Review

Bone morphogenetic proteins in destructive and remodeling arthritis

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Abstract

Joint destruction and tissue responses determine the outcome of chronic arthritis. Joint inflammation and damage are often the dominant clinical presentation. However, in some arthritic diseases, in particular the spondyloarthritides, joint remodeling is a prominent feature, with new cartilage and bone formation leading to ankylosis and contributing to loss of function. A role for bone morphogenetic proteins in joint remodeling has been demonstrated in the formation of both enthesophytes and osteophytes. Data from genetic models support a role for bone morphogenetic protein signaling in cartilage homeostasis. Finally, this signaling pathway is likely to play a steering role in the synovium.

Introduction

The classic signs and symptoms of arthritis - *rubor*, *tumor*, *calor*, *dolor et functio laesa* - cover a vast world of dynamic systemic and local processes with complex interactions between networks at the cellular and molecular levels. Major advances in our understanding of the pathology of chronic arthritis and new imaging techniques have highlighted distinct mechanisms of disease. In the joint, these include the development and persistence of an inflammatory and immune reaction, the activation of tissue destructive enzymes and cells, and the suppression or stimulation of molecular pathways regulating homeostasis, repair and remodeling (Figure 1).

Mechanisms of inflammation and auto-immunity have been studied most extensively, leading to the identification of key cell populations, such as T cells, B cells and macrophages, and of important messenger molecules, including cytokines such as tumor necrosis factor-α (TNFα). As a result, innovative targeted therapeutic strategies have an unprecedented effect on both rheumatoid arthritis (RA) and the spondyloarthritides (SpA). In addition, new immunological targets are identified at an amazing pace [1].

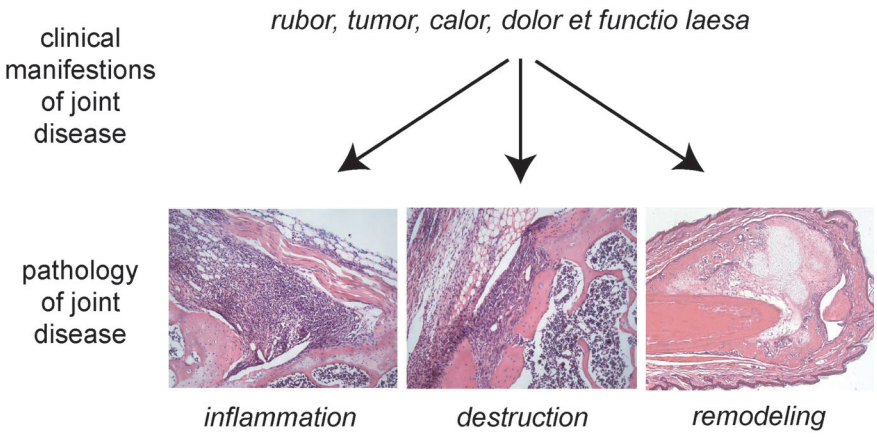
Two discoveries have recently opened up new paths of investigation for cartilage and bone destruction: the molecular characterization of osteoclast differentiation and activation [2] and the transformation of the synovium into tissue-destructive pannus tissue [3]. In addition, the success of the current treatment strategies has prompted new attention to be focused on repair and remodeling responses of joint tissues [4].

Tissue responses to inflammation or destruction in the joint can be physiological or pathological. Normal tissue responses include the regeneration or repair of soft and hard tissues, including cartilage and bone. Tissue regeneration involves a complete restoration of the original tissue with maintenance of function and homeostasis. This is perceived as a rare event. In tissue repair, the damaged tissue is replaced by a surrogate tissue with, at best, a partial restoration of its function. This is likely less durable and may evolve over time into functional failure. The articular cartilage has a very limited tissue restoration and repair capacity [5]. In bone, a tissue with a remarkable repair potential, such responses appear suppressed, probably by persistent inflammation [6]. In addition, abnormal tissue responses leading to joint remodeling, such as new cartilage and bone formation, may result in joint ankylosis and further loss of function [7].

