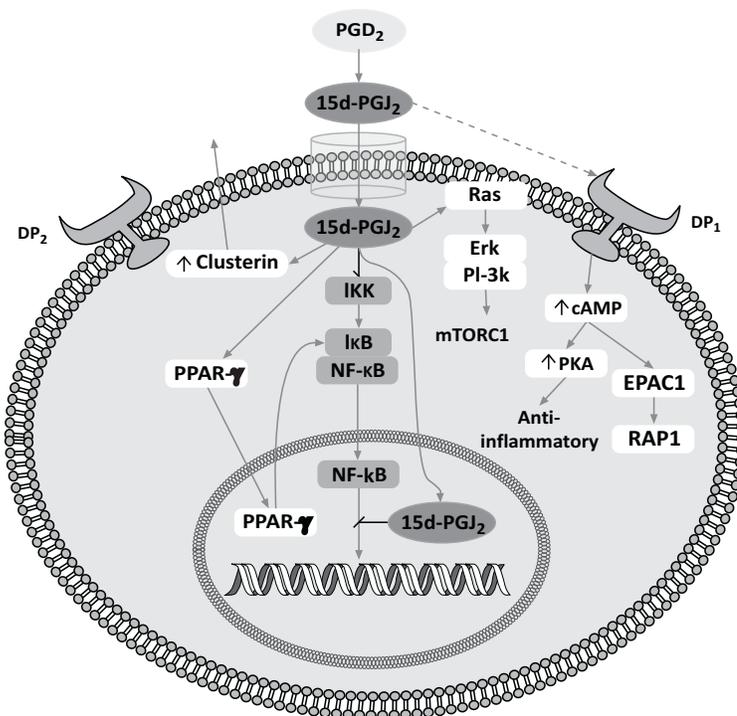
There is ample evidence that the transition from mPGES-1 to PGD synthase, with the subsequent synthesis of PGJ<sub>2</sub>-derived prostaglandins, is critical to initiating biochemical pathways associated with healing in OA (Fig. 3). As mentioned above, PPAR $\gamma$ -1 and -2 are part of a nuclear hormone superfamily. They derive from the same gene and produce slightly different proteins through alternative splicing of pre-mRNA with PPAR $\gamma$ -2 having 30 more amino acid residues at its amino terminus. It is the PPAR $\gamma$ -1 gene (up-regulated by osteoblast stretching (*vide supra*)) that is abundant in many tissues and immune cells. PPAR $\gamma$ -1 is expressed in human cartilage but is down regulated in OA. In articular chondrocytes, the gene is also down regulated by IL-1 $\beta$  [48]. 15d-PGJ<sub>2</sub> was the first endogenous agonist of PPAR $\gamma$  to be identified [49, 50]. Also, 15d-PGJ<sub>2</sub> completely suppressed NO and PGE<sub>2</sub> production in human OA chondrocytes. COX-2 was slightly inhibited, but substantial COX-2 activity remained allowing for PGD<sub>2</sub> synthesis during this anti-inflammatory phase [40, 51]. Finally, 15d-PGJ<sub>2</sub> completely inhibits mPGES-1 in both human and rat OA chondrocytes thus abolishing PGE<sub>2</sub> synthesis [34, 52, 53].

15d-PGJ<sub>2</sub> also inhibits the cartilage matrix degrading MMPs and aggrecanases. This is accomplished via the suppression of the NF- $\kappa$ B transcriptional activation system (Fig. 3). This suppression involves a number of mechanisms, including direct inhibition of I $\kappa$ B kinase which releases NF- $\kappa$ B allowing for nuclear translocation [54]. Other steps in the NF- $\kappa$ B transcriptional activation pathway are inhibited by 15d-PGJ<sub>2</sub> but are independent of PPAR $\gamma$  [55]. Therefore, it is important to note that the shutdown of this system leads to the anti-inflammatory effects of inhibiting MMP and aggrecanase synthesis. Since COX-2 is regulated by NF- $\kappa$ B and is also necessary for synthesis of PGJ<sub>2</sub>-derived prostaglandins, it may be that multiple levels of control of NF- $\kappa$ B are necessary to affect the mPGES to PGD synthase switch. Other anti-inflammatory properties of 15d-PGJ<sub>2</sub>, such as the inhibition of MMP 13 induced by TNF $\alpha$  treatment, are seen not only in chondrocytes but also in human synovial fibroblasts. This is also mediated by inhibition of the NF- $\kappa$ B system [56].

