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Review
Osteophytes: relevance and biology

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Summary

Objective: Osteophytes are common features of osteoarthritis. This review summarizes the current understanding of the clinical relevance and biology of osteophytes.

Method: This review summarizes peer-reviewed articles published in the PubMed database before May 2006. In addition this review is supplemented with own data and theoretical considerations with regard to osteophyte formation.

Results: Osteophytes can contribute both to the functional properties of affected joints and to clinical relevant symptoms. Osteophyte formation is highly associated with cartilage damage but osteophytes can develop without explicit cartilage damage. Osteophytes are mainly derived from precursor cells in the periosteum and growth factors of the TGFβ superfamily appear to play a crucial role in their induction.

Conclusion: Osteophyte formation is an integral component of OA pathogenesis and understanding the biology of osteophyte formation can give insights in the disturbed homeostasis in OA joints.

Key words: Osteophytes, Osteoarthritis, TGF beta, Periosteum, Mesenchymal stem cells.

Introduction

An osteophyte is ''a fibrocartilage-capped bony outgrowth''. Three types of osteophytes are known, the traction spur at the insertion of tendons and ligaments, the inflammatory spur, represented by the syndesmophyte at the insertion of ligaments and tendons to bone as can be seen in ankylosing spondylitis; and the genuine osteophyte or osteochondrophyte (chondro-osteophyte) arising in the periosteum overlying the bone at the junction between cartilage and bone[1]. In this article we discuss the clinical relevance, potential function and biology of primarily the osteochondrophyte, further called osteophyte, that can be found at the margins of diarthrodial joints, apophyseal joints and vertebral bodies and is a common feature of osteoarthritis (OA).

Clinical relevance

Osteophyte formation is in addition to joint space narrowing, subchondral sclerosis and subchondral cyst formation one of the main radiographic features of OA and an important criterium for this disease. Osteophytes can form early in the development of OA and can be seen prior to joint space narrowing.

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Osteophytes can have a significant clinical impact and can be a source of pain and loss of function. The latter mainly through nerve compression, limitation of joint mobility and obstruction of tissues and organs. For example, lumbar pain and abdominal pain caused by vertebral osteophytes have been reported by a number of authors[2–5]. Several studies have shown a clear relation between osteophytes of the spine and dysphagia and breathing problems[6–10]. In addition, osteophytes of the spine can be a cause of vocal cord paralysis[10–12] and vertebral artery compression by cervical osteophytes has been reported by Giroux *et al.*[13]

Also osteophytes at other sites can be a source of pain and dysfunction. An association between distally pointing osteophytes of the acromioclavicular joint and ruptures of the supraspinatus tendon has been proposed by Petersson *et al.*[14] A significant correlation between medial tibial condyle osteophytes and knee joint pain has been described in patients with knee OA[15] but this is not confirmed by others[16]. Moreover, a simple correlation between pain and osteophytes is not sufficient to demonstrate a causal relationship. Overall one can conclude that osteophytes can result in serious medical problems, mainly in the spine, but that osteophytes can also be present without negative effect or have even positive effects by increasing the joint surface.

Functional or bystander?

Are osteophytes a functional adaptation or a pathological phenomenon? It is unclear whether osteophytes develop because of pathological joint alterations or arise from normal remodeling processes secondary to joint changes,

such as OA? Can the development of an osteophyte be considered as an adaptation of a joint to instability or as a result of an altered internal joint milieu resulting in chondrogenesis of precursor cells in the periosteum and synovial lining?

The development of osteophytes in the vertebral column has been shown to be a general indicator of age[17]. However, it is difficult to strictly separate ageing form OA due to the high correlation between ageing and OA. The observation in humans and animal models that osteophyte formation can occur without overt cartilage damage seems to indicate that osteophyte formation and OA are not related one to one but that osteophyte formation can occur in "healthy" joints, maybe as a result of mechanical stimuli or ageing. Vise versa full thickness cartilage damage without osteophyte formation has also been shown[18]. However, in the latter study it was not clear whether the damage represents true OA or focal chondral defects. Although cartilage damage and osteophyte formation do not correlate completely, cartilage damage confirmed by joint space narrowing is reported to be highly associated with the presence of osteophytes[15].