We have used these tissue responses as a basis for an alternative mechanistic classification of chronic arthritis [8]. The disease can be defined as a 'destructive' arthritis, a 'steady-state' arthritis, and a 'remodeling' arthritis. In the first form, very little, if any, restoration or repair is observed, even with control of the inflammatory process. In the second form, local restoration or repair responses may be sufficient for many years, although ultimately joint homeostasis can be lost, resulting in joint failure. Finally, remodeling with neocartilage

BMP = bone morphogenetic protein; mBSA = methylated bovine serum albumin; OA = osteoarthritis; RA = rheumatoid arthritis; SpA = spondyloarthritides; TGFβ = transforming growth factor-β; TNFα = tumor necrosis factor-α.

Figure 1



The signs and symptoms of arthritis are caused by distinct processes in the joint. Synovitis with extensive inflammation is characteristic. Formation of pannus tissue and activation of osteoclasts contributes to joint destruction. Tissue remodeling is characterized by new cartilage and bone formation eventually leading to ankylosis. The images presented were obtained from mice with methylated bovine serum albumin-induced arthritis (inflammation and destruction) and from mice with spontaneous ankylosing enthesitis (remodeling).

and bone formation can be present. This may result in excessive responses, causing joint ankylosis, thereby directly contributing to loss of joint function and disability. In this concept, existing clinical boundaries are of less importance for the understanding of the molecular processes involved. More importantly, translation of this concept into animal models of disease could further strengthen our mechanistic approach to chronic arthritis.

Bone morphogenetic proteins

Reactivation of molecular signaling pathways that are critical for tissue formation during development and growth is increasingly recognized in the homeostasis, repair and remodeling of postnatal tissues. We have hypothesized that such signaling pathways including bone morphogenetic proteins (BMPs) may also be of importance in arthritis [4,8,9].

BMPs and closely related growth and differentiation factors comprise a large group of structurally related polypeptides that belong to the transforming growth factor-β (TGFβ) superfamily [10]. The original discovery of BMPs as protein factors that ectopically induce a cascade of endochondral bone formation *in vivo* [11] has strongly stimulated the study of their function in skeletal development (for a review, see [12]) and joint morphogenesis (for a review, see [13]). However, BMPs are involved in a wide array of biological processes, both during development and in postnatal life [14]. These include the specification of the dorso-ventral body axis and the development, growth and homeostasis of many organs. BMPs can act as morphogens, growth factors or cytokines depending on their spatio-temporal expression and target cells. Their downstream effects include cell lineage determination, differentiation, motility, adhesion and death [14].

BMPs induce ligand-dependent type I and type II receptor heterodimerization. These receptors are transmembrane serine-threonine kinases and phosphorylate intracellular receptor-smad signaling molecules (R-smad1/5) that bind common smad4 (co-smad4) and then translocate to the nucleus [10]. The diversity of cell responses to BMPs can at least partially be explained by differences in the affinities of different ligands for specific type I and II receptor combinations. BMP signaling is further regulated by extracellular antagonists such as noggin, chordin, gremlin, the DAN/Cerberus family, follistatin, follistatin-related protein and sclerostin (for a review, see [15]), by accessory receptors and by intracellular inhibitors. Transcriptional responses to BMP signaling are tightly controlled by different co-activators and co-repressors [10]. BMPs can also activate mitogen activated kinases such as p38 [16].

Bone morphogenetic proteins in 'remodeling arthritis'

Our group has been investigating the role of BMPs in an animal model of remodeling arthritis [17,18]. Spontaneous arthritis in aging male DBA/1 mice is characterized by new cartilage and bone formation at the entheses, progressively leading to joint ankylosis [19]. The proximal interphalangeal joints or ankles of the hindpaws are mainly involved. Other features of the model include dactylitis and nail lesions. We therefore consider this murine arthritis a model for tissue remodeling in SpA and, in particular, in psoriatic arthritis [19].

The exact trigger for entheseal new tissue formation is not clear. Injury, mechanical stress, hormones and activation of the immune system may all play a role [19-21]. Joint remodeling in this model is characterized by accumulation of spindle-shaped fibroblast-like cells, chondrogenic differentia-

tion, chondrocyte hypertrophy and replacement of the cartilage by bone. This is a typical cascade of endochondral bone formation. However, in continuity with the endochondral bone front, a small zone of direct bone formation is also recognized.

