TGF-β in dentin matrix extract induces osteoclastogenesis in vitro

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Abstrak

Previously, we have demonstrated that the extracellular matrix from dentin affects osteoclastic activity in co-culture between osteoclast and osteoblast-rich fraction from mouse marrow cells. In the present study, we aimed to investigate the mechanisms of dentin matrix extract-induced osteoclastogenesis in mouse bone marrow macrophages (BMMs). Dentin proteins were extracted from bovine incisor root dentin using 0.6 M HCl. BMMs were cultured in α-MEM containing macrophage colony-stimulating factor/receptor activator of nuclear factor kappa-B ligand in the presence or absence of dentin matrix extract. Tartrate-resistant acid phosphatase (TRAP)-positive cell number, total TRAP activity, and the mRNA levels of osteoclast-related genes, assayed by real-time RT-PCR, were determined as markers of osteoclastogenesis. A neutralizing antibody against transforming growth factor-β1 (TGF-β1), SB431542, a TGF-β receptor inhibitor, and ELISA were used to determine the role of TGF-β1. We observed increases in TRAP-positive cell number, TRAP activity, and the mRNA levels of osteoclast-related genes of BMMs cultured with dentin extract. The use of a neutralizing antibody against TGF-β1 or SB431542 inhibited the inductive effect of dentin extract, suggesting TGF-β1 involvement. The addition of exogenous TGF-β1, but not bone morphogenic protein-2, also increased osteoclastogenesis, corresponding to the ELISA determination of TGF-β1 in the dentin extract. In conclusion, our results indicate that proteins from dentin matrix have an inductive effect in osteoclastogenesis, which is mediated, in part, by TGF-β1.