INTRODUCTION:

Aseptic loosening is generally associated with the presence of wear particle-associated macrophages in the pseudomembrane commonly formed around the failed prosthetic implants. The extent of the macrophage response evoked by the wear particles has been shown to correlate with the amount of periprosthetic osteolysis. Numerous studies have shown that wear particle-associated macrophages contribute to osteolysis by (i) releasing inflammatory cytokines and/or (ii) differentiating into bone resorbing osteoclasts. Although macrophages and macrophage polykaryons are the main cell types in the pseudomembrane, numerous fibroblasts are also present in the connective tissue pseudomembrane. The recently identified molecule, receptor activator of NF-κB ligand (RANKL) has been shown to play a central role in the osteoclast formation and bone resorption observed in aseptic loosening. We have also shown that arthroplasty macrophages, which express RANK, the receptor for RANKL, are capable of osteoclast formation; this process is inhibited by osteoprotegerin (OPG), the soluble decoy receptor for RANKL. More recently, it has also been reported that TNFα (in the presence of M-CSF) could support macrophage-osteoclast differentiation from mouse marrow precursors and human mononuclear cells, in a manner independent of RANKL/RANK interaction. As fibroblasts are known to express RANKL, and as TNFα and other inflammatory cytokines are known to be abundant in periprosthetic tissues, the aim of the present study was to determine:

(i) whether fibroblasts, isolated from periprosthetic tissues, could generate bone resorbing osteoclasts, and
(ii) whether this process is through a RANKL-dependent and/or – independent mechanism.

METHODS:

Fibroblast-like cells were isolated by routine collagenase enzyme digestion of the arthroplasty-derived membrane specimens from 6 patients undergoing hip revision due to aseptic loosening. The isolated cells were seeded in tissue culture flasks for 2-4 weeks before being passaged for a further 3-4 times. Generated fibroblast-like cells (10⁵) were then co-cultured with 5x10⁷ human peripheral mononuclear cells (PBMC) (n=6) on glass coverslips and dentine slices in the presence of (i) no added factors, (ii) 25 ng/ml macrophage colony stimulating factor (M-CSF), (iii) M-CSF plus 100 ng/ml OPG and (iv) M-CSF plus 10 µg/ml anti TNFα antibody. All cultures were maintained for 1, 17 and 21 days after which the extent of osteoclast differentiation was determined by the expression of specific osteoclast markers including tartrate-resistant acid phosphatase (TRAP) and vitronectin receptor (VNR) on coverslips and evidence of lacunar resorption on dentine slices. Each experiment was repeated in triplicate for each treatment. The results are expressed as the mean number of lacunar resorption pits formed on 3 dentine slices ± standard error of the mean (SEM).

RESULTS:

In fibroblast cultures alone (i.e. in the absence of PBMC), in the presence of M-CSF, there was no evidence of TRAP or VNR positive multinucleated cells after 17 days incubation. In addition, lacunar pit formation was not evident in these cultures after 21 days incubation. In PBMC cultures alone (i.e. in the absence of fibroblasts), in the presence of M-CSF, small number of TRAP/VNR positive multinucleated cells were formed which were incapable of lacunar pit formation on dentine slices. These results indicate that pseudomembrane-derived fibroblasts and PBMC alone do not form resorption lacunae.

In fibroblast/PBMC co-cultures, in the absence M-CSF, no osteoclast formation was noted after 24 hours, 17 or 21 days incubation. However, in the presence of M-CSF, large numbers of TRAP+ and VNR+ multinucleated cells capable of lacunar resorption were noted in all the fibroblast/PBMC co-cultures (mean number of lacunar resorptions pits: 32 ± 4.5), thus suggesting that pseudomembrane-derived fibroblast-like cells are capable of expressing RANKL. This observation was further confirmed by RT-PCR techniques which indicated that pseudomembrane-derived fibroblasts express mRNA for human RANKL.

The addition of OPG, which is known to inhibit RANKL-mediated osteoclast formation, significantly reduced the extent of osteoclast formation and lacunar resorption in these co-cultures (mean number of lacunar resorptions: 10 ± 3.1). In order to determine if this incomplete inhibition was due to the involvement of TNFα-induced osteoclastogenesis, we sought to determine the role of this cytokine in this co-culture system. Addition of anti-TNFα antibody to fibroblast/PBMC co-cultures resulted in a reduction in osteoclast formation and lacunar resorption (mean number of lacunar resorption pits formed: 20 ± 4.2).

DISCUSSION:

These results indicate that fibroblasts in the arthroplasty membrane can directly contribute to macrophage-osteoclast differentiation commonly reported in periprosthetic loosening. Our findings indicate that one of the means by which this occurs is through expression of RANKL (either in a membrane-bound or soluble form) in these pseudomembrane-derived fibroblasts. Addition of RANK-expressing PBMC to these fibroblast cultures resulted in osteoclast formation and lacunar resorption which indicate the involvement of a RANKL/RANK interaction mechanism in this process of osteoclastogenesis.

Inhibition of RANKL/RANK-induced osteoclastogenesis by OPG was not complete, thus suggesting that other cellular mechanisms of macrophage-osteoclast differentiation (i.e. a RANKL-independent mechanism) are likely to be involved in this process. As TNFα and IL-1β have been shown to be abundant in periprosthetic tissues and as it has recently been shown that TNFα can directly induce osteoclast formation in vitro, we investigated the effect of addition of anti-TNFα antibody in these fibroblast/PBMC co-cultures. Our results suggest that in addition to RANK/RANKL interaction, fibroblasts may employ the TNFα-induced mechanism of osteoclast formation and bone resorption.

These findings in overall, indicate that fibroblasts directly contribute to osteolysis associated with aseptic loosening through both a RANKL-dependent and RANKL-independent mechanism. As such, suppression of osteoclast formation by OPG as well as anti-TNFα treatment could represent potential therapeutic strategies to be employed in controlling periprosthetic loosening.