TITANIUM PARTICLE SIZE EFFECTS ON RANKL EXPRESSION IN ST2 BONE MARROW STROMAL CELLS

#O'Connor, D; *Koh, HS; **Murphy TC; #Ka, A; **Rubin J; +*#Sung, KLP
University of California, San Diego, CA 92093, Dept. of *Orthopaedics and #Bioengineering. Phone: 858-534-5252, Fax: 858-534-6896, klsung@ucsd.edu

Introduction: In previous studies [1, 2, 3] we have shown that osteoblast functional parameters were regulated by Ti particles in a size dependent manner. We now report that bone cell expression of RANKL, a dominant signal for osteoclast recruitment, is also regulated by exposure to Ti particles.

Radiographs of the hip implant often indicate a loss of bone density in areas not directly adjacent to the joint such as along the implant stem in the case of hip arthroplasties. Micro-motion and fretting along the implant-bone interface can generate debris over the life of the implant yet little research has been done on the direct influence of implant debris on osteoclast functional parameters. We hypothesized that debris wear might also induce local bone remodeling through recruitment of osteoclasts through regulation of RANKL expression. The murine stromal cell line, ST2, is well known to respond to osteoactive factors such as vitamin D and TNFα with regulated RANKL mRNA expression. We thus explored RANKL mRNA expression of ST2 cells in response to particle loading using a quantitative real-time PCR assay.

Methods: Particle size separation: Mixed sizes of commercially pure titanium particles were obtained from Alfa Aesar and separated by several sedimentation and differential centrifugation rates into three size groups; < 1.5, 1.5-4, and 5-9 µm (group I, II, and III, respectively). All groups of particles were extensively washed and heated at 200°C for 2+ hours and carefully weighed and autoclaved before use. Cell Culture: ST2 cells were plated at 100K in 6-well plates and Ti particles added the next day for 24 or 48 hrs. Realtime assay for RANKL and 18S mRNA species: 0.5 µg total RNA from ST2 cells was reverse-transcribed with primers for RANKL or 18S and then PCR’d using the Biorad iCycler in the presence of SYBR-green from ST2 cells was reverse-transcribed with primers for RANKL or 18S and then PCR’d using the Biorad iCycler in the presence of SYBR-green and cycle threshold assessed. Standards and samples were run in triplicate. RANKL was normalized for amount of 18S in the RT sample, which was also standardized on a dilution curve from a control RT sample. (Forward and reverse primers were 5'-CAC CAT CAG C TG CAC CAT CAG C TG and 5'-CCA AGA TCT C TA ACA TGA C G respectively [4, 5]). The efficiencies of reactions for both RANKL and 18S were more than 90%.

Results: Particle size separation: Separation procedure was successful when > 97% of the particles visualized (mean of 500 particles) were within a size group (Fig. 1).

Real-time PCR analysis of ST2 cells for RANKL Expression: Real-time PCR has been used to measure the levels of RANKL in stromal cells treated with osteotropic and mechanical factors [4, 5]. In this preliminary study, the levels of RANKL expression in cells loaded with a 0.1 wt% titanium particle-loaded cell culture media were compared to a control, non-particle-loaded cell culture medium in each of three size groups; <1.5 µm, 1.5-4 µm, and 5-9 µm (groups I, II, and III, respectively). Our hypothesis was that higher levels of RANKL expression induced by certain sizes of particles would indicate greater levels of osteoclast recruitment and/or activation in vivo. Data from this study is represented in Table 1 and Figure 2. The number of assays measured for each data point ranges from n=10 to n=4. Note that this study indicates the lower levels of RANKL expression in stromal cells loaded with the <1.5 µm particles relative to the cells loaded with larger particle sizes. The levels attained by the largest size particles are equivalent to RANKL mRNA induced by 1 nM vitamin D.

Table 1: Collected real-time PCR data (RANKL mRNA levels) from size-separated commercially pure Ti particle-loading of rat stromal cells. Data represented as % control ± SEM.

<table>
<thead>
<tr>
<th>Particle Size</th>
<th>24 hours</th>
<th>48 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100 ± 3</td>
<td>100 ± 3</td>
</tr>
<tr>
<td>&lt;1.5 µm</td>
<td>118 ± 14</td>
<td>75 ± 2</td>
</tr>
<tr>
<td>1.5-4 µm</td>
<td>190 ± 28</td>
<td>232 ± 26</td>
</tr>
<tr>
<td>5-9 µm</td>
<td>264 ± 57</td>
<td>270 ± 53</td>
</tr>
</tbody>
</table>

Figure 2: Plot of real-time PCR data of RANKL levels following loading with 0.1 wt% Ti particles of three size-separat ed groups (relative to control) for 24 hours. Error bars represent ± SEM.

Discussion: The results of this study demonstrate a differential response of ST2 stromal cells to loading with particles of identical composition (commercially pure titanium) but different sizes. The RANKL expression study showed that particles in the 5-9 µm size group caused the highest RANKL mRNA expression (2.5 X control) while particles in the <1.5 µm size group caused no significant increase when compared with unloaded control cells. The mid-sized Ti particles (1.5-4 µm) caused a doubling in RANKL expression level compared to control cells. In conclusion, the particle size determined the degree to which RANKL mRNA expression was induced. This suggests that debris particles larger than 1.5 µm might induce local bone remodeling by recruitment of osteoclasts due to upregulation of RANKL expression by stromal/osteoblast cells in bone. Interestingly, particle sizes smaller than 1.5 µm have other impacts on osteoblast cell function. In an earlier study, particles (<1.5 µm) cause significant inhibition (more than 40% by 0.1% particle loading) of osteoclast proliferation as well as a decrease in viability (15% for 72 hr loading). Also, in our in vivo study, particles of this smaller sized group inhibited bone ingrowth and recovery by 50% after loading particles with pin insertion. Therefore, it is possible that debris particles stimulate variable signal transduction pathways dependent on their size, and that these signals result in different responses from the local bone environment.


Acknowledgements: KPS: NIH AR45635, OREF 59121; JR: AR42360

Additional Author Affiliation: **VAMC and Emory University, Atlanta GA 30033, Dept. of Medicine. Phone: 404-321-6111 x2080, Fax: 404-235-3011, jrub02@emory.edu

49th Annual Meeting of the Orthopaedic Research Society
Paper #0077