In knee joints it has been shown that after a tear of the anterior cruciate ligament, osteophytes develop anteriorly and posteriorly and limit translocation of the femur on the tibia, stabilizing the joint. In primary knee OA removal of marginal osteophytes significantly increased joint motion, indicating that osteophytes limit the movability of OA knees[19]. In hip OA, recuperation of the joint space has been linked to the development of large osteophytes[20]. However, the latter does not necessarily indicate resurfacing of the joint with functional cartilage or return of mobility. These data show that osteophytes are not non-functional bystanders in all cases but that they can have constructive roles in a number of OA affected joints.

Osteophyte biology

Osteophytes develop in diarthodial joints (Fig. 1). Diarthrodial joints are constrained by a ligamentous structure called the joint capsule. Synovium is a membrane containing macrophages and fibroblast-like cells that cover all the non-cartilaginous surfaces within the capsule. The synovium is separated from the capsule by the subsynovial layer that contains blood vessels and nerves. Synovium also covers the periosteum positioned on the outside of the cortical bone in the joint. The periosteum and synovial lining contain cells involved in osteophyte formation.

The development of a representative osteophyte originating at the medial tibia during murine experimental osteoarthritis (collagenase-induced OA) is presented in Fig. 2. Cells in the periosteum covering the bone at the cartilage and bone boundary are stimulated to proliferate [Fig. 2(b and c)]. Cells inside the developing osteophyte undergo chondrogenesis and deposit matrix molecules, such as aggrecan, in the tissue [Fig. 2(d)]. The osteophyte remains covered with a layer of fibroblast-like cells during development. Cells in this layer contribute to the growth of the osteophyte by proliferation and differentiation to chondrocytes inside this layer [Fig. 2(e)]. The most central chondrocytes further differentiate and hypertrophy [Fig. 2(e and f)]. Hypertrophy of the chondrocytes is followed by endochondral ossification, deposition of bone and formation of marrow cavities [Fig. 2(g)]. A fully developed osteophyte is integrated with the original subchondral bone and still shows a outer fibrous layer [Fig. 2(h)]. The top of the osteophyte is covered with cartilage expanding the original cartilage surface of the joint [Fig. 2(h)].

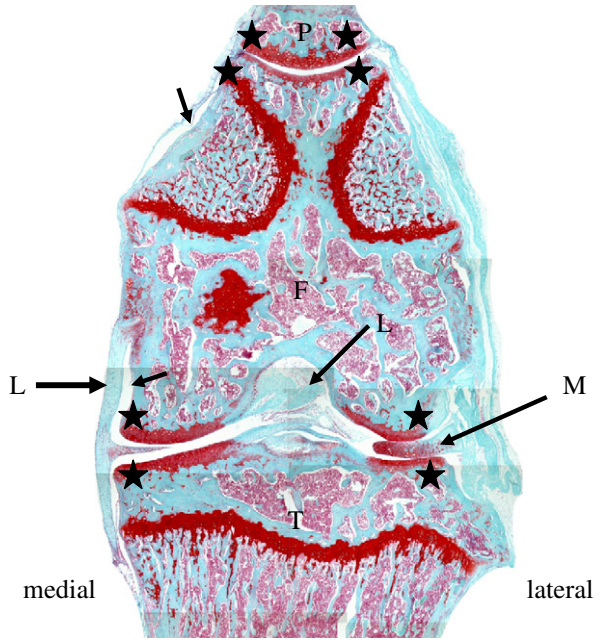

Fig. 1. Schematic representation of a murine knee joint. Shown are the sites were osteophytes develop during experimental OA (stars). T – tibia, P – patella, F – femur, L – ligament, M – menuscus, C – capsule. Synovium covers all non-articular surfaces within the joint cavity (◄).

OSTEOPHYTES CAN BE RAPIDLY INDUCED

Induction of osteophyte formation can be, at least in experimental models, a fast process. In murine models of OA the first signs of osteophyte formation can be seen within 2–3 days. This fast induction of osteophyte formation is not confined to small rodents. The development of periarticular osteophytes in experimental knee OA in dogs begins as early as 3 days after induction of the disease process[21]. This indicates that at least in these experimental models osteophyte formation and OA do not have a cause and effect relationship since overt cartilage damage will not have developed within this limited time span. Resident cells lying in the transition zone of bone and articular cartilage are rapidly triggered to undergo chondrogenesis.

