Review Article

Current understanding of cellular and molecular events in intervertebral disc degeneration: implications for therapy

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Abstract

Until recently, material removed from the intervertebral disc (IVD) at surgery consisted either of ‘loose bodies’ from the centre of the IVD or discal tissue displaced (prolapsed) into the intervertebral root or spinal canals. This material is best regarded as a by-product of disc degeneration and therefore not representative of the disease process itself. Recent advances in surgical techniques, particularly anterior fusion, in which large segments of the anterior part of the IVD are excised with the anatomical relationships between different components intact, have generated material that can be investigated with modern molecular and cell biological techniques. This is an important area of study because degeneration of the lumbar IVDs is associated, perhaps causally, with low back pain, one of the most common and debilitating conditions in the West. ‘Degeneration’ carries implications of inevitable progression of wear-and-tear associated conditions. Modern research on human IVD tissue has shown that this is far from the case and that disruption of the micro-anatomy described as degeneration is an active process, regulated by locally produced molecules. The exciting consequence of this observation is the possibility of being able to inhibit or even reverse the processes of degeneration using targeted therapy. Copyright © 2002 John Wiley & Sons, Ltd.

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Chronic low back pain and the IVD

Approximately 70% of the population will experience low back pain (LBP) during their lives [1] and it accounts for about 15% of all sickness leave in the UK [2]. Although LBP constitutes an important public health issue, little is known of its pathogenesis. The organs that together make up the spine and its accessory structures are so numerous and complex that the causes of LBP are undoubtedly multifactorial. However, clinical, interventional, and imaging studies on volunteers and patients [3–5] and therapeutic trials [6] have produced evidence implicating mechanical or traumatic disorders of the intervertebral disc (IVD) in up to 40% of cases. In almost every case when IVD tissue has been examined from patients with LBP, it has shown the features typical of discal degeneration (see below).

Structure and function of the IVD

The three components of the IVD are:

(i) The end-plate – This structure consists of a layer of cartilage, resembling articular cartilage that covers the central parts of the inferior and superior cortical bone surfaces of the vertebral bodies.

Ex vivo vertical loading experiments of isolated spinal segments show that this is the weakest part of the spinal column [7].

(ii) Nucleus pulposus (NP) – The space between the end-plates of adjacent vertebrae is filled by the nucleus pulposus, consisting of chondrocytes within a matrix of type II collagen and proteoglycan, mainly aggrecan [8]. Surprisingly little is known of the structure of the NP or the biology of its cells. The type II collagen fibres are not believed to give the same level of order to the structure or the same degree of mechanical stability to the matrix as in articular cartilage. The proteoglycans are hydrophilic, causing the NP to swell. The swelling pressure of the NP proteoglycans is constrained by the end-plates above and below and the annulus fibrosus (see below) around the periphery. On H&E sections, the NP appears homogeneous and pale lilac-blue, consistent with its complement of proteoglycans. In polarized light, it exhibits little birefringence.

(iii) Annulus fibrosus (AF) – This comprises dense sheets of highly orientated collagen fibres (mainly type I but also types II and III) in which are cells with the morphology and phenotype of fibroblasts. Our experience is that when cultured in alginate they can be transformed into chondrocytes. Functionally, the annulus fibrosus is a very...
strong ligament binding together the outer rims of adjacent vertebrae. On H&E sections, it exhibits fairly uniform eosinophilia and in polarized light, its constituent alternating bands of highly orien-
tated collagen fibres are clearly seen.

The IVD is a joint and, as such, allows movement between bones, in this case adjacent vertebral bodies. In its normal fully hydrated state, each IVD permits small degrees of flexion, extension, lateral bending, and twisting, but is resistant to compressive loads. When summated, the small movements at each IVD permit great mobility of the entire spine.

‘Degeneration’ of the IVD

It is believed that many of the disorders of the IVD are mechanical in origin. The erect spine is subjected to a range, rate, and degree of loading that arguably it is poorly designed to sustain. As a consequence, it suffers mechanical damage, which, in a high proportion of individuals, results in a pattern of morphological and histological changes, well characterized from autopsy studies, which together constitute discal degeneration. With the exception of the outer third of the AF, the normal adult IVD is avascular and aneural; there is a relatively clear demarcation between the AF and the NP; and the end-plate forms an intact layer of cartilage overlying cortical bone. In degeneration, the NP is disrupted, with changes in the proportion and types of proteoglycans and collagens (making the NP more eosinophilic), a reduction in the total number of lacunae containing viable chondrocytes, but the for-
mation of chondrocyte clusters in many lacunae, just as in osteoarthritis of articular cartilage, which may even increase the total number of cells present in the diseased tissue. In addition, there is breakdown of the matrix with the formation of permeative ‘slit-like’ spaces. There is often also disruption of the collagen fibre arrays in the AF, traumatic damage to the end plate, and vessel and nerve ingrowth into the inner AF and NP. Current understanding of the biology of connective tissues would causally implicate alterations in the function of local cells in these events.

