Effect of recombinant TRAIL in a murine co-culture system of osteoclastogenesis

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Although some experimental evidence has implicated the TRAIL/TRAIL-receptor system in the regulation of osteoclastogenesis, the only available studies performed so far have been performed on isolated pre-osteoclasts, induced to differentiate by the addition of recombinant RANKL and M-CSF. Using a more physiological co-culture system in the absence of exogenous cytokines, we have here demonstrated that recombinant TRAIL inhibits osteoclast formation, but only at relatively high concentrations (500 ng/mL).

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RAW264.7 macrophagic cell line were induced to differentiate into mature osteoclasts by the addition of recombinant RANKL+M-CSF. Since bone marrow stromal cells and osteoblasts are the major source of OPG within the bone marrow environment (Boyle et al., 2003), to shed light into the role of TRAIL in osteoclastogenesis, we have analyzed the effect of increasing concentrations of recombinant human TRAIL, prepared as described (Zauli et al., 2003), in a previously described model of co-cultures between mouse osteoblasts and RAW264.7 pre-osteoclastic cell line (Nicolin et al., 2006). This culture system shows the advantage that does not require the addition of exogenous cytokines, since it

![Figure 1. Effect of treatment with increasing concentrations of recombinant TRAIL on osteoclastic differentiation in a RAW264.7/osteoblast co-culture system. After 4 days of co-culture of mouse osteoblasts type CRL 12257 with mouse monocyteid RAW264.7 cells, the degree of differentiation was evaluated by scoring the number of the tartrate-resistant acid phosphatase (TRAP)-positive multinucleated cells. In A, data are expressed as means ± SD of three different experiments performed in duplicate (⁎p<0.05). In B, representative fields of co-cultures treated with vehicle (a), 5 ng/mL TRAIL (b), 50 ng/mL TRAIL (c) or 500 ng/mL TRAIL (d) are shown. Bars: 100 μm.](image_url)
spontaneously produces both RANKL and OPG (Nicolin et al., 2006). As shown in Figure 1 A, B, the addition of recombinant human TRAIL had no significant impact on the osteoclast formation up to 50 ng/mL. Only, at high concentrations (500 ng/mL), recombinant TRAIL significantly ($p<0.05$) inhibited the formation of osteoclasts. Although we are aware that mouse cells only express the homologue of human TRAIL-R2 while lacking the expression of TRAIL-R1, -R3, -R4 homologues (Zauli and Secchiero, 2006) and therefore experiments in mice cannot be immediately related to the human situation, our current observation, taken together with previous studies (Zauli et al., 2004; Zauli et al., 2008; Roux et al., 2005; Colucci et al., 2004; Colucci et al., 2007; Vitovski et al., 2007) indicate that the relative concentrations and the expression patterns of TRAIL and OPG in the local microenvironment are key determinant in the TRAIL/OPG interactions. Our findings also suggest that only at high but pharmacologically achievable concentrations (Cretney et al., 2007), TRAIL might efficiently inhibit osteoclastogenesis in vivo.

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**References**