To summarize, it is expected that 15d-PGJ<sub>2</sub>, and perhaps  $\Delta$ 12-PGJ<sub>2</sub>, might have anti-inflammatory properties *in vivo* (Fig. 3). The induction of these two prostaglandins might be part of the mechanism of action required in a new treatment for knee OA. Regarding the stability and direct administration of these two prostaglandins, anti-inflammatory effects were seen in a mouse model of inflammation, but only efficiently if 15d-PGJ<sub>2</sub> was encapsulated in nano-particles [57].

### STEM CELL THERAPY IN OA

In pursuing new treatments for OA, the biochemical pathways of halting inflammation and promoting healing in



**Fig. (3).** Schematic representation of the effect of 15d-PGJ<sub>2</sub>. 15d-PGJ<sub>2</sub> has a number of pathways by which it initiates an anti-inflammatory cascade. It can react with the PGD<sub>2</sub> receptors (DP<sub>1</sub> and DP<sub>2</sub>) to stimulate cyclic AMP (cAMP) production and increase protein kinase A (PKA) leading to downstream anti-inflammatory effects. More importantly, 15d-PGJ<sub>2</sub> can enter the cell directly and activate PPAR $\gamma$  resulting in the transcription of multiple anti-inflammatory mRNAs. Additionally, PPAR $\gamma$  inhibits the transcription factor NF- $\kappa$ B by binding to the inhibitor I $\kappa$ B thus protecting I $\kappa$ B degradation by I $\kappa$ B kinase (IKK). Finally, 15d-PGJ<sub>2</sub> can also inhibit IKK activity through possible covalent modification and, further downstream, can inhibit NF- $\kappa$ B nuclear translocation as well as impairing NF- $\kappa$ B binding to DNA.

the knee joint are important considerations. However, long term treatment is likely to be effective for severe cases of OA only when new cartilage, and perhaps bone, is made *de novo*. This is presumably accomplished from differentiation of mesenchymal stem cells (MSCs) that are most likely derived from bone marrow and, to a lesser extent, are blood derived.

MSCs primarily reside in bone marrow but can migrate to other tissues. They are able to differentiate into osteogenic, adipogenic, chondrogenic, myogenic, and endothelial cell lineages. The chondrogenic cell line is characterized by the presence of the surface antigen CD90 [58]. MSCs are used for a variety of tissue regeneration studies, and some OA clinical trials have been performed with mixed results. Although MSC administration is a promising form of therapy, there are difficulties that need to be overcome. One of the biggest obstacles is the administration of MSCs intravenously. Only a small portion of MSCs are able to home and engraft to the diseased tissue, making this potentially curative cell therapy very inefficient (reviewed by [59]). Most of the cell therapy experiments for OA and other cartilage defects have been performed using chondrocytes with mixed results. Positive results could take years to generate [60]. A variety of engraftment aids have been devised to make MSCs more effective in treating OA, but more work is required before this method is regarded as routine therapy for severe OA [61].

Rather than review all the research promoting the use of MSCs for standard OA therapy, focus on recent molecular

research suggests a pathway to stimulate endogenous MSCs to migrate to OA cartilage and bone thereby inducing repair via natural pathways. For example, high density lipoprotein (HDL), most often associated with protection of cardiac tissue, induces lamellophilia in MSCs and stimulates migratory capacity 2-fold. This up-regulation depends on the activity of phosphatidylinositol-3 kinase (PI3K) [62]. Moreover, HDL also protects MSCs from oxidative stress related apoptosis via manipulation of the PI3K-Akt pathway [63].