CELL SOURCE OF OSTEOPHYTE PRECURSORS?

What is the cellular source of the osteophyte? Mesenchymal stem cells (MSC) present in the periosteum or synovial lining are thought to be the precursors of the osteophytes. In murine experimental OA osteophytes originate primarily from the periosteum covering the bone at the cartilage–bone junction. However, also cell populations from the synovium can be triggered to form cartilage *in vitro* and synovium-derived MSC have been shown to be even more efficient in cartilage formation than bone marrow-derived MSC[22]. A comparative study of Sakaguchi *et al.* showed that synovium-derived cells were superior in cartilage formation compared to cells derived from bone marrow, periosteum, skeletal muscle, and adipose tissue[23]. It

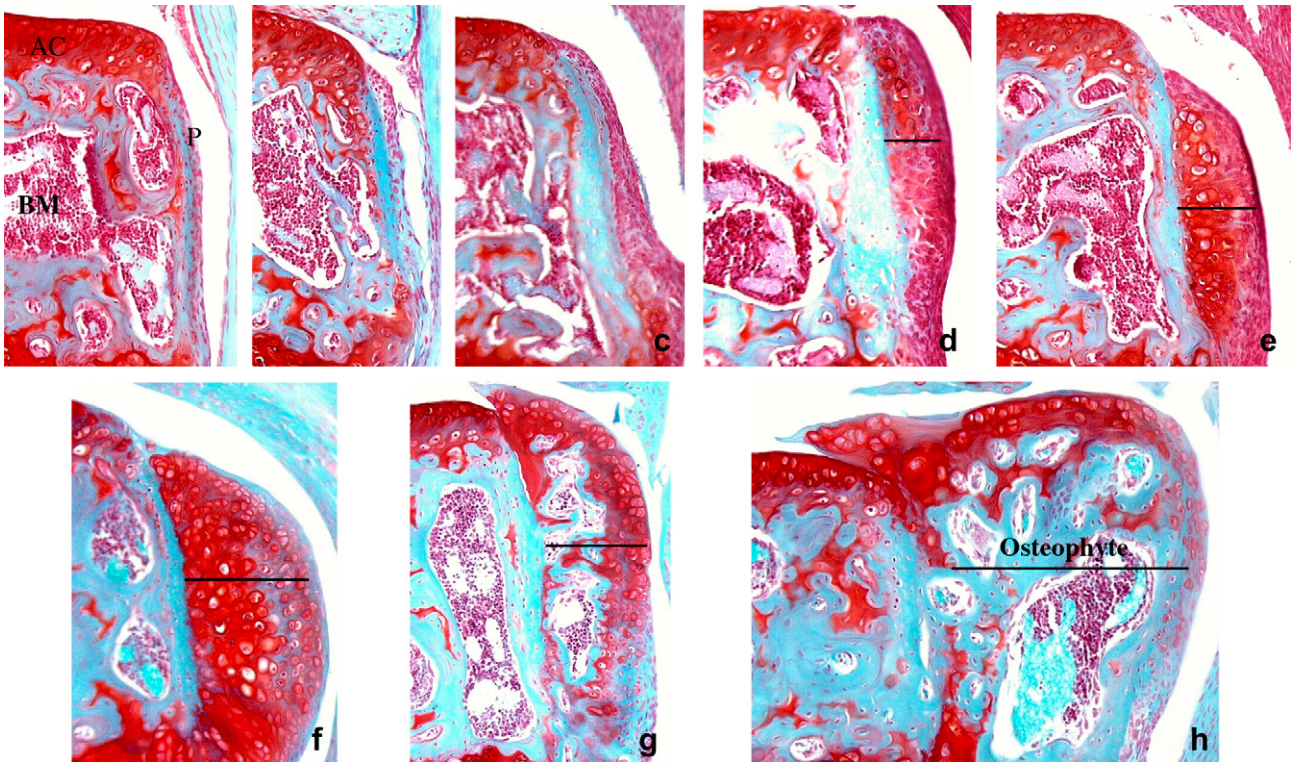

Fig. 2. Osteophyte formation in experimental murine OA (medial tibia). Cells in the synovial lining are stimulated to proliferate (b and c). Cells inside the developing osteophyte undergo chondrogenesis and deposit matrix molecules such as aggrecan (d). The most central chondrocytes further differentiate and hypertrophy (e and f). This is followed by endochondral ossification and formation of marrow cavities (g). A fully developed osteophyte is integrated with the original subchondral bone and the top of the osteophyte is covered with cartilage expanding the original cartilage surface of the joint (h) (p − periosteum, AC − articular cartilage, BM − bone marrow cavity).

has been shown that although the bone marrow-derived and synovium-derived MSC shared similar functional properties their differentiation capacities and transcriptional profiles were dissimilar[24]. However, De Bari *et al.* have shown that human periosteum-derived cells from young as well as older individuals still can undergo chondrogenesis while synovium-derived cells failed to form stable ectopic cartilage in nude mice indicating a functional difference between periosteum and synovium-derived cells[25–27].

Are osteophytes only the consequence of the recapitulation of endochondral bone formation as can be seen during development or are also other processes involved. In other words are osteophytes, as can be found at joint margins, totally derived from chondrocyte precursors or is there an attribution of other cells such as (pre)osteoblast? In animal models of OA it is observed that in addition to chondrogenesis and endochondral ossification intramembraneous lamellar bone formation contributes to the definitive osteophyte. In dogs osteophyte formation has been shown to be accompanied by hyperplasia of bone[21]. This bone develops eventually a mature trabecular structure and becomes confluent with