We studied the presence of different BMPs in this process [17]. BMP2 was associated with early events whereas BMP7 and BMP6 were mainly found in pre-hypertrophic and hypertrophic chondrocytes, respectively. Overexpression of noggin, a non-specific endogenous BMP antagonist, inhibited both clinical onset and severity of disease in a preventive and therapeutic strategy [17]. Detailed histomorphological analysis revealed that BMP signaling is critically important in the early stages of the disease processes, in particular in the commitment of progenitor cells to the chondrogenic lineage. Phosphorylation of smad1/5 molecules was used as a marker for activation of the BMP signaling pathway. Active BMP signaling was found in cells entering chondrogenic differentiation. These data were further corroborated by immunohistochemistry for phosphorylated smad molecules on human biopsies from entheseal lesions at the achilles tendon insertion of SpA patients [17].

However, the role of BMP signaling in the cascade of endochondral bone formation as seen in this model is more complex. Endogenous expression of noggin is important to counteract the BMP signal once the cells start chondrogenesis to allow progression towards chondrocyte hypertrophy and new bone formation [18]. Therefore, in noggin haploinsufficient mice, where endogenous noggin levels are reduced by about 50%, incidence of disease is not different from the wild type but progression of disease is delayed [18].

As for all animal models of disease, this model has both strengths and weaknesses. It allows the molecular analysis of ankylosis originating from the entheseal sites. However, the role of inflammation, innate and adaptive immunity in the murine disease is not yet clear and the specific relevance thereof for human SpA remains to be defined.

BMP and related TGFβ signaling have also been studied in osteophyte formation in mouse models of osteoarthritis (OA). Injection of recombinant BMP2 into healthy murine knees enhanced proteoglycan synthesis in the articular cartilage but also stimulated osteophyte formation. Interestingly, osteophytes induced by BMP2 injection were found predominantly in the regions where the growth plate met the joint space, whereas TGFβ-induced osteophytes originated from zones of the periosteum that were more remote from the growth plate [22,23]. Synovial macrophages appear to be critical in this process as osteophyte formation induced by TGFβ was reduced after depletion of macrophages by intra-articular liposomes. The number of BMP2 and BMP4 positive cells in these experiments declined upon deletion of the macrophages [24]. Similarly, depletion of macrophages also inhibited osteophyte formation in collagenase-induced arthritis, a mouse model of joint instability leading to osteoarthritis [25]. Papain-induced arthritis is a mouse model in which direct injection of papain depletes articular cartilage proteoglycans, leading to accelerated osteoarthritis-like lesions. Osteophyte formation in this model can be inhibited by adenoviral overexpression of both BMP and TGFβ antagonists. Again, expression of BMP2 and BMP4 in this model was markedly increased in the synovium [26]. Further analysis in this model and in a spontaneous model of osteoarthritis suggested that BMP2 expression occurs at later stages than $TGF\beta_3$ [27].

Two groups have studied expression of BMPs in human osteophytes [28,29]. Zoricic and colleagues [28] observed three different types of bone formation in the growing osteophyte: endochondral, and membranous from the periost and from the endosteum. Immunohistochemistry demonstrated BMP2 in both fibrous matrix and osteoblasts. BMP3 was found in osteoblasts and osteoclasts, BMP6 in osteocytes and osteoclasts, and BMP7 in hypertrophic chondrocytes, osteoblasts and osteocytes. Nakase and colleagues [29] demonstrated BMP2 in fibroblastic mesenchymal cells, fibrochondrocytes, chondrocytes and osteoblasts at both the mRNA and protein levels.

