IL-1 RECEPTOR ANTAGONIST INHIBITS TISSUE INGROWTH IN VIVO

+*Ma, T; *Miyanishi, K; *Trindade, MC; *Genovese, M; *Regula D; *Smith, RL; *Goodman SB
+*Stanford University, Stanford, CA

Introduction
Interleukin-1 (IL-1) is a pro-inflammatory cytokine that plays an important role in bone remodeling and homeostasis. Interleukin-1 receptor antagonist (IL-1ra) is a naturally occurring receptor antagonist which binds competitively to IL-1 receptors but doesn’t possess IL-1 agonist activity. The purpose of this study was to examine the effect of local infusion of IL-1ra over a 4-week period on bone ingrowth and tissue differentiation in rabbit Drug Test Chamber (DTC) model.

Methods:
Surgical Procedure: Institutional guidelines for the care and use of laboratory animals were strictly followed. The Drug Test Chamber (DTC) was implanted unilaterally in the proximal medial tibial metaphysis of 10 mature NZW rabbits (Figure 1). The chamber provides a continuous 1x1x5 mm cavity for tissue ingrowth. After a 5-week osseointegration period, the contents of the chambers were harvested and discarded. For continuous infusion, the DTC was connected to an Alzet osmotic diffusion pump via medical grade vinyl tubing. Animals received infusion of 0.05% bovine serum albumin (BSA) in phosphate buffered saline (PBS) and infusion of IL-1ra for 4 weeks, interspersed with 4-week controls with no treatment. Tissue inside the chamber, when present, was harvested and processed following each four-week period.

IL-1ra: Human recombinant IL-1ra (Amgen Inc.) at a concentration of 100 mg/ml was infused continuously at 5.0 ul/hr via Alzet diffusion pump for 4 weeks. The IL-1ra was endotoxin free as determined by a high sensitivity limulus amebocyte lysate assay. Each pump was replaced with a new one loaded with fresh IL-1ra solution every 2 weeks. For continuous infusion, the DTC was connected to an Alzet osmotic diffusion pump via medical grade vinyl tubing. Animals received infusion of 0.05% bovine serum albumin (BSA) in phosphate buffered saline (PBS) and infusion of IL-1ra for 4 weeks, interspersed with 4-week controls with no treatment. Tissue inside the chamber, when present, was harvested and processed following each four-week period.

Tissue Processing: The tissue harvested after each 4-week period was snap frozen in optimal cutting medium (OCT) and stored at ~70°C until processed. 4 um sections were obtained using a cryostat and stained with hematoxylin and eosin for general morphological and morphometric analysis. Osteoclast-like cells were identified by immunohistochemistry using a monoclonal antibody directed against the alpha chain of the vitronectin receptor. CD51. Osteoblasts were identified using alkaline phosphatase staining. Histomorphometric analysis was performed using NIH image. Statistical analyses included paired t-tests and Fisher’s Exact test.

Figure 1: The Drug Test Chamber

Essential results:
Chambers were filled with longitudinally oriented woven bone in a fibrovascular stroma following a 4-week infusion of 0.05% BSA in PBS and controls with no treatment. Four animals were excluded from the study after the first harvest due to acute/chronic infection. All the 6 remaining animals produced a tissue sample in the chamber every 4 weeks with a consistent percentage of bone area before IL-1ra treatment.

When IL-1ra was infused for four weeks, four of six animals yielded no tissue sample in the chamber (Fisher’s Exact p value=0.06) and lower levels of bone ingrowth were observed in the two samples available for harvesting (% bone = bone area in a tissue section divided by the tissue section area and expressed as a percentage (mean ± standard error). ** Tissue ingrowth is expressed as the number of tissue samples present in chambers / the number of animals undergone the treatment. Ψ four animals were excluded due to infection. ^ one animal had mal-assembled chamber making tissue ingrowth impossible. Δ p=0.023 compared with harvest 3. & p=0.0067 compared with harvest 1.

Table I

<table>
<thead>
<tr>
<th>Harvest</th>
<th>Treatment</th>
<th>Tissue ingrowth**</th>
<th>% Bone ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>None</td>
<td>10 / 10</td>
<td>29.8 ± 3.0</td>
</tr>
<tr>
<td>2</td>
<td>Infusion of 0.05% BSA</td>
<td>6 / 6 Ψ</td>
<td>27.4 ± 4.1</td>
</tr>
<tr>
<td>3</td>
<td>None</td>
<td>6 / 6</td>
<td>27.2 ± 3.9</td>
</tr>
<tr>
<td>4</td>
<td>Infusion of IL-1ra</td>
<td>2 / 6</td>
<td>see text</td>
</tr>
<tr>
<td>5</td>
<td>None</td>
<td>5 / 5 ^</td>
<td>20.4 ± 4.8</td>
</tr>
<tr>
<td>6</td>
<td>None</td>
<td>6 / 6</td>
<td>23.7 ±2.4 &amp;</td>
</tr>
</tbody>
</table>

* %Bone = bone area in a tissue section divided by the tissue section area and expressed as a percentage (mean ± standard error). ** Tissue ingrowth is expressed as the number of tissue samples present in chambers / the number of animals undergone the treatment. Ψ four animals were excluded due to infection. ^ one animal had mal-assembled chamber making tissue ingrowth impossible. Δ p=0.023 compared with harvest 3. & p=0.0067 compared with harvest 1.

Discussion:
IL-1 is involved in a wide spectrum of inflammatory responses, including particle-induced osteolysis following total joint replacement. In this study, local infusion of the IL-1ra over a 4-week period was associated with marked suppression of tissue ingrowth. The marked suppression of tissue ingrowth into DTC observed here may be associated with a number of functions of IL-1, that include mitogenic properties, anabolic activity and cellular recruitment and differentiation.

The results of this study with IL-1ra infusion demonstrated important temporal relationships between tissue ingrowth, bone formation and the presence of a natural inhibitor of the proinflammatory cytokine, IL-1. These data suggests that application of IL-1ra induces multiple effects that depend on tissue specific aspects under which it is being applied. These results may have important ramifications for treatment protocols for regulation of chronic inflammatory diseases.

This work was supported in parts by grants from Zimmer, Amgen, Durect and the Stanford Orthopaedic Research Fund.

49th Annual Meeting of the Orthopaedic Research Society
Poster #1368