the neighboring osteophyte. A similar pattern of osteophyte formation can be seen in mouse knee joints with experimental OA, even though not at all times intramembraneous bone formation significantly contributes to osteophyte formation in murine experimental OA. These observations suggest that osteophyte formation is primarily a process of neochondrogenesis of MSC present in the periosteum at the bone–cartilage junction but that cells derived from the synovial lining and intramembraneous bone formation can contribute to the definitive osteophyte.

WHICH FACTORS ARE EXPRESSED DURING THE DEVELOPMENT OF OSTEOPHYTES?

The expression of growth factors and extracellular matrix molecules has been studied in human osteophytes and animal models. Transforming growth factor β has been shown to be expressed by human osteophytes[28]. In human osteophytes TGFβ mRNA expression was found in the superficial cells in osteophyte cartilage, but it was scarcely present in the chondrocytes in the degenerative articular cartilage. We could demonstrate TGFβ expression and strong phosphorylation of SMAD2, an intracellular signaling molecule of TGFβ, in developing osteophytes in mice[29]. The fibrous outer layer of the developing osteophyte strongly expressed TGFβ while SMAD2P could be demonstrated also in more internal parts of the osteophyte. In hypertrophic chondrocytes BMP2 was expressed. These results not only indicate that TGFβ is involved in the initial stages of osteophyte formation but also that later developmental stages could be under the control of additional factors, like BMPs.

Besides growth factors of the TGFβ superfamily other factors such as Insulin-like Growth Factor-I (IGF-I) have been shown to be present in developing osteophytes. Okazaki *et al.* showed that in a murine OA model (collagenase-induced OA) both IGF-I and the type I IGF receptor were detectable in growing osteophytes. Both IGF-I and the IGF receptor mRNA were coexpressed by proliferating

perichondral cells, chondrocytes and osteoblasts[30]. Expression of the FGF receptor3, PTHrP and the PTH receptor was demonstrated in chondrocytes in human osteophytes[31]. In analogy with embryonic cartilage formation these factors likely play a role in osteophyte formation in adult individuals.

An interesting molecule demonstrated to be present in human osteophytes is leptin[32]. Serum leptin levels are related to body fat stores and is suggested to be involved in both obesity and OA. In animal experiments, leptin induced the synthesis of IGF-1 and TGFβ and strongly stimulated anabolic activity in chondrocytes[32]. Leptin can be a connecting factor between obesity on one hand and OA and osteophyte formation on the other.