### CLUSTERIN INVOLVEMENT IN OA HEALING

Does this activation and protection of MSCs relate to the healing switch in prostaglandin synthesis regarding inflammation in OA? Perhaps there is an intriguing link. One of the most important components of HDL is clusterin, or apolipoprotein J, which is a matrix heat shock protein. It has been demonstrated that HDL bound to clusterin is anti-apoptotic [64, 65]. In fact, the anti-apoptotic role of clusterin bound to HDL has been demonstrated using an endothelial cell survival model. In this study, the role of HDL was examined for protective effects on endothelial cell apoptosis both *in vivo* and *in vitro*. HDL particles isolated from healthy individuals were able to prevent endothelial cell apoptosis. However, when HDL particles were isolated from individuals who had recently had a myocardial infarct or who had chronic heart disease, these HDL particles did not protect against apoptosis. In examining the proteins bound to lipid in each of these particles, it was found that the “sick” HDL was deficient in clusterin. Remarkably, adding clusterin to the “sick” HDL

restored protection against apoptosis. Also, treating “healthy” HDL with an anti-clusterin antibody neutralized its anti-apoptotic capability. Thus, clusterin bound to HDL provides protection against a variety of apoptotic challenges [66].

This leads to the prostaglandin-clusterin link. In a rat pheochromocytoma cell line (PC12 cells), COX-2 expression induced clusterin production using a pathway that required 15d-PGJ<sub>2</sub>. This was also dependent on PPAR $\gamma$ . Basically, all of the anti-inflammatory components described after the prostaglandin switch from mPGES to PGD synthase (with subsequent transformation to PGJ<sub>2</sub>-derived prostaglandins) appear to be linked [67]. Clearly, these experiments need to be repeated in MSCs, chondrocytes, and synovial cells.

## CONCLUSION & FUTURE PROSPECT

Included in this review are the cellular and biochemical aspects of OA in order to provide a definition of some criteria used to judge new therapeutic agents for OA. Clearly, the primordial consideration will be clinical—does this therapeutic relieve the pain and immobility in the knees of these patients? To understand how such agents work, it might be important to assess how these agents may do one or, ideally, all of the following:

- Facilitate the switch of PGE<sub>2</sub> synthesis to PGD<sub>2</sub> and PGJ<sub>2</sub> synthesis (i.e. with subsequent conversion to 15d-PGJ<sub>2</sub> and/or  $\Delta$ 12-PGJ<sub>2</sub>).
- Inhibit NF- $\kappa$ B signaling.
- Stimulate PPAR $\gamma$  signaling.
- Inhibit MMPs, especially MMP13.
- Inhibit aggrecanase.
- Induce MSCs to migrate into and repair damaged cartilage.

## LIST OF ABBREVIATIONS

OA	=	Osteoarthritis
MMPs	=	Matrix metalloproteinases
TIMPs	=	Tissue inhibitor of metalloproteinase
IL-1 $\alpha$	=	Interleukin 1 alpha
IL-1 $\beta$	=	Interleukin 1 beta
TNF- $\alpha$	=	Tumor necrosis factor alpha
PAMPs	=	Pathogen-associated molecular pattern
DAMPs	=	Damage-associated molecular pattern molecules or danger-associated molecular pattern molecules
TLR-2	=	Toll-like receptor 2
TLR-4	=	Toll-like receptor 2
IL-6	=	Interleukin 6
IL-8	=	Interleukin 8
PLA2	=	Phospholipase A2

COX-1	=	Cyclo-oxygenase 1
COX-2	=	Cyclo-oxygenase 2
PGH <sub>2</sub>	=	Prostaglandin H <sub>2</sub>
PGE <sub>2</sub>	=	Prostaglandin E <sub>2</sub>
PGD <sub>2</sub>	=	Prostaglandin D <sub>2</sub>
PGJ <sub>2</sub>	=	Prostaglandin J <sub>2</sub>
mPGES-1	=	Microsomal-associated PGE synthase-1
15d-PGJ <sub>2</sub>	=	15-Deoxy- $\Delta$ 12,14-prostaglandin J <sub>2</sub>
$\Delta$ 12-PGJ <sub>2</sub>	=	$\Delta$ 12-Prostaglandin J <sub>2</sub>
PPAR $\gamma$	=	Peroxisome proliferator-activated receptor gamma
NO	=	Nitric oxide
NF- $\kappa$ B	=	Nuclear factor kappa-light-chain-enhancer of activated B cells
I $\kappa$ B	=	Inhibitor of kappa light polypeptide gene enhancer in B-cells
MSCs	=	Mesenchymal stem cells
HDL	=	High-density lipoprotein
PI3K	=	Phosphoinositide 3-kinase
Akt	=	Protein kinase B

## CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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