Review

Progress in spondylarthritis

Mechanisms of new bone formation in spondyloarthritis

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Published: 27 April 2009
This article is online at http://arthritis-research.com/content/11/2/221

Arthritis Research & Therapy 2009, **11:**221 (doi:10.1186/ar2642)

Abstract

Targeted therapies that neutralize tumour necrosis factor are often able to control the signs and symptoms of spondyloarthritis. However, recent animal model data and clinical observations indicate that control of inflammation may not be sufficient to impede disease progression toward ankylosis in these patients. Bone morphogenetic proteins and WNTs (wingless-type like) are likely to play an important role in ankylosis and could be therapeutic targets. The relationship between inflammation and new bone formation is still unclear. This review summarizes progress made in our understanding of ankylosis and offers an alternative view of the relationship between inflammation and ankylosis.

Introduction

The spondyloarthritides (SpAs) are a group of chronic inflammatory diseases of the skeleton and associated soft tissues. Different diagnostic entities that share clinical, pathological and genetic characteristics are integrated into this disease concept. These include ankylosing spondylitis (AS), psoriatic arthritis (PsA), inflammatory bowel disease-associated arthritis, reactive arthritis, juvenile SpA and undifferentiated SpA [1]. The prevalence and burden of SpAs, in particular AS and PsA, are at least as high as those of rheumatoid arthritis (RA) [1-3]. Sacroiliitis and spinal inflammation as well as peripheral arthritis and enthesitis, often with a nonsymmetrical distribution, are typical clinical features of these diseases. Extraskeletal manifestations include psoriasis, inflammatory bowel disease and acute anterior uveitis [1].

Clinical signs such as inflammatory pain, stiffness, swelling and loss of function are caused by enthesitis, bone edema, synovitis and joint effusion. The enthesis, an anatomical zone in which fibers of the tendons, ligaments and capsules insert into the bone through a fibrocartilaginous connection, is hypothesized to be the primary disease localization in SpA [4]. Entheses are found as a part of the joint organ or at extra-articular sites [5,6]. The synovium and the underlying bone marrow are in close contact and communication with the entheses [5-7]. Although compelling evidence is lacking, synovitis and osteitis in SpA can be understood by this close anatomical relationship. Chemotaxis and accumulation of inflammatory cells in combination with increased angiogenesis are more likely to occur in the easily accessible synovium and bone marrow than in the entheseal fibrocartilage, which is relatively resistant to cell invasion and neovascularization [6,7].

Although features of joint destruction can be dramatic, in particular in some forms of PsA, skeletal damage in SpA is only partially due to the loss of articular cartilage and bone erosion. In contrast, new cartilage and bone formation, presenting as ankylosing enthesopathy and leading to bony spurs, syndesmophytes, enthesophytes and eventually joint or spine ankylosis, are hallmark signs of these diseases. This process of ankylosis contributes significantly to the permanent disability of the patients, in particular in those suffering from AS [8].

The introduction of targeted therapies, in particular anti-tumour necrosis factor (TNF) drugs, has met unprecedented success in the treatment of signs and symptoms of SpA [9,10]. However, current radiographic follow-up data suggest that these drugs do not affect the process of ankylosis [11-13]. This apparent lack of structural effect is in sharp contrast to what is seen for the erosive destruction of joints in RA [14] and in PsA [15]. On the other hand, continuous treatment with celecoxib, a cyclo-oxygenase II specific nonsteroidal anti-

AS = ankylosing spondylitis; BMP = bone morphogenetic protein; DISH = diffuse idiopathic skeletal hyperostosis; DKK = dickkopf; MRI = magnetic resonance imaging; PsA = psoriatic arthritis; RA = rheumatoid arthritis; SpA = spondyloarthritis; TNF = tumour necrosis factor; WNT = wingless-type like.

inflammatory drug, as compared with on-demand treatment, does appear to influence ankylosis in AS [16].

