RANKL EMANTES FROM A MULTINUCLEAR CELL TYPE IN THE PERIPROSTHETIC MEMBRANE

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Introduction:
Aseptic osteolysis remains one major complication that leads to late failure of total joint arthroplasties (TJA), which cost billions of dollars each year in the United States. Periprosthetic membrane is usually found between the prosthesis and bone during revision TJA (TJR). It has been suggested that the active biological property of the membranes causes aseptic osteolysis around the prosthesis which may lead to failure of the arthroplasty and difficulty in reconstruction of the joint in the face of extreme bone loss. To date it has been shown that both cytokines such as IL-1 beta, IL-6, and TNF-alpha and the newly discovered triad protein RANK/RANKL/OPG are important in the differentiation of macrophages into osteoclasts (1). The interaction between RANKL presented by osteoblast/stromal cells and RANK presented by osteoclast precursor cells (usually referred to as monocyte/macrophage lineage cells) triggers the cascade of differentiation, maturation and activation of osteoclasts. Being the strongest osteoclast promoter, RANKL is studied extensively but the cellular origin of RANKL within periprosthetic membrane is not clearly understood. Our previous study has shown that the cellular expression pattern of RANKL within the periprosthetic membrane was closely related to fibroblast cells. In the process of further differentiating the cellular milieu of the osteocytic membrane our laboratory has identified a multinuclear cell of mesenchymal lineage. It has been noted previously that multinucleated foreign body cells have been seen in response to particulate debris (2-3). To date there is no literature reporting the characterization of fibroblast multinucleated giant cells in the periprosthetic membrane of patients with osteolysis. Using different markers, it is possible to identify the cellular origin of the multinucleated cells. It is our hypothesis that these multinucleated giant cells are of stromal/fibroblast in origin and are important in the differentiation and regulation of macrophage/osteoclast process.

Methods:
Retrieved osteolytic periprosthetic membranes were obtained from revision TJA. The protocol was reviewed and approved by an institutional review board and all patients were consented. All samples were harvested in a sterile aseptic fashion and kept on ice until processed at the laboratory. Upon arrival at the lab, specimens were embedded in Optimal Cutting Temperature (OCT; Sakura Finetek USA Inc., Torrance, CA) media and frozen at -20°C over the short term. Long-term storage was at -70°C. 12 patients (2 knees and 10 hips; 36-87 years old, average 59 years old) periprosthetic osteolytic membranes were retrieved. Osteolytic lesions were defined as a radiolucent space measuring 6 mm or greater on X-ray. The membranes were cut into 16μm thick sections on a cryostat (HM 500 M; Microm International, Waldorf Germany) and placed onto charged slides (SuperFrost Plus; Fischer Scientific, Pittsburgh, PA) at two sections per slide. Macrophages were detected using the Ber-MAC3 antibody (Dako); fibroblasts were detected using the 5B5 fibroblast specific antibody (Dako). Slides were thawed at room temperature and then washed twice with Tris-buffered saline (TBS). Tissues were fixed with ice cold methanol and then rinsed twice with TBS. Tissues were permeabilized and blocked by adding a solution of 0.1% Triton X-100 and 3% normal goat serum (Vector Laboratories, Burlingame, CA) in TBS and then washed twice with TBS. Slides were then incubated with primary antibody for one hour. A fluorescent DNA binding stain (Hoechst 34580; Molecular Probes, Eugene, OR) was used at a concentration of 5μg/ml and incubated for 30 minutes to visualize nuclei. Fluorophore-conjugated secondary antibodies were incubated for one hour at a dilution of 1:200 with either chicken anti-rabbit Alexa Fluor 488, chicken anti-rabbit Alexa Fluor 594, donkey anti-goat Alexa Fluor 488, or chicken anti-mouse Alexa Fluor 594 (Santa Cruz Biotechnology Inc., Santa Cruz, CA), as specified in respective figure legends. Slides were viewed and images stored using a laser scanning confocal microscope (model LSM 520 Meta; Carl Zeiss Inc, Thornwood, NY).

Results/Discussion:
Using immunohistochemical studies we have identified a cell in the interfacial membrane (IFM) that stains positive for RANKL, displays the 5B5 fibroblast marker while also being multinucleated. The fibroblast-like characteristics of the multinuclear cell in our study suggest that it is distinct from previously described multinucleated cells present in the IFM (osteoclast, foreign body giant cell). Strong colocalization was found between 5B5 and RANKL in the multinucleated cells. Macrophage cell marker Ber-MAC3 was not colocalized with the multinucleated structure but was found to reside right next to these cells. RANKL seems to be a characteristic staining of the multinucleated cells in all 12 patients. Future studies would attempt to isolate this cell from the IFM and study both its formation and osteoclastogenic effects on myeloid osteoclast precursors in an attempt to understand fully the contribution of these cells to periprosthetic osteolysis.

The role of the RANKL/RANK/OPG triad in bone resorptive pathologies has been well documented (4-6). IFM Fibroblasts are capable of making RANKL and have been implicated in the osteolysis surrounding TJA by altering the RANKL/OPG ratio. The levels of RANKL present on the surface of these cells are higher than other mononuclear RANKL-positive cells in the IFM suggesting a key role for these cells in promoting osteoclastogenesis and elevated bone resorption in periprosthetic osteolysis. The high level of RANKL produced suggested this multinuclear cell could be an important factor in promoting osteolysis. Generation of this multinucleated cell of mesenchymal lineage in response to the surrounding cytokine environment and wear debris from the joint prosthesis may be a critical step in the pathology of periprosthetic osteolysis. An understanding of the conditions necessary for formation of this multinucleated cell as well as the causes of the increased RANKL production are crucial to the understanding of the osteolysis as well as providing possible therapeutic targets.

References