INCREASED INNERVATION OF THE INTERVERTEBRAL DISC IS ASSOCIATED WITH GLIAL CELL GROWTH.

+*Johnson, W (A-Action Research Charity); **El Haj, A; *Evans, H; *Eisenstein, S; *Roberts, S  
+*Centre for Spinal Studies, Oswestry. Centre for Spinal Studies, Robert Jones and Agnes Hunt Orthopaedic Hospital, Oswestry, Shrophire SY10 7AG, U.K., (44)  
1691 404660, Fax: (44) 1691 404054, w.b.johnson@keele.ac.uk

Introduction: In health, the intervertebral disc is generally avascular and sparsely innervated. Those few nerves and blood vessels present are generally restricted to the outer areas of the annulus fibrosus where they lie predominantly between concentric layers of matrix (called lamellae). During disc degeneration, however, many more nerves are seen within the annulus, often in association with an increased vasculature. In addition, these nerves penetrate further into the disc, towards the inner annulus and nucleus pulposus (1-3). Such new nerve growth into degenerate discs, particularly nerves immunopositive for GAP-43 entering into deeper regions of the disc, has been associated with discogenic low back pain (3). We are currently investigating the mechanisms that regulate the capacity of nerves to penetrate the intervertebral disc. One aspect of this study has been to determine the presence of nerve support cells within discs and here we show that cells that are immunopositive for the glial cell marker, GFAP, are commonly seen in association with nerves.

Methods: Human disc samples (anterior or antero-lateral) were obtained within 60 minutes of excision following discectomy for discogenic back pain. Each sample was macroscopically graded for degeneration using criteria based on those of Thompson and co-workers (4) and snap-frozen. Serial sections (30µm) were immunostained for the general nerve marker PGP 9.5 (rabbit polyclonal 1/1000, Ultraclone, U.K.), for neurofilament 200kD (NF200, clone RT97, 1/100, Novacastra, U.K.) and for glial fibrillary acidic protein (GFAP, clone GA5, 1/100, Novacastra). For single immunostaining protocols, visualisation was achieved using a commercial system (Vectastain ABC-AP kit, Vector Laboratories, Peterborough, U.K.) and DAB. Sections were also dual stained for PGP 9.5 and GFAP. PGP 9.5 immunopositivity was revealed using a biotin-labelled secondary step then FITC-labelled strepatavidin (Vectastain ABC-AP kit) and GFAP staining was revealed using a Texas Red labelled secondary (Vectastain ABC-AP kit). Irrelevant isotype-matched antibodies or antisera were used to stain parallel sections as controls. These control sections were all negative. Double staining was analysed using confocal microscopy. General histology of discs was noted following H/E staining of serial sections.

Results: PGP 9.5 and/or NF200 immunopositive processes were present in 8 out of 10 discs examined and were more prevalent in degenerate discs than non-degenerate. These neuronal processes commonly ran between the lamellae of the outer annulus. However, nerves also crossed lamellae and penetrated into the deeper areas of the more degenerate discs. Much, but not all, of the innervation was in the proximity of blood vessels. Immunostaining for GFAP demonstrated that glial cells were present within the disc also. The distribution of GFAP was akin to that of the neuronal markers. Thus, GFAP immunopositive processes were absent in those discs that were anueral, but were observed with a similar frequency as neuronal markers in all innervated tissue (Table 1).

Table 1. The incidence of neuronal and glial cell markers in human intervertebral disc samples.

<table>
<thead>
<tr>
<th>Spine Level</th>
<th>Grade</th>
<th>NF 200kD</th>
<th>PGP 9.5</th>
<th>GFAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>L3-L4</td>
<td>II</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>L4-L5</td>
<td>II</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>L5-S1</td>
<td>II</td>
<td>+</td>
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<td>+</td>
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<td>L5-S1</td>
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</tr>
<tr>
<td>L5-S1</td>
<td>IV</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

Furthermore, GFAP and PGP 9.5 or NF200 immunopositivity was seen in similar areas of serial disc sections. Like nerves within the disc, GFAP positive processes were often in association with blood vessels (Figure 1). Double staining for PGP 9.5 and GFAP confirmed that much of the immunopositivity for these markers co-localised, i.e. that nerve and glial cell processes were in very close proximity to each other within the disc. However, this double staining also revealed that glial cell processes could be seen independently penetrating the disc matrix (Figure 2). In addition, PGP 9.5 immunopositive neuronal processes were observed within the disc in the absence of GFAP.

Discussion: This study provides further evidence that nerve growth into the intervertebral disc is associated with disc degeneration. The precise mechanisms that regulate nerve growth within discs remain to be elucidated. However, the finding of GFAP immunopositive processes is of interest. GFAP is an intermediary filament and a marker of cells of the glial cell lineage (5). In peripheral tissues, for example, GFAP immunopositivity indicates the presence of non-myelinating Schwann cells (6). We have shown for the first time, therefore, that Schwann or glial cell growth into the disc is also a feature of its degeneration. Furthermore, the observed pattern of immunostaining for both neuronal and glial cell markers suggests that the ingrowth of these cell types is associated with each other and possibly with neovascularisation.

Studies in other tissues, including keratodermal grafts (7), denervated muscle (8) and grafted tendon for regenerate sciatic nerves (9), have shown that Schwann cells may act as pioneer cells growing into aneural tissue, both leading and directing nerve processes. Indeed, there is increasing realisation that these cells play a critical regulatory role in nerve process outgrowth, guidance and survival (10). We conclude, therefore, that innervation of degenerate intervertebral discs is likely to be affected by the presence of glial cells. Indeed, glial cells may act as pioneers of innervation within this tissue and further studies will determine how glial cell growth, disc innervation and disc vascularisation are inter-related.

References:  

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**C.S.T.M., Keele University, Keele. U.K..