HUMAN OSTEOBLASTIC CELLS SUPPORT OSTEOCLAST FORMATION FROM ARTHROPLASTY-DERIVED MACROPHAGES

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Introduction:

A predominant histological feature of periprosthetic tissue surrounding aseptically loose implant components is the presence of a prominent foreign body macrophage response to prosthesis derived wear. A number of potent bone resorbing mediators have been identified in periprosthetic tissue from around loose prostheses and these mediators have been shown to be released from wear particle stimulated macrophages both *in vivo* and *in vitro.* However, the cellular mechanisms underlying aseptic loosening of implant components and the manner in which this foreign body macrophage infiltrate in periprosthetic tissues contributes to osteolysis remain uncertain. Recent studies have shown that murine monocytes and inflammatory macrophages responding to prosthesis wear particles are capable of differentiating into osteoclastic bone resorbing cells. Thus, one cellular mechanism whereby bone resorption and implant loosening may occur is by osteoclastic differentiation of precursor cells present in periprosthetic tissue. Recently it was reported that macrophages isolated from the pseudomembrane surrounding failed joint arthroplasties, when co-cultured with rat osteoblast-like cells, UMR106, in the presence of $1,25(OH)_2D_3$, were capable of differentiating into osteoclastic bone resorbing cells. In order to develop an *in vitro* system of human macrophage-osteoclast differentiation which more closely parallels what occurs *in vivo*, we sought to determine if human bone derived stromal cells were capable of supporting the differentiation of arthroplasty-derived macrophages into osteoclastic bone resorbing cells.

Material and Methods:

Human osteoblast-like cells, positive for alkaline phosphatase and exhibiting matrix mineralisation, were derived from cell outgrowths of human trabecular bone. Macrophages were isolated from the pseudomembrane obtained at revision surgery of 6 aseptically loose hip arthroplasties and added to either cortical bone slices or glass coverslips previously seeded with human osteoblastic cells. The non-adherent cells were removed and the cultures incubated for 1, 10 and 14 days [± $1,25(OH)_2D_3$ and dexamethasone]. Specific markers of macrophages (CD11b and CD14) and osteoclast associated markers (TRAP, VNR and lacunar bone resorption) were used to characterise the cultured cells. Bone resorption was measured as the number of resorption pits per bone slice. Each treatment was studied in triplicate for each tissue specimen.

Results:

After 24 hours incubation, numerous adherent cells in periprosthetic macrophage-human bone stromal cell cocultures on glass coverslips were found to express strongly the macrophage cell surface antigens, CD11b and CD14. These 24 hour cocultures were largely negative for TRAP and VNR multinucleated cells although scattered TRAP and VNR positive mononuclear cells were present in most preparations. In 3 of the 6 arthroplasty tissue specimens, a few TRAP and VNR positive multinucleated cells were also noted (<5 per coverslip). Very few resorption pits were seen on the bone slices after 24 hours co-culture. The mean number of total resorption pits in each experiment (n=6 experiments; 3 bone slices per experiment) ranged from 0 to 12 pits per bone slice.

In the 10 day co-cultures of periprosthetic macrophages and human bone stromal cells on glass coverslips, in the absence of $1,25(OH)_2D_3$ and dexamethasone, numerous large TRAP positive cells and clusters of smaller TRAP positive mononuclear cells were seen. Numerous large VNR positive multinucleated cells were also present (>30 per coverslip). After 14 days co-culture on bone slices, extensive lacunar bone resorption was evident on all the bone slices studied. The mean number of resorption pits (mean ± SEM) was 76.1 ± 17.7 pits per bone slice for the 6 experiments using human bone stromal cells. It was found that $1,25(OH)_2D_3$ and dexamethasone were not essential requirement for osteoclast formation and bone resorption in periprosthetic macrophages-human bone stromal cell co-cultures.

In the control cultures where periprosthetic macrophages were incubated for 10 days on glass coverslips in the absence of human bone stromal cells, scattered TRAP and VNR positive mononuclear cells were seen. In 3 of the 6 experiments, a few TRAP and VNR positive multinucleated cells were also seen. These multinucleated cells are most likely derived from pieces of bone which are attached to or embedded in the arthroplasty pseudomembrane. The mean number of total resorption pits for each experiment (n=6 experiments; 3 bone slices per experiment) ranged from 0 to 16 pits per bone slice in these control cultures.

Discussion:

The results of this study have shown that human bone-derived stromal cells are capable of supporting osteoclast formation from cells present in the macrophage-rich periprosthetic tissues surrounding a loose implant. This human macrophage-human osteoblastic cell co-culture system shows striking differences in the requirements for osteoclast formation i.e. osteoclast differentiation supported by human osteoblasts does not require exogenous $1,25(OH)_2D_3$. This in vitro human osteoblast-human macrophage co-culture system of osteoclast formation is likely to reflect more closely what occurs in vivo in aseptic loosening.

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