Expression of growth factors and other soluble mediators appear to be the driving force of osteophyte formation but expression of extracellular matrix molecules reflects the developmental stage of the cells and tissues involved. In human osteophytes cellular differentiation has been analyzed by investigating collagen expression on mRNA and protein level[33,34]. Developing osteophytes are composed of fibroblasts, mesenchymal prechondrocytes, maturing chondrocytes, hypertrophic chondrocytes and osteoblast. Early precartilaginous tissue expressed both mRNA and protein for type I and type II collagen. The cartilaginous zones of the osteophytes were characterized by strong staining of type II collagen, comparable to staining in fetal cartilage. Mesenchymal prechondrocytes expressed type IIA procollagen mRNA and maturing chondrocytes expressed type IIB procollagen mRNA[34]. Fibrocartilaginous areas expressed type II as well as type III collagen while the strongest expression of type I was in bone and in the superficial fibrous layer of the osteophytes. Hypertrophic chondrocytes, expressing type X collagen, were found in areas of endochondral ossification. Specific stages of differentiation can be recognized in developing osteophytes based on collagen expression[33].

TRIGGERS OF OSTEOPHYTE FORMATION?

What elicits osteophyte formation can be learned from *in vitro* studies on chondrogenesis by MSC or isolated periosteum and from *in vivo* studies. Are osteophytes induced as a result of mechanical or biochemical stimulation or by a combination of both factors? Are mechanical stimuli transferred to biochemical signals that actually trigger chondrogenesis? Studies have indicated that cartilage can be formed by periosteum even in paralyzed limbs and immobilized joints[35]. This demonstrates that mechanical factors are not indispensable in the process of chondrogenesis but does not proof that the initiation of osteophyte formation is independent of mechanical factors. Most likely mechanical stimuli that are transcribed to biochemical factors on a cellular level or autonomous biochemical stimuli initiate the process of chondrogenesis in residing MSC.

Studies from our own group demonstrate that not only the MSC in the periosteum and synovial membrane are involved in osteophyte formation but that also macrophage-like cells contribute to this process. In murine collagenase-induced OA synovial lining macrophages were selectively removed from the synovial lining using chlodronate liposomes. This resulted in a significant reduction of synovial activation and osteophyte formation in the treated joints. Production of growth factors by synovial macrophages appears to contribute to the process of osteophyte formation[36,37].

FACTORS THAT INDUCE OSTEOPHYTES *IN VIVO*

Many of the factors that have been shown to be expressed during osteophyte development appear to be able to induce chondrogenesis, suggesting a role for these factors during the process of osteophyte formation. Several factors belonging to the TGFβ superfamily have been shown to induce osteophyte formation *in vivo*. The most potent factor inducing osteophytes appears to be TGFβ. Triple injection of TGFβ, 20–200 ng per knee joint per injection, resulted in impressive osteophyte formation. Interestingly, the localization of TGFβ1-induced osteophytes was very similar to that of osteophytes observed in murine OA models, suggesting a role for endogenous TGFβ in osteophyte formation during OA[38]. No differences in osteophyte formation were observed between TGFβ1, β2, β3. Also adenoviral overexpression of active TGFβ clearly stimulated osteophyte formation although adenoviral overexpression resulted in relatively more marked synovial fibroplasia than direct injection of TGFβ[39]. This difference can be attributed to the difference in concentration and duration of TGFβ exposure in the two different experimental settings.

In addition to repeated TGFβ injection also repeated injections of BMP2 or BMP9, induced osteophyte formation[40]. Osteophytes induced by BMP2 were found at sites in the joint where the growth plates meet the joint space while osteophytes induced by TGFβ originated from the periosteum at sites remote from the growth plates. This shows that BMP2 targets different types of cells than TGFβ. Apparently TGFβ activates MSC in the periosteum while BMP2 stimulates cells that are already committed to the chondro-osteogenic pathway. Localization and developmental pattern of osteophytes induced by TGFβ were more similar to OA-related osteophytes than BMP-induced osteophytes. However, this does not exclude that BMPs are important in osteophyte formation at specific developmental stages of this structure. Exposing joints to high levels of IGF-I or Connective Tissue Growth Factor (CTGF) did not induce the formation of osteophytes in murine knee joints[29,41].

