OSTEOCHONDRAL PROGENITOR CELLS IN ACUTE AND CHRONIC CANINE NONUNIONS

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Introduction: During normal fracture healing, mesenchymal progenitor cells are recruited into the defect which differentiate and form bone as well as other mesenchymal tissues (1). The healing process involves site-specific factors which orchestrate a cascade of events including the proliferation of mesenchymal progenitor cells, subsequent differentiation, and the formation of calcified cartilage that is replaced by cartilage and bone (2). The question remains as to whether multipotent mesenchymal cells are present in nonunion tissue, and if so, whether they respond to factors involved in bone healing. The aim of the present study was to examine the ability of cells isolated from early healing segmental defects and from chronic nonunion tissue, to support bone and cartilage formation in vivo and their response to transforming growth factor beta-1 (TGF-β) in vitro.

Materials and Methods: Osteotomies (3mm) were created in the radial diaphysis of four dogs. Dogs were splinted 3-5 days post-operatively and then allowed to bear full weight. At 7 days, tissue in the defect was removed and cells released by enzymatic digestion. Dogs were reimplanted and allowed to bear full weight for 12 weeks. Radiographs confirmed a persistent nonunion in all dogs. Defect tissue was again removed and cells were isolated. Cells were also obtained by nonenzymatic means using explant cultures. One half of the tissue, one half of any preconfluent first passage cultures, and frozen were harvested six weeks after implantation into nude mice. At second passage, these cells were loaded onto ceramic cubes and implanted into nude mice for 3 or 6 weeks. Harvested cubes were examined histologically for cartilage and bone and scored using a semi-quantitative system. A score of “0” indicated that no bone or cartilage was present in the section. When bone or cartilage was present in up to 25% of the total number of pores, the section was awarded a score of “1”, while a section with greater than 75% of bone-positive pores was assigned a score of “4”. Confluent fourth passage cultures of 7-day and 84-day defect tissue cells were cultured with 0.03 to 0.88 ng/ml TGF-β for 24 hours and [3H]-thymidine incorporation and alkaline phosphatase activity were determined. Data were analyzed by ANOVA to determine if statistical differences existed. Post hoc testing was performed using Bonferroni’s modification of the Student’s t-test with P-values < 0.05 considered significant.

Results: Donor-dependent differences were noted in the rate at which defect cells achieved confluence; in general, cells from 7-day tissue divided most rapidly. 7-day defect cells formed less bone and at a slower rate than was seen in the ceramic cubes containing 84-day samples. Cells derived enzymatically (Table 1) behaved similarly to cells from explant cultures (data not shown). Ceramic cubes contained fibrous connective tissue, cartilage, bone, and fat, indicating that pluripotent cells were present (Figure 1). Stimulation of [3H]-thymidine incorporation in response to TGF-β was donor-dependent and variable. Only two of the six batches of cells examined for alkaline phosphatase had measurable activity, and this was relatively low; none of the cultures exhibited an increase in response to TGF-β at 24 hours. Discussion: The present study indicates that multipotent mesenchymal cells are present in the healing defect tissue at 7 and 84 days and that the number of osteochondroprogenitor cells is greater at the later time. Moreover, although these cells are part of an established nonunion they have the ability to induce bone and cartilage if they get the appropriate stimulus. The response to TGF-β was typical of multipotent cells but not of committed chondrocytes or osteoblasts, indicating that these latter cells are not present in early stages of healing and suggesting that their differentiation is inhibited in chronic nonunion.

Table 1: Ceramic cube assay: semi-quantitative scores.

<table>
<thead>
<tr>
<th>Animal Identification Number</th>
<th>7-Day Tissues</th>
<th>84-Day Tissues</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Three Weeks</td>
<td>Six Weeks</td>
</tr>
<tr>
<td>2733</td>
<td>0</td>
<td>2.0</td>
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<tr>
<td>2962</td>
<td>0; 0</td>
<td>0.0</td>
</tr>
<tr>
<td>2970</td>
<td>0; 0</td>
<td>1.8; 2.0</td>
</tr>
<tr>
<td>2974</td>
<td>0</td>
<td>2.5</td>
</tr>
</tbody>
</table>

C = Cartilage was present in some pores.
Cubes scores separated by a semicolon indicate that two or more cubes were implanted at separate sites in a host animal.

Figure 1: Photomicrographs of typical histologic sections of tricalcium phosphate-hydroxyapatite porous ceramic cubes which had been filled with cultured cells derived from nonunion tissue removed seven days (a) or 84 days (b) after creation of a gap defect in canine tibiae; cell-loaded ceramic cubes were harvested six weeks after implantation into nude mice.

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