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Soluble TRAIL does not impair the anti-osteoclastic activity of osteoprotegerin

Dear Editor:

It has been extensively documented that osteoprotegerin (OPG), a soluble member of the TNF receptor superfamily, inhibits osteoclastogenesis by binding to receptor activator of NF-kB ligand (RANKL) and preventing interaction with its cognate transmembrane receptor RANK [reviewed in 1]. However, OPG can also interact with, and neutralize, TNF-related apoptosis inducing ligand (TRAIL), whose extracellular domain shares a 30% homology with the extracellular domain of RANKL [reviewed in 2]. Although some inconsistencies on the differential binding affinity of OPG for RANKL *versus* TRAIL were present in initial studies [2, 3], it has been recently demonstrated that the affinity of native OPG for native TRAIL is comparable to that for RANKL (45 nM *versus* 23 nM, respectively) at 37°C, as determined by plasmon surface resonance analysis [4]. Consistently with this biochemical study, OPG has been shown to act in a paracrine and autocrine manner by binding TRAIL and promoting the survival of multiple myeloma [5], prostate cancer [6], ameloblastoma cells [7] and synovial fibroblasts [8]. Interestingly, previous data from different groups have shown that recombinant TRAIL modulates the differentiation of erythroid and myeloid precursors [9-10], while inhibits both human and mouse osteoclastogenesis when added to pre-osteoclast cultures induced to differentiate with recombinant macrophage colony-stimulating factor (M-CSF) +RANKL as well as to mature osteoclasts [11–14]. On the other hand, a couple of studies suggested that recombinant soluble TRAIL might promote osteoclastogenesis [4, 15], and the proposed molecular mechanism to explain such observation was a competition between TRAIL and RANKL for OPG binding. However, it should be noticed that Vitovsky *et al.* used much higher concentrations of TRAIL (500 ng/ml) than RANKL (30 ng/ml) or OPG (50 ng/ml) and more importantly used mouse bone marrow pre-osteoclasts [4]. In this respect, it has been clearly shown that mouse pre-osteoclasts only express TRAIL-R2 [2], while human peripheral blood-derived pre-osteoclasts express both death receptors TRAIL-R1 and TRAIL-R2 as well as TRAIL-R4 [11–14].

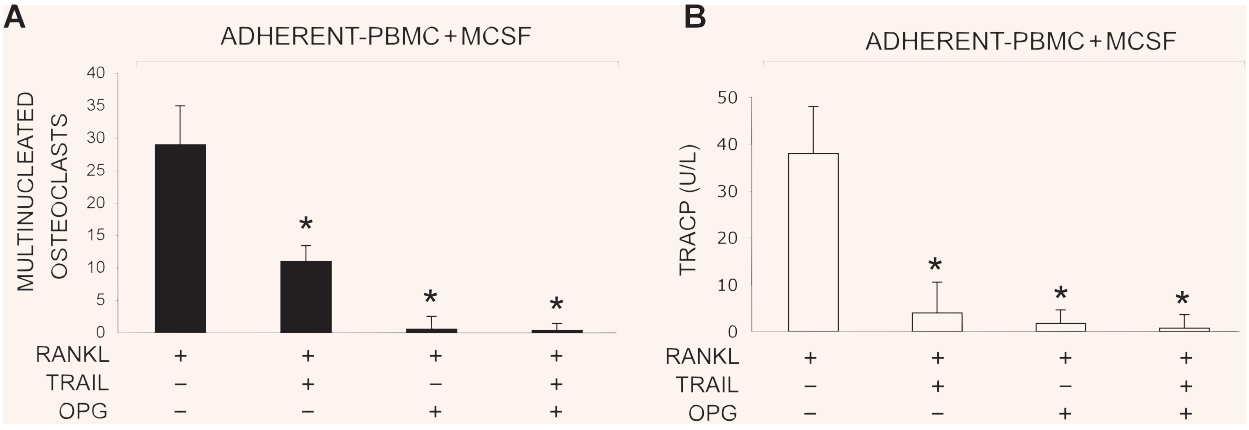

Fig. 1 Effect of combined treatment of RANKL, TRAIL and OPG on osteoclastic differentiation. Adherent PBMC were cultured with M-CSF alone for 6 days. Then cells were cultured for additional 14 days with the addition of RANKL in the absence or presence of TRAIL and/or OPG, as indicated. Cultures were analysed for osteoclastic differentiation by scoring the number of $TRAP^+$ multinucleated cells (**A**), and measuring the levels of TRAP 5b (**B**), a specific marker of resorption activity, in culture supernatants by ELISA. Data represent the means ± SD of three different experiments performed in duplicate.

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Therefore, to further elucidate the important issue of the interplay between RANKL, TRAIL and OPG in human osteoclastogenesis, we have cultured adherent PBMC with M-CSF+RANKL for 14 days in the absence or presence of recombinant OPG and recombinant TRAIL, prepared as previously described [16]. TRAIL and OPG were added alone or in combination. Importantly, all cytokines were used at the same concentration (50 ng/ml). As expected [11–14], the addition of TRAIL to M-CSF+RANKL significantly ($P < 0.05$) inhibited osteoclast formation (Fig. 1A and B). Of note, recombinant OPG completely abrogated ($P < 0.05$) osteoclast formation irrespectively of the presence of recombinant TRAIL in culture (Fig. 1A and B). The anti-osteoclastic activity of OPG could not be ascribed to a low affinity of OPG for TRAIL since OPG (50 ng/ml) efficiently inhibited the apoptosis induced by TRAIL (50 ng/ml) in HL-60 leukemic cells (data not shown).

Our current observations on one hand confirm that TRAIL has anti-osteoclastic activity and on the other hand indicate that it does not affect the potent anti-osteoclastic activity of OPG at least in the simplified model of osteoclastogenesis represented by human PBMC induced to differentiate by M-CSF+RANKL. Taken together with previous studies [4, 11–15], these data also suggest that the relative concentrations of TRAIL, RANKL and OPG in the local microenvironment are likely key determinant for the regulation of osteoclastogenesis.

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