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).

Key words: TRAIL, osteoclastogenesis, OPG.

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A number of studies have suggested a potential role of the tumor necrosis factor (TNF)-related apoptosis inducing ligand (TRAIL) in modulating the differentiation of hematopoietic progenitors towards different lineages, including osteoclastogenesis (Secchiero *et al.*, 2002; Secchiero *et al.*, 2004; Zauli *et al.*, 2004). In particular, previous studies have shown that recombinant TRAIL either inhibits both human and mouse osteoclastogenesis when added to isolated pre-osteoclasts induced to differentiate with soluble recombinant macrophage colony stimulating factor (M-CSF) + receptor activator of NF-kB ligand (RANKL) (Zauli *et al.*, 2004; Zauli *et al.*, 2008) or induce apoptosis of mature osteoclasts (Roux *et al.*, 2005, Colucci *et al.* 2007). On the other hand, another study performed on peripheral blood cells purified from patients affected by multiple myeloma showed that the addition of recombinant TRAIL paradoxically induced osteoclastogenesis (Colucci *et al.*, 2004). The suggested mechanism proposed by Colucci *et al.* (2004) to explain the pro-osteoclastic activity of TRAIL was that TRAIL likely precluded osteoprotegerin (OPG) produced by the peripheral blood cells of patients affected by multiple myeloma from inhibiting the pro-osteoclastic activity of RANKL. In this respect, it has been recently shown that the affinity of OPG for native TRAIL was comparable to that for RANKL at 37°C, as determined by plasmon surface resonance analysis (Vitovski *et al.*, 2007). Moreover, *in vitro* studies have convincingly demonstrated that OPG acts in a paracrine or autocrine manner by binding TRAIL and promoting the survival of prostate cancer cells (Holen *et al.*, 2002) and multiple myeloma cells (Shipman and Croucher, 2003). Thus, the exact role of TRAIL in modulating osteoclastogenesis is uncertain.

In our opinion, the major limitation of these previous studies is that the effect of TRAIL was evaluated in culture systems in which isolated human peripheral blood mononuclear cells or the

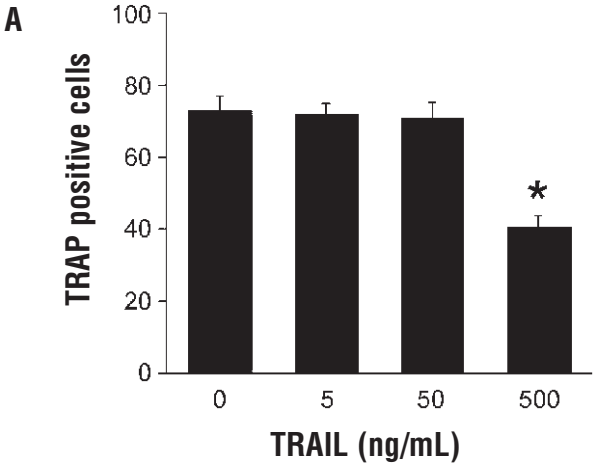
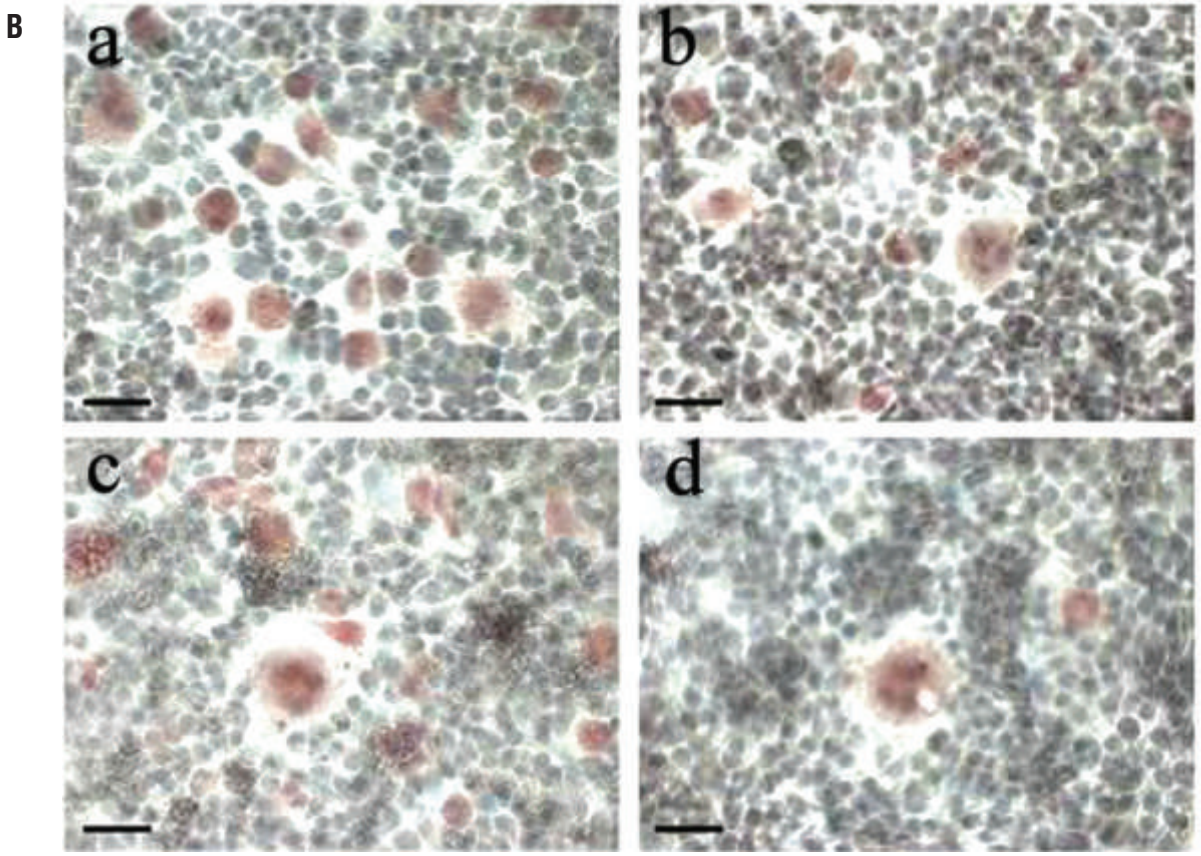


Figure 1. Effect of treatment with increasing concentrations of recombinant TRAIL on osteoclastic differentiation in a RAW264.7/osteoblast co-culture system. After 4 days of co-culture of mouse osteoblasts type CRL 12257 with mouse monocytoid RAW264.7 cells, the degree of differentiation was evaluate 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/mLTRAIL (d) are shown. Bars: 100 µm.



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

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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