The role of fibroblasts and fibroblast-derived factors in periprosthetic osteolysis

Periprosthetic osteolysis following total joint arthroplasty (TJA) is a major clinical problem in both cemented and cementless reconstructions.

One of the pathological features of periprosthetic osteolysis in failed TJAs is the formation of a (pseudo)membranous tissue (interfacial membrane) at the bone/prostheses or bone/cement interface. The interfacial membrane (IFM) is a granulomatous tissue consisting predominantly of fibroblasts, macrophages, and foreign body giant cells. It is believed that particulate wear debris via phagocytosis activates cells which then proliferate and release inflammatory mediators, such as TNF-α, IL-1β and IL-6. These “bone-resorbing” agents activate eventually all cell types in the IFM in either a paracrine or autocrine manner.

In an earlier study, we found that fibroblasts isolated from IFMs of patients with failed total joint replacements, when challenged with small-sized (<3 µm) titanium (Ti) particles, responded with enhanced expressions of collagenase, stromelysin and, to a lesser extent, tissue inhibitor of metalloproteinases. In this study we focused on the fibroblast response measuring the expression of “fibroblast activation markers” such as MCP-1, IL-1β, IL-6, VEGF (see abbreviations in Fig. 1 legend), RANKL and OPG in response to stimulation with Ti particles and/or the conditioned media of IFM of loosened TJAs. We concluded that fibroblasts may significantly contribute to the diminished bone remodeling in periprosthetic osteolysis.

METHODS. The collection of human samples was approved by the Institutional Review Board in consent with the patients. Conditioned media (CM) of explant cultures of IFM (CM-IFM) were collected, and used for stimulation of isolated fibroblasts in the presence or absence of Ti particles. The effect of CM-IFM and/or Ti particles on fibroblast gene expression was determined by (i) standard Ribonuase Protection Assay (RPA) Systems (for various cytokines and chemokines (BD Pharmingen) and (ii) two additional custom-made templates (Fig. 1). In addition, (iii) RANKL (receptor of activated nuclear factor-kappa B [NF-xB] ligand) and osteoprotegrin (OPG: a decoy receptor of RANKL) were quantified by real-time PCR. Protein expression (secretion) in CM-IFM and CM of stimulated fibroblasts were determined by ELISA and Western blot hybridization, while the cell surface expression of RANKL was determined by flow cytometry (Fig. 2).

To measure the effect of activated fibroblast-expressed RANKL on osteoclastogenesis, human fibroblast/bone marrow co-cultures (in the presence of macrophage-colony-stimulating factor [M-CSF]) were stimulated with IFM-derived CM and/or Ti wear debris, and multinucleated tartrate-resistant acid phosphatase positive (TRAP+) cells counted.

RESULTS. Fibroblasts phagocytosed particulate wear debris, and responded to cytokine/chemokine stimulation. The most dominant cytokines measured in CM of IFM were: TNF-α, MCP-1, IL-1β, IL-6, IL-8 and VEGF. The most prominent upregulated genes, and secreted proteins by fibroblasts in response to stimulation (with particles and/or cytokines/chemokines) were MMP-1, MCP-1, IL-1β, IL-6, IL-8, cyclo-oxygenases (Cox-1 and Cox-2), ILF-1, TGF-β1 and its receptor-1 (TGF[RI]) (Fig. 1). In addition, IFM fibroblasts expressed large amounts of RANKL (Fig. 2) and OPG in response to CM-, TNF-α, or IL-1β-stimulation. Particulate wear debris, in the presence of proinflammatory cytokines in CM, even had a synergistic effect on RANKL expression. Fibroblast pre-stimulated with Ti wear debris expressed RANKL, and in co-cultures, induced the formation of multinucleated TRAP+ cells from adherent bone marrow cells in the presence of M-CSF.

CONCLUSION: IFM fibroblasts respond directly to particulate wear debris, possibly via phagocytosis, express proinflammatory cytokines, RANKL and M-CSF (osteoastrogenic factors). Thus, these cells may actively be involved in pathological (periprosthetic) bone resorption.

Clinical Relevance: Systematically administered drugs, such as those (TNF-α and Cox inhibitors) used for treatment of rheumatoid arthritis, may suppress periprosthetic osteolysis via the suppression of RANKL expression not only by macrophages and osteoblasts, but also by fibroblasts.

Figure 1. The effect of Ti particles and conditioned media of interfacial membranes (CM-IFM) on gene expression by fibroblasts isolated from the IFM. The hCK4 and two custom-made templates show representative RPA using radiolabeled cRNA probes from untreated, Ti- and/or CM-treated fibroblasts of IFM after 48 hr treatment. 32P-labeled transcripts of known size were generated by in vitro transcription from RNA templates and were used as size markers.

**Abbreviations:** Template 65184: TNF-α = tumor necrosis factor-α; IL-1β = interleukin 1; IL-1RI = IL-1 receptor-1; MMP-1 = matrix metalloproteinase-1 (collagenase); MCP-1 = monocyte chemoattractant protein-1; TGF-β = transforming growth factor-β; TGF-RI = TGF-β receptor-1; IFN-γ = interferon-γ. Template 65120: RANTES: a CXC chemokine regulated upon activation normally T-cell expressed and secreted; IP-10 = CXCL-10 = CXC chemokine; Cox-1/2 = cyclooxygenase 1 and 2; bFGF = basic fibroblast growth factor; GFR-K = FGF receptor; Ang-1 = angiogenic protein-1; VEGF = vascular endothelial growth factor; c-myc = oncogene. Template hCK4: M-CSF = macrophage colony-stimulatory factor; IL-6 = interleukin-6; LIF = leukemia inhibitory factor-1; SCF = stem cell factor; OSM = oncostatin M. Dashes indicate the corresponding gene’s position (occasionally two or three transcripts can be seen), whereas the intensities of bands show the level of mRNA expression. L32 and GAPDH (glyceraldehyde-3-phosphate-dehydrogenase) (housekeeping genes) correlated (>95%) with 18S ribosomal RNA and was used for the normalization of all experiments. Treatments are indicated underneath the templates. Representative panels of over 15 hybridization experiments are shown.

Figure 2. Flow cytometry results, when IFM fibroblasts were stimulated for 24 hr (as indicated) or left untreated (Untr) and stained with mouse mAb (clone 70513; R&D Systems) for RANKL expression.

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tglant@rush.edu