These observations emphasize that insights into the molecular mechanisms of ankylosis and into the relationship between inflammation and new tissue formation in SpA are essential. Ankylosis is a fairly slow process and may not be seen in all patients [11-13,16]. In addition, the human tissue samples that are needed to study these processes are difficult to obtain, in particular in patients with axial disease. Current understanding and further progress into the nature and mechanisms of pathological new bone formation in SpA are therefore largely based on data obtained in different animal models, in imaging and biomarker studies.

Types of new bone formation

Two different types of physiological bone formation that take place during embryonic development and growth are recognized. Most skeletal elements are formed by a process of endochondral bone formation. Mesenchymal cells condensate into a so-called 'anlagen' and subsequently undergo chondrogenic differentiation. Cells within this cartilaginous mould of the skeletal element then differentiate into hypertrophic chondrocytes, their matrix is invaded by vessels and the cartilage tissue is progressively replaced by bone matrix synthesized by osteoblasts. Some bones, such as the calvaria, form through membranous bone formation as mesenchymal cells directly differentiate into osteoblasts that produce the bone matrix.

Endochondral bone and membranous bone formation remain important during postnatal growth. The growth plate is a strictly organized process of endochondral bone formation. The cortical bone further thickens through direct bone formation. Bone homeostasis is determined by lifelong cycles of local bone resorption by osteoclasts and new bone synthesis by osteoblasts.

New bone formation can be required under pathological circumstances [17]. Tissue responses to damage can lead to tissue regeneration or repair, with the former resulting in complete restoration and maintenance of function and homeostasis. Tissue repair results in a surrogate tissue, which at least partially restores function but which may expose the patient to risk for functional failure in the future. Abnormal or exaggerated tissue responses may lead to further loss of function instead of restoration. These concepts apply in particular to skeletal pathology, not only in SpA but also in fracture healing, osteoarthritis, RA, diffuse idiopathic skeletal hyperostosis (DISH, or Forestier's disease) and rare genetic disorders such as fibrodysplasia ossificans progressiva.

Fracture healing occurs through callus formation, which is a process of mainly endochondral and partially direct bone formation. This leads to healing and later remodelling in such a way that the bone more or less regains its original shape. In SpA, osteoarthritis, different forms of juvenile arthritis and DISH, new bone formation is mainly orthotopic (in continuity with existing bone) and appears to originate from the cartilage-bone edge (osteoarthritis), the growth plate (juvenile arthritis), or the enthesis and periosteum (SpA and DISH). Although most of the bone formation appears to be endochondral, direct bone formation also contributes.

Molecular mechanisms of new bone formation: data from animal models

Bone formation during development and growth relies on a number of molecular signalling pathways and their complex interactions [18]. Increasing evidence supports the concept that similar pathways are important during cartilage and bone pathology, particularly with regard to new bone formation. These pathways include bone morphogenetic protein (BMP), wingless-type like (WNT), hedgehog, fibroblast growth factors, notch and parathyroid hormone-like peptide signalling.

The potential roles played by BMP and WNT signalling in the process of ankylosis in SpA were recently studied in various animal models. Our group has used the spontaneous arthritis model in ageing male DBA/1 mice to study molecular mechanisms of ankylosing enthesitis [19]. These immunologically normal mice develop oligoarthritis, especially in the toes of the hind limbs, from the age of 12 weeks onward after grouped caging of males from different litters. The disease process is not characterized by primary synovitis but rather by entheseal cell proliferation, cartilage and bone differentiation, leading to peripheral joint ankylosis through orthotopic endochondral bone formation. The model also presents with dactylitis and destructive onychoperiostitis, which are well recognized features of human PsA. This model also has its limitations. Entheseal new cartilage and bone formation are only seen in peripheral joints and not in the spine. Inflammation with infiltration of immune populations into the joint tissues is only of short duration and does not appear to become a chronic process. These features are in contrast to what is commonly seen in SpA. Nevertheless, the model allows one to study molecular mechanisms of new tissue formation and may provide some information about the relationship between inflammation and ankylosis.

