INVolvEMENT OF TWO DISTINCT CELLULAR MECHANISMS OF OSTEOCLASTOGENESIS IN ASEPTIC LOOSENING

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INTRODUCTION:

The current paradigm to explain the osteolysis of aseptic loosening is that wear debris generated from implant components is phagocytosed by macrophages stimulating the activation and secretion of pro-inflammatory cytokines (e.g. TNFα and IL-1). TNFα and other cytokines are known to induce receptor activator of NF-κB (RANK) ligand (RANKL) expression on osteoblasts and bone stromal cells. Interaction of RANKL with RANK, expressed on osteoclast precursors, is now known to promote osteoclast development and activation. We have shown that particle-associated macrophages isolated from failed joint arthroplasties can differentiate into mature functionally active osteoclastic cells in the presence of soluble RANKL and M-CSF. We have also shown that RANKL-induced osteoclast differentiation in these tissues can be significantly inhibited by osteoprotegerin (OPG). Recently it has been reported that TNFα (in the presence of M-CSF) could support macrophage-osteoclast differentiation in periprosthetic tissues in a manner independent of RANKL/RANK mechanism.

METHODS:

Pseudomembrane specimens obtained from 6 patients undergoing hip revision due to aseptic loosening, were treated with collagenase and Versine®. As the pseudomembrane contains a heterogeneous population of macrophages, macrophage polykaryons, T-cells and fibroblasts, CD14+ macrophages were separated using the MiniMACS magnetic cell sorting system. CD14+ populations were cultured on coverslips and dentine slices in the presence of: (i) 25 ng/ml M-CSF, (ii) M-CSF with 30 ng/ml soluble RANKL (iii) M-CSF plus 10 ng/ml TNFα, (iv) M-CSF, TNFα plus 10 ng/ml IL-1α, (v) M-CSF, TNFα, IL-1α plus 100 ng/ml OPG and (vi) M-CSF, TNFα, IL-1α plus 5 μg/ml anti-TNF receptor p55 or p75 antibody. All cultures were maintained for 1-17 days after which time the extent of osteoclast markers including TRAP and VNR on coverslips and evidence of lacunar resorption on dentine slices.

RESULTS:

Extensive osteoclast formation and lacunar resorption was evident in macrophage cultures in the presence of soluble RANKL and M-CSF (Figure 1). In the absence of soluble RANKL, but in the presence of TNFα macrophages isolated from periprosthetic tissues were capable of differentiating into active bone resorbing osteoclasts (Figure 1). Addition of IL-1α to cultures containing TNFα and M-CSF resulted in a marked increase in extent of lacunar resorption. Addition of OPG, however, did not reduce the extent of TNFα/IL-1α-mediated osteoclast differentiation by periprosthetic macrophages (Figure 2).

In order to determine through which TNF receptor subunit the TNFα-induced osteoclastogenesis occurred, we used specific antibodies directed against human TNF receptor p55 and p75 subunits. Addition of either of these antibodies, significantly, but not completely, reduced the extent of osteoclast formation and lacunar resorption in CD14+ cultures (Figure 2).

DISCUSSION:

These results indicate that two different cellular mechanisms of osteoclast formation are associated with periprosthetic loosening. Our results clearly indicate that both RANKL-dependent and RANKL-independent (TNFα-induced) mechanisms induce the differentiation of arthroplasty pseudomembrane-derived macrophages to osteoclasts capable of lacunar resorption. In addition, we have shown that IL-1 significantly enhances the process of TNFα-induced osteoclastogenesis.

Periprosthetic tissues contain a heavy inflammatory macrophage (CD14+) infiltrate in response to wear particles. As abundant pro-inflammatory cytokines (e.g. TNFα, IL-1, IL-6) are known to be present in this inflammatory microenvironment, it is therefore likely that RANKL-independent mechanisms of osteoclast formation are likely to play an important role in the pathological bone resorption of aseptic loosening.

Based on these findings, in order to control the osteolysis associated with aseptic loosening, the use of specific reagents which block or reduce the RANKL-dependent and RANKL-independent/cytokine-induced processes of osteoclast formation (i.e. OPG and anti TNFα receptor antibodies) are likely to be required. In addition the use of other inhibitors which could block the signalling pathways of these two mechanisms should also be pursued in future studies.