Osteoarthritis pain and inflammation – the new science

In part two of a two-part series, Duncan Lascelles BSc BVSc PhD FRCVS CertVA DSAS(ST) DECVS DACVS; Scott Knauer MS; Katherine Walker BS; and Cynthia North DVM MS continue the exploration of the underdiagnosis of osteoarthritis in dogs. Following on from the impact of chronic pain and treatment options available, this sequel addresses issues relating to inflammation and pain

INFLAMMATION

Expanded understanding of the role of joint tissues

- **Synovium (synoviocytes)**

  Synoviocytes exist as a two-to-three-cell deep lining of the interior of the capsule that normally provides continual support through the production of synovial fluid and other factors in support of a healthy joint. Synovitis involves the infiltration of mononuclear cells into the synovial membrane and production of proinflammatory mediators including interleukin 1β (IL-1β), tumor necrosis factor-α (TNF-α), chemokines and nerve growth factor (NGF). Matrix metalloproteinase (MMP)-1, MMP-3, and MMP-13 as well as IL-6, can be detected in osteoarthritis synovial fluid samples31 (see Figure 1).

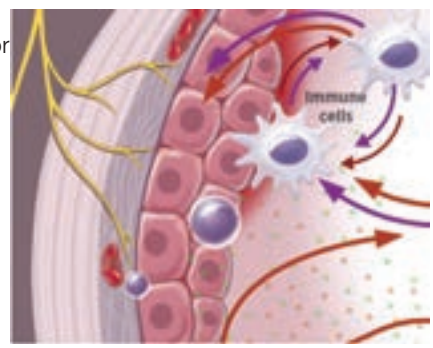

Figure 1.

- **Cartilage (Chondrocytes)**

  Chondrocytes are the only cells found in healthy cartilage and produce and maintain the cartilaginous matrix, which consists mainly of collagens and proteoglycans. Normally, NGF is expressed at low levels by articular cartilage, but previous studies have shown that both NGF and its receptors (TrkA and P75NTR) are increased in OA articular cartilage.[32]

  Increased expression and secretion of NGF by synovial chondrocytes can be regulated by several factors in the osteoarthritis (OA) environment, including biomechanical stress and the upregulation of pro-inflammatory mediators such as TNF-α and IL-1β.[33]

  Recent studies indicate that these changes can cause the normally quiescent articular chondrocytes to undergo a phenotypic shift, leading to the disruption of homeostasis and, ultimately, to the aberrant expression of further pro-inflammatory and catabolic genes including aggrecanases and collagenases, especially MMP-13.

Many *in vitro* models are currently in use by researchers to study OA, but there is no consensus on the most appropriate one. Models attempt to mimic factors and conditions that initiate OA, and elucidate pathways active in the disease. However, the aetiology of OA is very complex, with many factors contributing to the disease, and the response of isolated cells and tissues are likely very different from the naturally occurring disease. The development of combinatorial models encompassing different physiological and molecular aspects of the disease will, more accurately, reflect the pathogenesis of the naturally occurring disease.[34] As new therapies are developed which target the low-grade, chronic inflammation seen in OA, a critical review of the models or techniques being used to measure low-grade, chronic inflammation may be needed.

Subchondral bone

Changes in the subchondral bone are extensive in OA, and a meta-analysis conducted by Barr et al, points to the existence of a robust crosstalk between subchondral bone and articular cartilage in the pathophysiology of joint diseases.[35,36] Thickening of the subchondral bone and osteophyte formation in OA is familiar to clinicians. Changes in the mechanical properties of the subchondral bone, and the production of inflammatory mediators by subchondral bone, can both contribute to degradation of articular cartilage.[23] Largely because of NGF, nerves in the subchondral bone exhibit neurite sprouting and NGF, itself and via neurogenic inflammation (and release of CGRP), induces angiogenesis. Both neurite sprouting and angiogenesis contribute to the pain and inflammation associated with the disease (see Figure 2).