BMPs were originally identified as protein factors that can induce an ectopic cascade of endochondral bone formation *in vivo*, and are members of the transforming growth factor-β superfamily. We demonstrated that different BMPs are expressed during the process of ankylosis in male DBA/1 mice [20]. BMP2 is typically found in proliferating cells and entheseal cells that commit their differentiation fate to chondrogenesis. BMP7 is recognized in prehypertrophic chondrocytes, whereas BMP6 is associated with hypertrophic chondrocytes.

In the spontaneous ankylosing enthesitis model, systemic over-expression of noggin, a BMP antagonist with broad

ligand affinity, inhibited the incidence, and clinical and histomorphological severity of arthritis in a dose-dependent manner in both preventive and therapeutic experiments [20]. Progenitor cells committing to chondrogenic differentiation were recognized as BMP target cells. The histomorphological and molecular analysis of the experiments strongly suggested that BMPs play a role in these initial phases of the disease process.

However, the process of entheseal endochondral bone formation is highly regulated at different stages. Endogenous noggin is expressed in prehypertrophic and hypertrophic chondrocytes and appears to play a role in reducing some BMP signals in the replacement of hypertrophic chondrocytes by bone. A reduction in these endogenous noggin levels in noggin haplo-insufficient mice was associated with slower progression of ankylosis without affecting the initial stages of the disease [21]. These data are consistent with the complex role played by the BMP signalling pathway and its antagonists as regulators of endochondral bone formation, with different effects at distinct stages [18].

Interestingly, in a recent study, presented as an abstract, the investigators used a similar strategy to inhibit BMP signalling in aggrecan-induced spondylitis [22]. As our group demonstrated for peripheral arthritis, over-expression of noggin resulted in reduced spinal ankylosis, a feature of this murine disease model. Different BMPs were found at similar disease stages, and the target cells in this model appeared to be identical to those in our earlier work. We also described such BMP target cells in human entheseal lesions of the Achilles' tendon insertion [20].

Another study identified dickkopf (DKK)1, an antagonist of the WNT signalling pathway, as a potential key regulator of the balance between erosive joint destruction and new bone formation in inflammatory arthritis. Diarra and coworkers [23] demonstrated that inhibition of DKK1 with specific antibodies changed the histomorphological appearance of arthritis in human TNF transgenic mice and other models, such as collagen-induced and glucose-6-phosphate isomerase-induced arthritis. The anti-DKK treated mice exhibited osteophyte formation, which was absent in control antibody treated mice. *Dkk1* is a TNF target gene through p38 mitogen-activated protein kinase. Inhibition of DKK1 results in higher osteoprotegerin levels, which block the activation of osteoclasts and hence bone erosion. In addition, bone formation appears to be directly enhanced by stimulating WNT signalling both *in vitro* and *in vivo* [23].

Both observations, blocking BMPs to inhibit ankylosis and a WNT antagonist to stimulate it, albeit in different models, raise questions about the potential interactions or primary roles of these specific pathways. As mentioned above, BMPs were originally identified as proteins that can induce endochondral bone formation. In our studies, we identified BMP2 as an early mediator of chondrogenesis in ankylosing enthesopathy. Similar observations were reported in other models of chondrogenesis and osteogenesis. Tsuji and coworkers [24] demonstrated that limb-specific BMP2 knockout mice develop a normal skeleton but fail to maintain bone growth and homeostasis in the limb after birth. Limb-specific osteoporosis and spontaneous fractures occur, and the natural healing process is absent. In addition, these limb-specific BMP2 knockout mice fail to heal fractures in a fracture model [24]. The authors hypothesize that before birth loss of BMP2 in the limb can be compensated for by other BMPs, whereas this seems no longer the case postnatally. These findings indicate that developmental and postnatal processes may have many similarities but can be different at the molecular level. BMPs also play a critical role in the development of osteophytes in models of osteoarthritis [25].