Mesenchymal stem cells can be isolated from synovial membranes[24]. Exposure of human MSC from synovium to TGFβ activates the chondrogenic pathway in these cells. However, it is not clear if TGFβ alone is sufficient for induction of chondrogenesis. Addition of BMP2 and dexamethasone to TGFβ noticeably increased cartilage formation and cartilage matrix synthesis[22–24].

CHONDROGENESIS IN PERIOSTEUM

Chondrogenesis has been studied in detail in periosteum and a lot can be learned from those studies. Joyce *et al.* have injected TGFβ directly into the periosteum of newborn rat femurs[42]. Daily injections of TGFβ induced chondrogenesis that was followed by endochondral bone formation at these sites. Interestingly, besides induction of cartilage formation also stimulation of intramembraneous bone formation was observed. The dose of TGFβ applied influenced the ratio of cartilage to intramembraneous bone formation: high dosage mainly supported cartilage induction and lower dosage supported more intramembraneous bone formation[42].

In vitro studies with isolated periosteum show that TGFβ is the most important factor inducing cartilage formation in these tissues[43–49]. Miura showed that a short pulse, only 30 min, with a high dosage TGFβ was sufficient to induce chondrogenesis in rabbit periosteum[47]. However, in a study

with rat periosteal cells TGFβ alone was unable to induce chondrogenesis[50].

An important component of media known to stimulate chondrogenesis *in vitro* are glucocorticosteroids[51–53]. Recently it has been shown that expression of SCRG1, a molecule expressed during chondrogenesis, suppressed proliferation, and stimulated chondrogenesis of the murine MSC cell line C3H10T1/2 cells. Expression of SCRG1 was dependent on the presence of dexamethasone in the culture medium[52].

Besides glucocorticoids other factors modulate chondrogenesis in periosteum. Bone morphogenetic proteins show conflicting results. In rat periosteal cells TGFβ alone did not induce chondrogenesis but BMP2 treatment resulted in type II collagen and aggrecan expression. During culture the aggregates expressed type X collagen and osteocalcin, markers of chondrocyte hypertrophy. Aggregates treated by BMP2 consisted of numerous hypertrophic chondrocytes while culturing with both BMP2 and TGFβ blocked the generation of hypertrophic chondrocytes, indicating that TGFβ modulates chondrocyte terminal differentiation[50]. Uusitalo and co-workers demonstrated that adenoviral overexpression of BMP2 in mouse periosteal cells was sufficient to induce cartilage formation by mesenchymal cells and subsequent endochondral ossification[54]. In chicken periosteal cells BMP2 had no effect on chondrogenesis but enhanced osteogenesis[55]. These studies appear to indicate that the effect of BMP2 on chondrogenesis is species dependent but that in general BMP2 can contribute to chondrogenesis and osteogenesis. This suggests an essential role for TGFβ in the initiation of chondrogenesis which can be taken over by BMPs during terminal differentiation of chondrocytes and endochondral ossification of the osteophyte.

IGF-I is an essential growth factor in the metabolism of differentiated articular cartilage[56]. In addition to a role of IGF-I in differentiated cartilage, in rabbit periosteum it has been shown that IGF-1 increases chondrogenesis in a dose-dependent manner[43,57]. A combination of IGF-I and TGFβ gives the strongest stimulation of chondrogenesis. A short term incubation with IGF-I increases cartilage formation and continued IGF-I exposure enhanced cartilage growth[43,57]. It appears that IGF-I and TGFβ can act in concert to stimulate proliferation and differentiation of periosteal MSC during chondrogenesis.

Exposure of periosteum to basic Fibroblast Growth Factor (FGF-2), in the presence of TGFβ, also enhances cartilage formation[43,44,57,58]. Addition of FGF-2 during early stages of culture enhances cell proliferation which results in increased cartilage formation at later stages. Iwasaki *et al.* stimulated periosteum-derived cells form chickens with TGFβ and FGF-2[44]. Basic Fibroblast Growth Factor clearly stimulated the proliferation of peristeum-derived cells but inhibited osteochondrogenic differentiation. TGFβ has the opposite effects in these cells. These data indicate that the main role of FGF-2 in the stimulation of cartilage formation is the expansion of a pool of cells that can be triggered to undergo chondrogenesis by other factors, such as TGFβ.