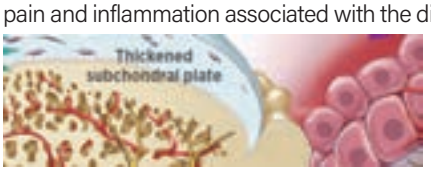

Figure 2.

- **Angiogenesis**

  Both neurogenic inflammation and NGF itself contribute to new blood vessel development in osteoarthritis. Fusco et al, wrote that experimental models 'show increased vessel density in calcified cartilage, which is especially pronounced in older animals (+100%) compared to young adults (+50%).' Angiogenesis occurs in parallel with thickening of subchondral bone.[28] In OA, blood vessel growth has also been shown to be increased in articular cartilage, synovium and at the osteochondral junction, and angiogenesis contributes to structural progression, tissue differentiation and pain

associated with disease.[37] The increased angiogenesis found in the stifle joint is associated with chronic inflammation, which is characterized by the release of proangiogenic factors such as VEGF, angiogenic neuropeptides and NGF.[37-39] As angiogenesis and sensory nerve growth are closely linked, processes that contribute to pain, the increased neovascularisation likely accompanies sensory innervation into structures that are not normally innervated. Furthermore, increased binding of NGF to TrkA receptors on sensory nerve terminals may further stimulate angiogenesis, which has been demonstrated in a variety of animal models.[40,41]

- **Neuron sprouting**
  NGF promotes neuronal sprouting which may increase overall sensitivity of the joint.
  NGF can initiate inappropriate nerve sprouting in sensory and sympathetic nerve fibres that innervate the knee joint, contributing to increased sensitivity of individual nerves, and overall sensitivity of the joint.[42] Several animal model studies have demonstrated that administration of NGF stimulates axonal sprouting leading to painful neuronal-like structures within the dorsal root ganglion (DRG) and dorsal horn, suggesting that NGF mediated nerve remodeling may contribute to chronic pain sensitivity in OA.[30]
  Angiogenesis and sensory nerve growth are closely linked processes, both occurring in the tissues of OA, and both contributing to pain and inflammation. There is strong crosstalk between nerves and blood vessels, and increased binding of NGF to TrkA receptors on sensory nerve terminals may further stimulate angiogenesis30 (see Figure 3).

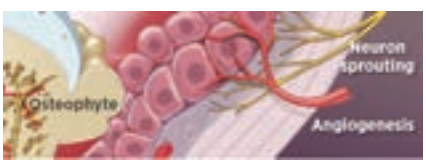

Figure 3.

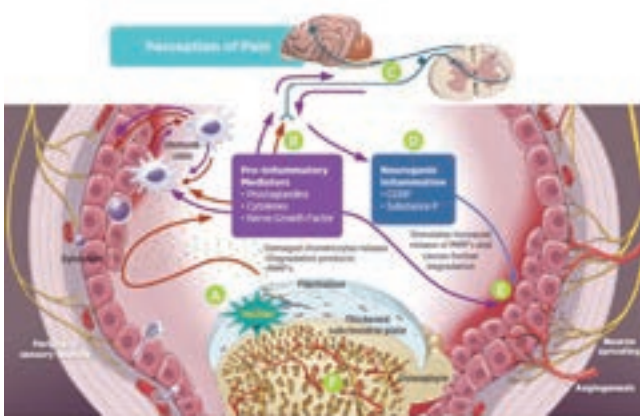

Figure 4: Cycle of osteoarthritis – pain and inflammation.

A. Inciter/s – factors such as conformational abnormalities, trauma or metabolic changes initiate damage to cartilage. This damage results in the release of inflammatory mediators from chondrocytes, degradation products and MMP.

B. Pro-inflammatory mediators – the products released from damaged cartilage, in turn, induce synovitis which results in the release of pro-inflammatory mediators including cytokines, chemokines, PGE2 and NGF. NGF causes further inflammation of the synovium and activation of inflammatory cells. It also sensitises nerve endings.