The effects of WNT signalling on bone formation appear more complex. WNTs are a family of glycoproteins with an array of functions during development, growth, tissue homeostasis and disease. Some of the WNT ligands, in particular WNT3A and WNT10B, are associated with direct membranous bone formation during development and growth, most likely by activation of the so-called canonical WNT signalling pathway in which the nuclear translocation of β-catenin acts as a downstream mediator [26]. The roles of WNTs in endochondral bone formation are more difficult to understand. WNT3A and WNT7A have been shown to inhibit chondrogenesis in endochondral bone formation in developmental models [26]. Other ligands, WNT5A and WNT5B, appear to play opposite roles in determining the pace of chondrocyte differentiation [27].

The complex and contrasting effects of WNT proteins are further highlighted by studies of intracellular mediator β-catenin. Over-expression of a constitutively active form of this molecule in developing skeletal elements, mimicking enhanced WNT signalling, inhibited the early stages of chondrogenesis, whereas over-expression in later stages stimulated maturation of the chondrocytes and bone formation [28]. These observations are in accordance with a study in which the progression of BMP2-induced endochondral bone formation was found to be dependent on β-catenin [29].

Taken together, current evidence therefore suggests that WNTs are most important in the later stages of endochondral bone formation. WNTs signals stimulate progenitor cells into the bone lineage and may inhibit early cartilage differentiation. This negative effect on chondrogenic differentiation may also be important postnatally, because WNTs appear to have a negative effect on articular cartilage homeostasis. For instance, mice that are deficient in the secreted WNT antagonist frizzled related protein (FRZB) develop more severe cartilage damage in osteoarthritis models, which is associated with enhanced WNT signalling and increased expression of WNT target genes [30]. Specific activation of

β-catenin in articular cartilage in a genetic mouse model also leads to an osteoarthritic phenotype [31]. Surprisingly, the same group also reported that lack of β-catenin *in vivo* leads to loss of articular cartilage [32].

Based upon these data, we hypothesize that BMP family members are critical in the early phases of ankylosis in SpA and that WNT signalling through β-catenin plays a crucial supportive role in this process, in particular in the progression of endochondral bone formation (Figure 1).

Molecular mechanisms of new bone formation in spondyloarthritis: human data

Progress in SpA research has been hindered by the relative lack of human materials to study. Biopsies of the spine or bone from peripheral joints are difficult to obtain. Corrective surgical interventions are only rarely performed because the balance between benefits and risks is unpredictable. Moreover, surgical and autopsy materials are usually obtained from patients with long-standing or end-stage disease.

Historical studies have demonstrated that both endochondral and direct bone formation contribute to ankylosis in SpA [33]. New bone formation in SpA occurs mainly in continuity with the existing skeleton. The different stages of the disease process are more difficult to appreciate fully. Activation of entheseal progenitor cells appears to play an important role. A number of histology samples suggest that direct ossification takes place in the spine. More recently, surgical samples of spine and hip have been extensively studied. Although most attention has been given to the involvement of inflammatory cells in AS, areas of endochondral and direct bone formation were also recognized [34-36].

Molecular analysis of pathology materials from SpA patients is not only limited by the amount of tissue available but also to some extent by the extensive processing of the calcified tissues that is required. Transforming growth factor-β has been detected in some samples, including biopsies of the sacroiliac joints [37]. The specific involvement of this pleiotropic cytokine, which can have chondrogenic and osteogenic effects but is also an important immune modulator, remains to be demonstrated. Our group has demonstrated the presence of BMPs and activation of the BMP signalling pathway in peripheral entheseal lesions in SpA [20].

Imaging studies appear very useful for further studying the progression of SpA. Current approaches, in particular nuclear magnetic resonance imaging (MRI), have mainly focused on the detection of inflammatory changes. Progression of ankylosis is studied using conventional radiography. Radionuclide scans do not provide the required spatial resolution to permit bone formation to be studied dynamically in humans. It remains an open question whether approaches in animal models, including enzyme-activated probes, will find their way into clinical and translational patient imaging.

