Rethinking Periprosthetic Osteolysis through OsteoImmunology: Peripheral Blood Spontaneous Osteoclastogenesis and Effector T-Cells

Poster No. 2159 • 56th Annual Meeting of the Orthopaedic Research Society

Introduction.
Hip arthroplasty is one of the most successful surgical procedures in all medical assets, dramatically increasing quality of life of people affected by hip arthritis worldwide (1). Failures depend mainly on aseptic loosening, caused by periprosthetic osteolysis (Figure 1), an inflammatory reaction to prosthetic wear debris leading to massive local bone resorption (2). Although retrieved in periprosthetic tissues, T cells are generally believed not to play any role in the osteolysis; the assumption is based on the absence of traditional activation markers in periprosthetic tissue T cells (3, 4).

It was previously described that patients with osteolytic bone metastases show spontaneous osteoclastogenesis: their peripheral blood mononuclear cell (PBMC) differentiate into osteoclast (OC) in vitro without adding any exogenous factors (5). Spontaneous osteoclastogenesis is characterized by the presence in the bloodstream of committed circulating T cells, capable to support and promote osteolysis. In these patients, affected by different solid tumors, it was also detected an abnormal serum level of interleukin-7 (IL-7) (6). IL-7 was shown to be involved in osteoclastogenesis (6).

Our goal is to investigate the presence of spontaneous osteoclastogenesis and abnormal IL-7 serum levels in peripheral blood from patients affected by periprosthetic osteolysis; we will also test the role of peripheral T cells and their activation profile.

Material and Methods
45 subjects were selected and included in either one of the following groups: periprosthetic osteolysis (PO) study group, stable prosthesis (SP) control group and "healthy" control (HC) group. After obtaining informed consent, we collected peripheral blood for testing. PBMCs were isolated after centrifugation over a density gradient; Cells were resuspended in α-MEM supplemented with FCS, 100 IU/ml penicillin, and 100 μg/ml streptomycin then counted and plated. To obtain fully differentiated human OCs, PBMCs were then cultured for 15–17 days; culture medium was refreshed every 3–4 days. At the end of the culture period, mature OCs were identified as tartrate-resistant acid phosphatase positive (TRAP+) multinucleated cells.

During in vitro osteoclastogenesis we studied the effects of RANKL inhibition, adding Rank-Fc (a RANKL inhibitor), to some of the cultures. To study the effect of Tcell depletion, T cells were removed by “panning” method from part of the cultures.

Lymphocyte immunophenotypes were examined upon collection of the blood sample and at different culture times using flow cytometry to assess the frequencies of CD4(+)/CD90(+), CD4(+)/CD25(+) and CD8(+)/CD25(+) cells.

IL-7 serum levels were determined by ELISA kit.
In addition we analysed 19 periprosthetic tissue samples by immunofluorescence to detect Tcell-osteoclast interactions.

Our data are consistent with previous findings of nonactive periprosthetic T cells, suggesting a different interpretation of their role; it is possible to speculate that “non-active effector” T cell may play a previously undetected role in osteolysis progression. Those findings suggest potential ineffectiveness for some medical therapies proposed in literature such as the use of RANKL inhibitors (7).

Discussion.
Peripheral Blood Spontaneous osteoclastogenesis was demonstrated in periprosthetic osteolysis patients; osteoclastogenesis was partially RANKL-independent, as previously shown in metastatic osteolysis.

Committed T cells, detected in peripheral blood of PO patients, support spontaneous osteoclastogenesis. Thus, we demonstrated for the first time that T cells, “committed” and supporting osteolysis, can be negative for some of the classic activation markers.

Our data are consistent with previous findings of nonactive periprosthetic T cells, suggesting a different interpretation of their role; it is possible to speculate that “non-active effector” T cell may play a previously undetected role in osteolysis progression. Those findings suggest potential ineffectiveness for some medical therapies proposed in literature such as the use of RANKL inhibitors (7).

Periprosthetic osteoclasts may be involved in priming T cells in periprosthetic tissues. The exact mechanism of “priming” need to be further investigated.

Spontaneous osteoclastogenesis and IL-7 are potential blood markers of periprosthetic osteolysis, as in bone metastasis; their clinical usefulness need to be tested in specifically designed studies.

Table 1: group composition and osteoclastogenesis, averages (SD)

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of subjects</th>
<th>Age Years from implant</th>
<th>OC Day 15</th>
<th>OC T dep</th>
<th>OC RANKF</th>
<th>c</th>
</tr>
</thead>
<tbody>
<tr>
<td>SO</td>
<td>15</td>
<td>76 (6)</td>
<td>12 (4)</td>
<td>131.9 (65.3)</td>
<td>39.8 (38.9)</td>
<td>74.2 (39.3)</td>
</tr>
<tr>
<td>PTA</td>
<td>15</td>
<td>75 (8)</td>
<td>4 (4)</td>
<td>22.0 (22.0)</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>HC</td>
<td>15</td>
<td>59 (18)</td>
<td>---</td>
<td>32.5 (21.6)</td>
<td>nd</td>
<td>nd</td>
</tr>
</tbody>
</table>

Figure 1: Periprosthetic Osteolysis

References
3. L. Baldwin et al., Biomaterials 23, 3007 (Jul, 2002).
5. I. Roato et al., FASEB J 19, 228 (Feb, 2005).