C. Upregulation of the sensory nerve – transport of the NGF/TrkA receptor complex to the cell nucleus causes changes in transcription within the sensory nerve, which results in enhanced pain signaling at both ends (peripheral and central) of the sensory nerve.

D. Neurogenic inflammation – NGF-induced changes in the function of the peripheral nerve result in the release of pro-inflammatory mediators such as CGRP and Substance P locally from the ends of the nerve when they are activated. Release of these substances occurs at the ends of the nerve in the joint, and these substances cause inflammation – this process is called neurogenic inflammation.

E. Angiogenesis and neuronal sprouting – both neurogenic inflammation and NGF itself contribute to new blood-vessel development. NGF promotes neuronal sprouting which may increase overall sensitivity of the joint. Together, these processes promote deleterious remodeling of the joint and increased joint sensitivity.

F. Cycle of degradation – these ongoing processes lead to gradual deterioration in all components of the joint, including cartilage degradation, subchondral bone deterioration and osteophytosis.

New science of OA – summary

- OA is a highly prevalent disease in dogs and the chronic pain associated with OA negatively impacts many areas of a dog's health. Early detection and management are key.
- Inflammation in OA is low-grade and chronic, perpetuated by the loss of homeostasis leading to progressive joint destruction.
- Initially considered cartilage driven, OA is now known to be a much more complex disease with inflammatory mediators released by cartilage, bone, neurons and synovium.
- Significant crosstalk occurs between all these component tissues of the joint.
- NGF is elevated in osteoarthritic joints, and its receptors are found on immune cells. NGF is involved in neurogenic inflammation.
- Although much knowledge about inflammatory mediators in osteoarthritis has been gained in the last decade, further studies are needed to better define the mechanisms by which these factors upset the normal homeostatic processes in the joint and result in processes favoring joint degradation.

PAIN

As we look clinically at OA, pain remains the underlying factor that pet owners are looking to manage to improve their dog's quality of life. Nociception is part of an early-warning mechanism for the protection of the animal particularly in the acute scenario.

Nociceptors for mechanical, thermal and chemical stimuli in the periphery transduce these stimuli, initiating transmission of the signals to the brain, and resulting in a withdrawal reflex from the noxious stimuli (see Figure 5). However, in the context of chronic diseases such as OA, pain does not serve a protective function, and indeed, the changes induced in the pain transmission system are considered maladaptive, and non-useful.

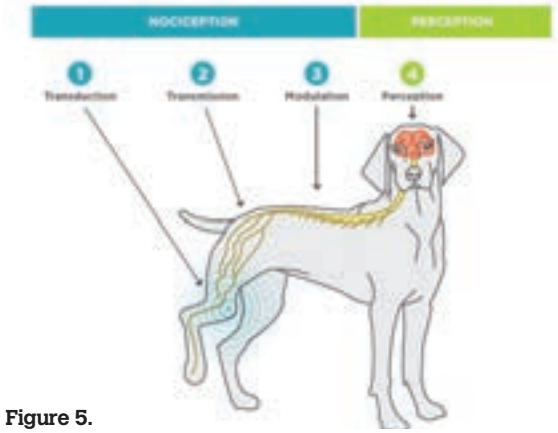

Figure 5.

In addition, immune cells play a key role in pain signaling (see Figure 6) in chronic OA. The balance of activity of the different signaling pathways and immune cell dysregulation result in the individual experience of pain. With so many signaling pathways and cells involved in OA pain, pain alleviation is only effective if the target is a key player amongst the multitude of processes. NGF has been found to be a key player in pain associated with OA and is a target for both human and veterinary medicine.