Serum biomarkers provide another means with which to study the process of ankylosis. In their original study, Diarra and coworkers [23] found that serum levels of DKK1 are very low to absent in patients with AS as compared with those who have RA. However, studies in other cohorts have yielded conflicting results [38,39]. Markers of bone metabolism suggest an upregulation of alkaline phosphatase activity in AS patients treated with anti-TNF [40-42]. It is not clear whether this increase is caused by enhanced trabecular bone formation to restore inflammation-induced general bone loss or by the specific development of syndesmophytes.

A relationship between inflammation and new tissue formation

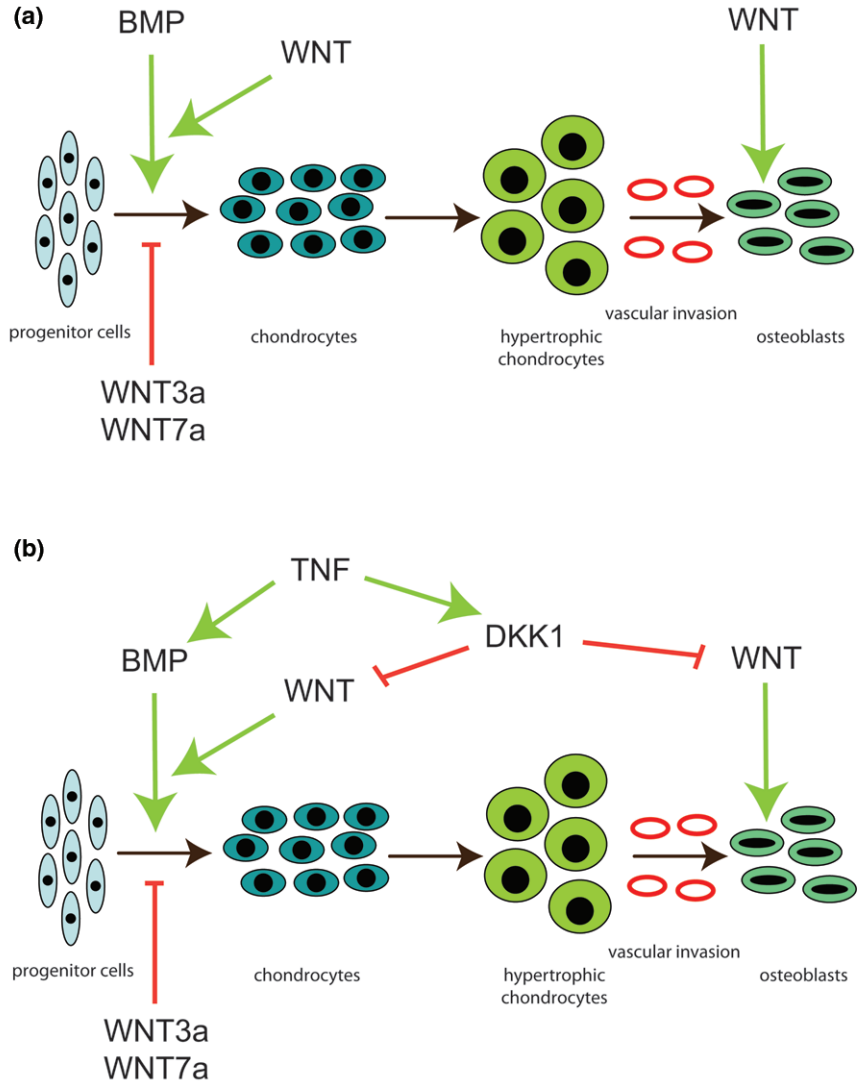
The existence or the nature of an eventual relationship between inflammation and ankylosis has become a central focus of research during the past couple of years. Pro-inflammatory cytokines such as TNF have a negative effect on chondrogenesis in *in vitro* systems [43]. We have demonstrated that etanercept, a soluble TNF receptor, does not affect ankylosing enthesopathy in the spontaneous arthritis model in DBA/1 mice [43]. As indicated above, 2-year follow-up cohorts suggested that, despite control of signs and symptoms of the disease with anti-TNF, ankylosis may progress [11-13].