Because man is the only obligate bipedal vertebrate, replicating human disorders of the IVD in other animals has proved problematic. As a consequence, there are no satisfactory animal models of human IVD disease, which has inhibited the study of this group of disorders. An alternative strategy is to study human tissue removed from cadavers or from patients at surgery. This has been facilitated by the development of modern tissue-probing techniques [9–12] that can be performed on histological sections of human tissue, such as immunohistochemistry, in situ RT-PCR, and lectin histochemistry, as well as by new surgical approaches to the management of IVD disease. The recent advent of an anterior approach to the diseased IVD to facilitate disc replacement or spinal fusion has generated large wedges of discal tissue for study, often, in the case of spinal fusion, from multiple levels. These are a valuable source of material because they consist of primary diseased tissue in which the components of the IVD are in the same anatomical relationship to one another as in vivo. There has also been a trend towards pre-operative discography, in which IVDs are injected with a radio-opaque medium. This is performed under ‘aware state anaesthesia’ so that the patient can report if discography recapitulates symptoms. Either ‘pain level’ or ‘non-pain level’ (or both) IVDs may be removed depending on the clinical indications, particularly in multi-level fusion surgery, and this gives the pathologist the opportunity to relate findings to symptomatology.

In scientific terms, studies of random samples of human tissue cannot be as satisfactory as controlled experiments. Nevertheless, they have generated useful data from which has come the realization that the origins of IVD degeneration are neither as passive nor as inevitable as the term implies. This has, in turn, opened the way for a re-evaluation of the management of IVD disease and LBP.

Recent advances in the understanding of disc degeneration

The problems of controls

Two major issues have dictated the pace and direction of research into the processes of IVD degeneration: the adequacy or otherwise of control material and the difficulty in defining a recognized starting point from which to assess degenerative change.

Tissue acquisition

Until recently, difficulty in accessing relatively intact normal human IVD, other than from cadavers, has meant that a greater emphasis has been placed on those aspects of the IVD that do not change significantly in the first few hours after death, such as matrix chemistry and disc biomechanics. As spinal surgery, particularly spinal reconstruction for metastatic malignancy, has advanced, this situation has changed and with it, opportunities have developed to obtain an improved understanding of the biology of the IVD cells in discal degeneration.

Ageing and load

The nature of collagenous tissues means that their structure changes with time and with the organism’s age. Continuing post-synthetic changes in the structure of many matrix molecules and alteration in the nutritional status of these poorly vascularized tissues with age lead to modifications in the collagen and proteoglycan composition of the IVD [13]. As the incidence of discal degeneration also increases with age, distinguishing ‘normal ageing’ from ‘disease’ [14] becomes paramount. This is complicated by the high
frequency of disc degeneration at some spinal levels (e.g. L3–4, L4–5, and L5–S1), making the definition of 'normality' problematic.

In almost every connective tissue there are cells that respond to differential loading by changing their biology [15] and the IVD is no different [16]. Lifestyle, body weight, and age are major factors that influence the load environment of the normal IVD. In patients with LBP, pain and stiffness also play a part, through the natural protective reaction of abnormal prolonged contraction of specific muscle groups. Such factors further complicate the starting point from which to assess IVD degeneration.

**Altered matrix composition and integrity**

When compared with age- and sex-matched controls, there are profound alterations in the cell biology of chondrocytes in degenerate IVD. Normal discal chondrocytes are characterized by expression of type II collagen and proteoglycans [17] and we have derived evidence that this is regulated by the 'master chondro-regulatory gene' SOX-9 (unpublished observations). In discal degeneration, chondrocyte synthesis of matrix molecules changes differentially with the degree of degeneration [18], leading to an increase in the synthesis of collagens I and III and decreased production of aggrecan. Exactly what effect these changes have on IVD function is unknown. Furthermore, the regulation of matrix turnover is deranged, affecting both synthesis and degradation [19]. In degeneration, there is a net increase in matrix-degrading enzyme activity over natural inhibitors of such activity, which leads to loss of discal matrix. Particular attention has been paid to the role of matrix metalloproteinases (MMPs) in these processes [20–23], making them a potential target for therapy designed to inhibit discal degeneration. More recently, following work on the mechanism of aggrecan degradation in articular cartilage, interest has grown in the possible role of aggrecanases in IVD degeneration [19]. Aggrecan has two cleavage sites, one acted upon by MMPs and the other by members of a group of enzymes called the ADAMs family, after their hybrid function ‘A Disintegrin And Metalloproteinase’. In fact, the aggrecanases are two enzymes, ADAMTS4 and 5, that in addition to their disintegrin and metalloproteinase function also have ‘ThromboSpondin’ motifs. From the pathological perspective, these studies have largely been undertaken, not by looking for the enzymes themselves, but through the application of antibodies targeted at the breakdown product of aggrecan formed by enzyme action at the specific site [19].

**Reduced cell number**

The reduction in chondrocytes that typifies IVD degeneration has been ascribed to apoptosis [24]. Indeed, there is some evidence of a ‘dose-dependent’ relationship between apoptosis and excessive load (lifestyle, body weight, age) [25]. As in other chondroid tissues, nitric oxide has been implicated in the induction of apoptosis [26].