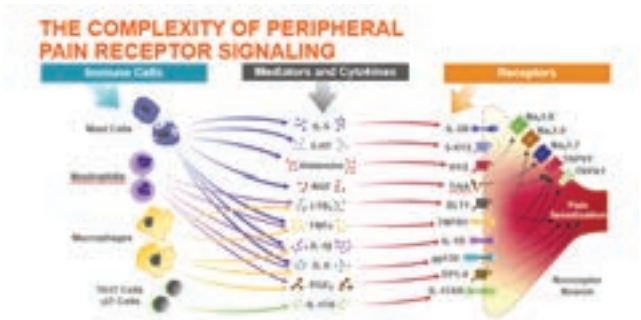

Figure 6: Image adapted from Trends in Immunology, January 2017, Vol. 38, No. 1 http://dx.doi.org/10.1016/j.it.2016.10.001

NGF plays pivotal role in production of pain

OA pain is a complex process, mediated by many factors, including prostaglandins as well as NGF, a signaling protein that is produced by injured tissues. During development, NGF aids the normal development of the sensory and sympathetic nervous system and it plays an important role in ensuring that the nervous system develops normally. But in the adult, the primary role of nerve growth factor is pro-nociceptive, that is the production of signals which will be interpreted as painful.

How does NGF work compared to other factors, such as prostaglandin E2 (PGE2)? PGE2 doesn't generate action potentials (nociceptive signals), but it sensitises nerves to other molecules and that's one of the reasons inhibiting prostaglandin E2 results in effective pain relief. If you dampen down the sensitisation of nerves, you're going to dampen down the ability of these other molecules to activate those nerves.

NGF does a very similar thing. It sensitises nerves, but in addition, it also alters the phenotype of nerves. It alters the expression of pain receptors and the number of neurotransmitters these nerves are producing. So, you can think of NGF as very similar in its actions to prostaglandin E2 with the addition that it also changes the nerves in a way that makes them more responsive and that makes them respond more strongly to pain signals. New scientific innovations allow for the creation of monoclonal antibody therapy (mAbs) designed specifically for feline and canine use. Clinical researchers have found that it is now possible to lower NGF's negative influence on pain with anti-NGF antibody therapies.[43,44] These species-specific therapies are long-acting (about a month) and are delivered via subcutaneous injection. Antibodies are metabolised differently than small molecules – they are metabolised to peptides and amino acids within cells. As such, they are expected to have a different safety profile than traditional drug therapies.[45]

Anti-NGF antibody therapy may provide vets with a powerful new alternative for the treatment of OA pain:

- Anti-NGF mAbs effectively reduces pain signals by preventing NGF from binding to, and activating TrkA receptors;
- Lowers the amount of NGF within the joint available to bind to immune cells;
- Non-narcotic, non-sedating; and
- Sustained pain reduction delivered for about a month in both canine and feline proof of concept studies.[43,44]

A new class of pain therapy

The potential for anti-NGF antibody therapy to control OA pain is an exciting new development and represents the first innovation identified to block pain outside the prostaglandin pain pathway in over 20 years. Anti-NGF antibody therapy may come to represent a new class of veterinary medication and may be an effective new way for veterinarians to provide safe, long-lasting control of chronic pain to both cats and dogs.

Overall summary

- OA continues to be a significant disease for humans and dogs alike.
- There has been a lack of new products to help manage the pain associated with OA for over 20 years in both the human and veterinary professions.
- OA in dogs is often under-diagnosed. Early identification and treatment of OA pain can help improve a dog's life quality.
- Inflammation in OA is low-grade and chronic, perpetuated by the loss of homeostasis leading to progressive joint destruction.
- Initially considered cartilage driven, OA is now known to be a much more complex disease with inflammatory mediators released by cartilage, bone, neurons and synovium.
- NGF is elevated in osteoarthritic joints, and its receptors are found on immune cells. NGF is also involved in neurogenic inflammation.
- NGF has been found to be a key player in pain associated with OA and is a potential new target for both human and veterinary medicine.
- NGF sensitises nerves but also changes the nerves in a way that makes them more responsive and that makes them respond more strongly to pain signals.
- Anti-NGF antibody therapies are in development. They may be an effective new alternative for veterinarians to provide safe, long-lasting control of chronic pain to both dogs and cats.

This article has been adapted from a technical bulletin published by Zoetis called The New Science of Osteoarthritis (OA) Pain and Inflammation.