These observations clearly highlight the critical question whether inflammation and new tissue formation in SpA are linked or uncoupled processes. The typical presentation of the disease - with signs and symptoms caused by inflammation prominent in the early phases, and ankylosis and the resulting disability in the later stages - may suggest a chronological order of events, but this is not supported by specific evidence. Because human tissues, in particular specimens from the spine, are not easily available, imaging methods may help us to understand the nature of the relationship between inflammation and ankylosis.

MRI can dynamically visualize the extent of inflammation in patients. Different cohorts have recently been studied and the conclusions about the relationship with tissue remodelling are certainly not unequivocal [44,45]. Sites with active inflammation appear to be more prone to later development of syndesmophytes, but on the other hand syndesmophytes are not adequately predicted by inflammation, as determined by MRI.

Probable mediators of new bone formation such as BMP2 are induced in different cell types (including synovial fibroblasts and cartilage cells) by pro-inflammatory cytokines such as TNF and interleukin-1 [46,47]. However, the direct effect of BMP2, which was identified in early stages of ankylosis in mice [20,22], may be counteracted by lack of supportive WNT signalling, because DKK1 production is also stimulated by TNF [23]. Of interest, downstream mediators of TNF and interleukin-1 signalling such as nuclear factor-κB and mitogen-activated protein kinases can also be triggered

Figure 1



Roles of BMPs and WNTs in endochondral bone formation. **(a)** Physiological endochondral bone formation is stimulated by bone morphogenetic proteins (BMPs). Wingless-type like (WNT) signaling plays a supportive role in relation to BMPs. However, some WNTs have a negative effect on early chondrocyte differentiation. **(b)** In the presence of inflammation, tumour necrosis factor (TNF) may stimulate BMP signalling but also the expression of DKK1, which acts a WNT antagonist. The balance between TNF, BMP and WNT signalling may determine the onset and progression of ankylosis. DKK, dickkopf.

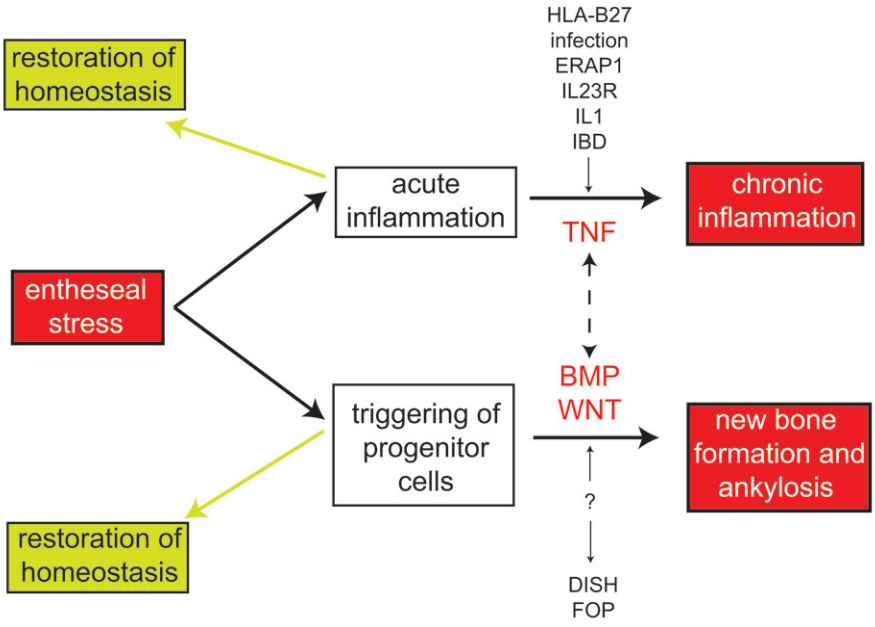
by mechanical stress, which is likely to be important in the enthesis.

