INTRODUCTION

Dense avascular connective tissues such as the intervertebral disc (IVD) have a poor healing capability, a greater understanding of this repair response would clearly be of great clinical value. The aim of this study was to evaluate the expression of osteonectin (SPARC, BM-40), TGF-beta and FGF-2, and assess how these effector molecules might play a role in annular repair processes in an ovine annular lesion model of experimental disc degeneration. SPARC is a high affinity Ca²⁺-binding protein that induces a variety of biological effects on connective tissue cells including modulation of spreading, proliferation, migration and matrix protein synthesis. SPARC expression is significantly elevated in synovial fibroblasts, chondrocytes and synovial lining cells of osteoarthritic (OA) and rheumatoid arthritis (RA) joints suggesting a role in the progression of these conditions. TGF-beta and FGF-2 have well defined roles in tissue repair and matrix remodelling and should be of relevance to annular repair processes.

METHODS

All procedures received institutional approval from The Animal Research Ethics Committee of The University of Sydney. Pure-bred merino wethers (n=32) aged 4 years received a controlled 4mm deep anterolateral surgical incision (extending over 10mm) parallel and adjacent to the inferior cartilaginous end plate of the superior vertebral body of their L1L2 and L3L4 IVDs (Fig 1A, B). Sham operated sheep (n=32) were also subjected to the same retro-peritoneal surgical approach to the annulus. Eight sheep from each animal group were sacrificed at 3, 6, 12 and 26 mth. post-operation (PO), lumbar spinal segments were removed within 30 min of death and individual IVDs isolated by cutting through adjacent vertebral bodies using a bone saw. IVD specimens were fixed in 10% neutral buffered formalin and decalcified in 10% formic acid in 5% neutral buffered formalin. The IVD specimens were embedded in paraffin and 4 micrometre vertical sagittal sections were cut. These were stained with toluidine blue/fast green, haematoxylin and eosin (H & E), Masson-trichrome and picrosirius-red (See Fig 1). TGF-beta, FGF-2, SPARC and type IV collagen were immunolocalised using specific antibodies. The slides were examined by plane polarised and normal light microscopy using a Leica MPS 60 photomicroscope system.

RESULTS

By 3 mth PO, penetration of granulation tissue and blood vessels was evident along the plane of the original surgical defect as were a large influx of fibroblastic cells along the margins of the defect. Cells of a more rounded chondrocytic morphology and a few larger macrophage type cells were also present adjacent to blood vessels. Type IV collagen localisation clearly delineated blood vessels in the vicinity of the defect. By 3mth PO, the outermost margins of the AF had undergone re-organisation consistent with an active repair process. Synthesis of collagen was evident, however it lacked a mature level of organisation when observed by polarised light microscopy using picro-sirius red staining (Fig 1E, F). By 6 mth PO, repair of the outer defect and infiltration of blood vessels and cells into the inner defect was even more advanced. By 12 mth PO, blood vessels had commenced resorption in the inner AF and cell numbers had also decreased. Macrophages were also more prominent and were presumably involved in this resorption process. Even after 12 mth PO the inner AF defect had still not undergone repair. By 26mth PO the defect extended through the NP, or it had propagated perpendicularly, leading to radial tears involving separation of adjacent annular lamellae (Fig 1C, D). Both types of defects have previously been described in degenerate human IVDs. FGF-2, TGF-beta and SPARC reactivity were strongly associated with infiltrating blood vessels, cells which penetrated the defect site and with areas of tissue remodelling.

DISCUSSION

A notable feature in this model was that blood vessels penetrated only as far as the inner AF. This also demarcated the extent of cellular infiltration into the defect and the region of the AF capable of undergoing a reparative response. IVD cells have formerly been shown to be responsive to a range of growth factors in-vitro including TGF-beta and FGF-2 which have been suggested as potential agents for therapeutic intervention. The association of these growth factors with areas of the outer AF which underwent repair in our experimental model is totally consistent with such a proposal. The association of SPARC with annular defects undergoing matrix re-modelling/repair however is a novel finding. SPARC expression has formerly been shown to be up-regulated in synovial lining cells and chondrocytes in RA and OA. Moreover, since SPARC can stimulate synovial fibroblasts and articular chondrocytes to produce matrix metalloproteases it may have an important role to play in the tissue remodelling events which are evident in our experimental model and with repair of annular lesions in vivo.

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