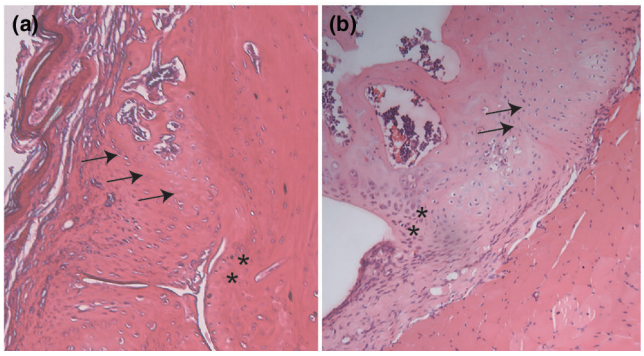
A key question is whether remodeling in SpA and OA are different (Figure 2). The enthesis has been suggested as the primary site of disease in SpA [30]. New tissue formation at the enthesis is a factor that contributes to pathology in SpA. The exact nature of the process is controversial. A classic point of view suggests that the formation of enthesophytes is a repair phenomenon [31]. However, the tissue response is excessive, suggesting that the process contributes more to pathology than to restoration of tissue function.

Osteophyte formation as typically seen in osteoarthritis may be of a different nature. It does not arise from the insertion sites but at the junctional zone where the synovium overlies the bone [32] (Figure 2). There is no evidence that the osteophyte contributes to the signs and symptoms in peripheral joints. Rather, it is hypothesized that osteophytes represent an attempt at repair and a stabilizing effort in a damaged joint [33]. Ankylosis is rarely, if ever, seen. Therefore, the nature of osteophytes in OA and enthesophytes in SpA is very different. Enthesophyte formation in SpA is a potential therapeutic target, in particular since new tissue formation and inflammation appear to be at least partially uncoupled events [34].

Bone morphogenetic proteins in 'steady-state' arthritis

The articular cartilage is a highly specialized tissue with unique properties. Its function is critically dependent on the interaction between the cells (chondrocytes) and their extracellular matrix and it is resistant to vascular invasion and mineralization. The complex regulation of extracellular matrix synthesis suggests that the articular chondrocytes can retain

Figure 2



Enthesophytes and osteophytes are different. **(a)** The enthesophyte originates from the insertion of capsule and tendons (arrows). The chondrosynovial border of the articular cartilage is not involved (asterisks). **(b)** Osteophyte originating from the border of the articular cartilage (asterisks). In contrast, the enthesis is normal (arrows).

cartilage homeostasis to a certain degree or for a limited period in case of chronic or progressive strain such as seen in OA. This homeostasis is critically dependent on the balance between, and the magnitude of, anabolic and catabolic molecular pathways. However, the restoration and repair capacity of the articular chondrocytes is limited [5]. Chondral lesions without injury to the subchondral bone do not heal spontaneously and gradually worsen. Osteochondral defects penetrate into the bone and show some attempts at repair, with invasion of mesenchymal progenitor cells from the subchondral bone marrow cavities. However, fibrocartilaginous rather than articular cartilage tissue is formed.

The role of BMPs in articular cartilage homeostasis and repair has been extensively studied *in vitro* and *ex vivo* (for a review, see [8]). More recently, the positive or anabolic effects of BMPs in this context have been further corroborated by *in vivo* data [18,35] (Table 1). Rountree and colleagues [35] developed a conditional gene deletion system that takes advantage of the expression of *Gdf5*, the murine homolog of cartilage derived morphogenetic protein-1 in the joint interzone during morphogenesis. Heterozygous BMP-receptor *(Bmpr)-Ia*$^{+/-}$ mice, engineered to express a Cre recombinase in the *Gdf5* locus (*Gdf5*$^{Cre/Cre}$;*Bmprla*$^{+/-}$) were crossed with mice that carry a floxed *Bmprla* allele (*Gdf5*$^{+/+}$;*Bmprla*$^{floxP/floxP}$). The *Gdf5*$^{+/Cre}$;*Bmprla*$^{-/floxP}$ conditional knockout progeny were viable and showed some mild developmental defects (short ears, soft tissue syndactyly between digit 1 and 2 and tarsal joint ankylosis). Importantly, *Gdf5*$^{+/Cre}$;*Bmprla*$^{-/floxP}$ mice failed postnatally to maintain articular cartilage in many joints compared to litter mate 'control' (*Gdf5*$^{+/Cre}$;*Bmprla*$^{+/floxP}$) mice. At birth the digit joints appeared normal, with high expression of both aggrecan and collagen type II mRNA in the two groups. As soon as one week after birth and more clearly by two weeks, changes in the articular cartilage had occurred. Expression of proteoglycans and collagen type II was greatly reduced. In other joints of forefeet and hindfeet similar changes were observed at seven weeks. By nine months of age, many regions of the cartilage were severely damaged. Progressive degenerative changes were also observed in the knee joints and triggered a loss of function.