PHYSICAL FACTORS

Growth factors appear to play a major role in chondrogenesis and consequently osteophyte formation. However, other factors appear to modulate this process. Oxygen tension and dynamic fluid pressure have been shown to effect chondrogenesis. Chondrogenesis appears to be, somewhat surprisingly, maximal at oxygen levels of 12–15% and no significant differences were observed in a range between 12 and 45%[59]. Only at very high (90%) and very low (1–5%) oxygen concentrations chondrogenesis was impaired. The actual oxygen levels in the joint are in the low range. Although *in vivo* chondrogenesis during osteophyte formation takes place at relatively low oxygen levels these levels do not appear to stimulate this process. Dynamic fluid pressure was applied to periosteal explants grown in agarose. Low levels of dynamic fluid pressure enhanced chondrogenesis while high levels totally blocked this process[60]. The authors did not use TGFβ in their culture system to induce chondrogenesis so the observed chondrogenesis was a result of the applied pressure. The applied pressure can have a direct effect on the cellular differentiation, induce chondrogenesis-promoting growth factors or modulate growth factor binding to the cells.

INHIBITION OF OSTEOPHYTE FORMATION

Chondrogenesis from periosteal MSC is thought to be primary step in the initiation of osteophyte formation. *In vivo* and *in vitro* studies have shown that TGFβ has the potential to induce chondrogenesis in these cells. However, this does not prove that TGFβ also has this role during OA. To study the role of endogenous TGFβ we blocked its activity, using the Latency Associated Peptide (LAP), a soluble form of the type II TGFβ receptor and SMAD7 as specific TGFβ inhibitors. Blocking TGFβ during murine papain-induced OA resulted in significant inhibition of osteophyte formation[61,62]. These studies show that TGFβ is essential in authentic osteophyte formation *in vivo*, it is until now not clear what other factors contribute to this process *in vivo* and which are the crucial factors in human osteophyte formation. In analogy with other species a role for TGFβ in human osteophyte formation can be expected. However, species differences are known with respect to osteophyte formation. Small rodents such as mice not only develop osteophytes during OA but also during experimental arthritis. This indicates that either the factors present in human and murine joints with arthritis are dissimilar or that human MSC react differently to these factors than rodent MSC. The fact that in human OA osteophytes appear indicates that adult human MSC have a general ability to develop osteophytes.

PHARMACOLOGIC INHIBITION OF OSTEOPHYTE FORMATION

Several drugs have been reported to inhibit osteophyte formation in models of experimental osteoarthritis. However, in most models it appears that inhibition of osteophyte formation goes hand in hand with reduction in cartilage damage. The group of Pelletier *et al.* has reported that in the canine ACL transection model the NSAIDs tenidap and carprofen significantly reduced osteophyte formation with a concomitant reduction in the size of the cartilage lesions[63–66]. A similar effect was seen when treating dogs with the nitric oxide synthase inhibitor L-NIL. The histological severity of cartilage lesions and osteophyte formation was reduced by L-NIL treatment[67,68]. A theoretical very interesting class of drug in the inhibition of osteophyte formation is the bisphosphonates. In dogs with ipsilateral ACL transection it has been shown that treatment with the bisphosphonate NE-10035 markedly reduced formation and resorption of cancellous bone but had no effect on cartilage damage or osteophyte formation[69]. In contrast, treatment of rats in which OA was induced by transection the ACL with

alendronate reduced osteophyte formation in a dose-dependent manner and inhibited cartilage damage[70]. The latter indicates that effects of bisphosphonates are compound or species dependent.

Conclusions

Osteophyte formation closely resembles the process of chondrogenesis and endochondral bone formation as can be seen during embryogenesis. Moreover, cytomorphology and gene expression patterns indicated that chondrogenesis and bone deposition associated with osteophyte formation bear a close resemblance to healing fracture callus[34]. Although the inflammatory element of fracture healing is absent in osteophyte formation it can be concluded that during osteophyte formation similar signaling pathways are activated as in healing callus. Moreover, as has been discussed above factors from activated synovium can contribute to osteophyte formation. Understanding the biology of osteophyte formation can give insights in the disturbed homeostasis of OA joints with OA and ultimately directions for OA therapy.

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