**Nerve and blood vessel ingrowth**

Although the normal adult IVD is avascular and aneural, nerves [27] and blood vessels [28,29] grow into diseased IVD. One avenue of investigation has been the local production of angiogenic and neurogenic molecules within degenerate IVD. Expression of the potent angiogenic factor vascular endothelial growth factor (VEGF) [30] has been demonstrated within the IVD. We have just completed an investigation of the active (β) chain of nerve growth factor (NGF) expression by cells in the IVD. NGF is synthesized by blood vessels in discs showing nerve ingrowth. Furthermore, the small nerves adjacent to the vessels express the high-affinity receptor for NGF, Trk-A. The implication of these data is that while either angiogenesis or neuronogenesis could be targets for therapy, angiogenesis drives nerve ingrowth and may be the more important from a therapeutic perspective.

**Cytokines as regulators of disease processes**

Recently there has been growing interest in the possible role of cytokines in regulating the connective tissue degradation, nerve and vessel ingrowth, and macrophage accumulation that characterize IVD degeneration. A number of cytokines have been implicated, including TNF, IL-1, PDGF, VEGF, IL-6, IGF-1, TGF-β, EGF, FGF, and IL-10, amongst others [23,30,31]. Of these, IL-1 is particularly interesting, because in other settings there is evidence that it is involved in cartilage homeostasis [32], largely through its ability to switch chondrocytes from anabolism to catabolism, inducing cartilage breakdown at molecular and morphological levels [33,34]. It has also been shown to be a regulator of angiogenesis in joints and non-articular cartilage, possibly by acting as a promoter of the potent angiogenic factor VEGF [35,36]. In the IVD, both its isoforms, IL-1α and IL-1β have been shown [37–39] to increase proteoglycan release and degradative enzyme production. IL-1β also increased production of MMPs and pain mediators, such as the eicosanoid prostaglandin E2, by human IVD cells [40]. IL-1 production by human IVD cells has also been reported [41]. Although not by any means conclusive, these studies implicate IL-1 both in matrix degradation and in pain. In addition, there is evidence that IL-1 could mediate some of the other characteristics of IVD disease, such as nerve and vessel ingrowth [42].

If IL-1 is the regulator of cartilage catabolism, the transforming growth factor beta (TGF-β) superfamily is the regulator of cartilage anabolism. Although there have been few experiments investigating the presence or effects of TGF-β on discal cartilage metabolism,
Nishida et al. [43] induced a two-fold increase in proteoglycan synthesis by rabbit nucleus pulposus cells following injection of rabbit discs with an adenoviral vector carrying a human TGF-β transgene. This will undoubtedly turn out to be a key experiment in this area.

There is a considerable amount still to be discovered about the role of cytokines in discal degeneration. However, these pioneering experiments hint at possibilities that have not previously been considered, both for improving understanding of the processes leading to IVD degeneration and for its management. The exciting aspect of this work is that the interest in the cytokine biology of the IVD comes at a time when advancing technology makes it possible to advance the study more rapidly and comprehensively than has hitherto been possible. For instance, we have undertaken cytokine microarray analysis of cDNA derived from RNA extracted from diseased human IVD. The results, showing expression of at least ten different cytokines working through two separate intracellular signalling pathways, are an indication of the extent of cytokine activity in the diseased IVD. The question still remains as to what initiates cytokine-mediated events and how this might be linked to the association between load and disc degeneration.

Clinical implications of these studies

Currently, the mainstays of treatment for damaged IVD are either symptomatic medical therapy or surgery, both of which have limited success. The novel data that are starting to accumulate hold out the possibility of managing IVD degeneration by normalizing the cell biology of the NP and the AF. A working hypothesis is that discal degeneration is an active process, induced by abnormal load and mediated by cytokines.

We and others are currently examining the possibility of using genes introduced into target cells to produce proteins within the degenerate disc that will create a chemical environment that is conducive to restoring cell function towards normality. This is helped by the relatively immune privileged and hypovascular nature of the IVD, which allows the use

Figure 1. The pattern of cellular events that lead from 'normality' to 'degeneration' as currently understood. Abnormal load (usually repetitive) sometimes associated with an abnormal chemical environment leads to cytokine synthesis by the chondrocytes within the nucleus pulposus. Either through direct cytokine-mediated events (e.g. angiogenesis and nerve ingrowth) or through secondary phenomena, the changes that histopathologists recognize as degeneration [e.g. cell proliferation (and apoptosis), matrix degradation, and changes in the balance between collagen and proteoglycan synthesis] occur.
of viral vectors for gene delivery whilst minimizing the risk of an immune response against infected cells and of dispersal of the gene product widely throughout the body [43,44]. The problem still remains of establishing a normal load environment within the damaged IVD in which genetically engineered cells might thrive, but combinations of physiotherapy and/or modern implant technology, with the potential to fashion support devices with biodegradable smart matrices that act as scaffold, load normalizer and cell stimulator, make this an opportune time to be a connective tissue pathologist with a personal interest in the management of back pain.

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