Table 1

***In vivo* evidence supporting a role for BMPs in cartilage homeostasis**

Pro-homeostatic effects	Normal BMP receptor type Ia [35]
	Noggin haploinsufficiency [18]
	Injection of BMP2 [22]
Anti-homeostatic effects	Noggin overexpression [18]

BMP, bone morphogenetic protein.

Our group studied the effect of noggin (*Nog*) haploinsufficiency on joint destruction in two different models of arthritis, collagen-induced arthritis and methylated bovine serum albumin (mBSA) induced arthritis [18]. Noggin is expressed in articular cartilage. Reduction of noggin levels by about 50% (haploinsufficient *Nog*$^{+/LacZ}$ mice) did not affect severity of inflammation in both models. However, reduced noggin levels in *Nog*$^{+/LacZ}$ mice protected the articular cartilage in mBSA arthritis (Table 1). This was associated with enhanced BMP signaling in the articular cartilage as demonstrated by immunohistochemistry for phosphorylated smad1/5. Overexpression of noggin in wild-type mice in both models increased cartilage damage, probably by reducing BMP activity [18].

Intra-articular injections of BMP2 in the mouse knee have been used to assess the effect of this BMP on articular cartilage *in vivo*. BMP2 stimulates proteoglycan synthesis in normal knees but cannot do this in a model of destructive arthritis [36].

Bone morphogenetic proteins in joint destruction

The role of BMPs in the normal and inflamed synovium, in particular in a destructive arthritis such as RA, is less clear. The increasing interest in mesenchymal populations in the synovium and the role of stem cells in arthritis [37-39] has stimulated research into embryonic signaling pathways that typically guide mesenchymal stem cell behavior [4,40] (Table 2). We have demonstrated that *BMP2* and *BMP6* are expressed in synovial biopsies obtained from patients with chronic arthritis [9]. Protein levels of BMP2 and BMP6 were significantly higher in patients with RA and SpA compared to non-inflammatory controls. BMP2 and BMP6 protein was found in both macrophages and fibroblast-like synoviocytes as demonstrated by immunohistochemistry [9]. *BMP2* and *BMP6* expression in fibroblast-like synoviocytes *in vitro* was

Table 2

BMP signaling in synovitis

Ex vivo human biopsies	Increased expression of BMP2 and BMP6 [9]
	Decreased expression of BMP4 and BMP5 [41]
	Presence of BMP receptor Ia positive cells in RA [38]
Animal model data	Influx of BMP receptor Ia positive cells precedes onset of arthritis [39]

BMP, bone morphogenetic protein; RA, rheumatoid arthritis.

upregulated by pro-inflammatory cytokines such as IL1 and TNFα. We also demonstrated that BMP2 is associated with fibroblast-like synoviocyte apoptosis *in vitro* and *in vivo* [9]. In contrast, *BMP4* and *BMP5* were downregulated at the mRNA level in RA and OA samples versus normal controls as demonstrated by Bramlage and colleagues [41]. In normal synovium, BMP4 and BMP5 positive cells were found mainly in the lining layer, whereas in RA these cells were more scattered.

It is noteworthy that the presence of BMPs in the synovium is not associated with local cartilage or bone formation at these sites. This again highlights the complex biology of BMPs that should be considered as pleiotropic cytokines and growth factors with distinct effects on different cell types. Identification of target cells for BMP signaling in synovium and their biological relevance is, therefore, an important challenge. Our preliminary observations suggest that both blood vessel associated cells and mesenchymal cells in the synovium can be activated by BMPs (unpublished observations). Expression of different BMP receptors is present in fibroblast-like synoviocyte cultures [42]. Again, the local balance with antagonists and the processing of pro-peptides into mature forms will ultimately determine the impact of BMP signaling at the single cell and tissue level.

Further evidence may again come from animal models. BMP-RIa positive cells have been identified as potential mesenchymal stem cells in both RA [38] and joints from mice with collagen-induced arthritis, a model of RA [39]. Surprisingly, infiltration of cells into the synovium from the bone marrow apparently precedes the onset of symptoms in the induced model and a specific role for this cell population in disease pathogenesis has been hypothesized [39].