Further support for an uncoupling of inflammation and new tissue formation may come from the observation that inhibition of osteoclasts, preventing bone erosion, does not affect ankylosis in a mouse model [48]. This suggests that bone erosion caused by osteoclasts is not necessary to trigger the process of entheseal new bone formation. This is further supported by human ultrasound data, which suggest that erosions and spurs occur in anatomically different sites [49]. In this sense, ankylosis is not by default a repair process initiated by damage to bone. However, damage to the fibrous or cartilaginous enthesis could be the primary event.

A broader view on new bone formation in spondyloarthritis

The apparent lack of effect on structural disease progression in AS has provided impetus to consider different hypotheses that apply to the relationship between inflammation and new bone formation. The traditional concept that ankylosis is a form of (excessive) repair has been translated into a new paradigm in which a distinction is made between the chronic active state of inflammation assumed to be typical for RA and

Figure 2



A view on the relationship between inflammation and ankylosis in SpA. The primary event is considered 'entheseal stress'. Biomechanical factors and microdamage are likely to play roles in this. Entheseal stress leads to triggering of an acute inflammatory reaction and of progenitor cells. In most instances, the acute events go unnoticed and homeostasis is restored. Under specific circumstances, the acute events can turn into a chronic situation in which inflammation and/or ankylosis are prominent. Different pathways regulate chronic inflammation and new tissue formation, but these pathways are likely to influence each other. Genetic factors are likely to steer chronic inflammation and new tissue formation. For the latter aspects, clues may be found in other bone-forming diseases. ERAP1, endoplasmic reticulum aminopeptidase 1; IBD, inflammatory bowel disease; IL23R, interleukin-23 receptor.

a more relapsing/remitting type of inflammation in SpA [50]. During this local remission phase, attempts at tissue repair could occur and result in ankylosis. This hypothesis has two important implications: first, early treatment could be useful to prevent structural damage; and second, anti-TNF treatment may lead to accelerated ankylosis in the short term but would in the long term be beneficial to avoid structural disease progression.

We propose an alternative hypothesis (Figure 2) based on the assumption that the primary event that triggers SpA is still unknown. We refer to this event as 'entheseal stress'. Activation of entheseal cells could lead to a double phenomenon: triggering of new tissue formation and production of pro-inflammatory molecules. The former can lead to restoration of tissue integrity or tissue remodelling. The latter phenomenon can develop into a chronic inflammatory process in which cytokines such as TNF play a pivotal role. A number of known factors may contribute to chronicity: the structural properties of HLA-B27; activation of the immune system by the presence of inflammatory bowel disease or infection; and polymorphisms in cytokines and cytokine processing molecules that lead to either more severe inflammation or delayed clearance of inflammation. However, under most circumstances, in particular in the absence of genetic predisposition, entheseal stress may not lead to chronic changes and homeostasis is likely to be restored.

In this paradigm, the development of SpA is dependent on a multi-step process that leads to chronic or recurrent inflammation but also to the triggering of new tissue formation, completely or partially independent of inflammation. The role of biomechanical factors that lead to stress responses or microdamage in the enthesis should therefore be further explored in this concept. Also, genetic factors, not yet identified and different from those that determine disease susceptibility, may have an impact on ankylosis. These genetic factors may be shared with other bone-forming diseases such as DISH and fibrodysplasia ossificans progressiva. Accordingly, additional strategies will be required to control new tissue formation in order to treat AS and other SpA patients adequately in the long term.

Conclusions

Despite the enormous progress that has been made to control signs and symptoms of disease in SpA, it remains unclear whether these strategies will also result in reduced disability by prevention of spinal or joint ankylosis. Observations in animal models point in another direction, and we therefore propose an alternative view of the relationship between inflammation and ankylosis in SpA. Current data

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suggest that targeting of pathways such as BMPs and WNTs is more likely to lead to prevention of structural damage and its consequences.

Competing interests

RL and FL hold a patent of the use of noggin for the treatment of SpA.

Acknowledgements

RL is the recipient of a postdoctoral fellowship from the Scientific Research Foundation Flanders (FWO Vlaanderen). The work of RL and FL is supported by grant from FWO Vlaanderen and a GOA grant from KU Leuven.

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