Of particular interest are recent data on the epitheloid character of the lining layer and its transformation towards a more typical mesenchymal cell type in arthritis [43]. RA synovial fluid stimulated this so-called epithelial-mesenchymal transition of normal fibroblast-like synoviocytes, an effect that could be inhibited *in vitro* by BMP7.

All these data provide further evidence that BMPs may act as regulatory molecules within the healthy and inflamed synovium (Table 2).

Perspectives

There is accumulating evidence that the tissue-resident cells of the normal synovium are critically involved in chronic arthritis [44]. These cells include both the mesenchymal fibroblast-like cells, macrophages and endothelial cells. Little is known about the role of these cell populations in joint remodeling - some of them may be targets for BMP signaling. Different hypotheses have been formulated to explain the role of such populations in arthritis.

The 'transformation hypothesis' proposes that fibroblast-like synoviocyte are stably transformed by the chronic inflammatory processes in the synovium. This results in a more aggressive cell type, pannocytes, with distinct morphological characteristics and the ability to attach to and invade the articular cartilage, as elegantly demonstrated in *in vivo* models of cartilage and synoviocyte co-implantation in SCID mice [45]. Mutations in tumor suppressor genes such as that encoding p53 have been documented and could explain some aspect of this altered cell behavior [46]. An alternative view suggests that low activity fibroblast-like synoviocyte/mesenchymal stem cells from the sublining zone acquire phenotypical characteristics of lining layer cells but lack positional information with overgrowth and invasion of cartilage and bone [47].

The transformation hypothesis was incorporated in the 'effector cell hypothesis'. The late destructive phase of RA, typically characterized by pannus formation, osteoclast activation and secretion of tissue-destructive enzymes, is considered mainly T-cell independent as it seems to be driven by an 'autonomous' fibroblast-like synoviocyte population, as suggested by the transformation hypothesis. Expansion and influx of mesenchymal cell populations are considered as a contributing factor in these processes [48].

These two hypotheses clearly focus on the tissue-destructive aspect of arthritis. There is also increasing evidence that the tissue-resident cell populations (mesenchymal cells, macrophages and endothelial cells) and embryonic signaling pathways play a part in the initiation and progression of arthritis. The 'stromal code' hypothesis [49] states that the stromal cell population of an organ provides differentiation, retention and exit signals for immune cells. The endothelium defines a stromal address code regulating cell entry by a number of selectins, integrins and chemokines. The code within the tissue further steers behavior of cells that have invaded the synovium.

Based on these theories and new experimental evidence from both developmental biology and arthritis research, we have proposed the 'signaling center hypothesis' [37]. Inflammation

and tissue destruction trigger a reaction aimed at repairing and conserving tissue function. However, in some cases this process is ill-coordinated within an inflammatory environment and leads to changes in the tissue-resident cell populations. Mesenchymal cells accumulate either by local proliferation, transdifferentiation or influx from other compartments such as blood or bone marrow. These cell populations can typically form signaling centers that regulate the behavior of surrounding cells. This concept from developmental biology places the stromal code hypothesis in a broader biological context. It enables understanding of not only the destructive but also the remodeling processes as the molecular signaling centers can guide both, dependent on the balance between tissue-destructive and homeostatic/reparative molecular signaling. As summarized above, there is increasing evidence that BMPs are involved in these processes. Moreover, interactions between mesenchymal cells and immune cells are likely to be critical in this process and may contribute to the differences between destructive and remodeling arthritis. In this context it is noteworthy that we and others identified macrophages as a source of BMPs in the joint [9,24].

Conclusion

BMPs are pleiotropic cytokines, growth factors and morphogens. Increasing evidence supports a critical role for BMP signaling in joint remodeling, particularly in enthesophyte formation in SpA. In addition, BMPs support cartilage homeostasis and repair. The role of BMP signaling in synovitis is still unclear, but a role as regulatory molecules is hypothesized.

Competing interests

The authors have filed a patent on the use of BMP inhibitors for the treatment of spondyloarthritis.

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