Advances in Brief

Osteoprotegerin Is a Soluble Decoy Receptor for Tumor Necrosis Factor-related Apoptosis-inducing Ligand/Apo2 Ligand and Can Function as a Paracrine Survival Factor for Human Myeloma Cells[1]

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Abstract

Myeloma cells grow only in the bone marrow closely associated with bone, suggesting that this microenvironment provides critical signals for their growth and survival. Osteoprotegerin (OPG) is a member of the tumor necrosis factor (TNF) receptor family, which binds to the ligand for receptor activator of nuclear factor κB and inhibits bone resorption. However, it is unclear whether OPG can also bind to other TNF family members, such as TNF-related apoptosis-inducing ligand/Apo2 ligand (TRAIL/Apo2L), and, by inhibiting their activity, function as a survival factor for myeloma cells. In the present study MG63 osteoblast-like cells and primary bone marrow stromal cells were both shown to produce OPG, whereas human myeloma cells did not produce OPG but down-regulated release of OPG from MG63 cells. TRAIL/Apo2L induced apoptosis in myeloma cells, and this could be prevented with the addition of recombinant OPG. Medium conditioned by MG63 cells was also shown to inhibit TRAIL/Apo2L-induced apoptosis, an effect that was reversed by the addition of soluble receptor activator of nuclear factor κB ligand. Medium conditioned by cocultures of MG63 cells with myeloma cells had a reduced effect on TRAIL/Apo2L-induced apoptosis, reflecting the decreased concentrations of OPG in cocultures of myeloma cells with bone cells. These observations suggest that OPG may function as a paracrine survival factor in the bone marrow microenvironment in multiple myeloma.

Introduction

Multiple myeloma is a hematological malignancy characterized by the clonal expansion of malignant plasma cells in the local bone marrow microenvironment. Myeloma cells grow predominantly in this specialized environment, where the tumor cells interact with a range of cells including BMSCs,[3] hematopoietic cells, and the cells of bone (1, 2). These interactions are critical not only for the growth and survival of the tumor cells, but also for the development of the characteristic bone disease, which is mediated by an increase in the number and activity of osteoclasts (1, 3, 4). The cellular and molecular mechanisms responsible for this activity are unclear; however, recent studies have suggested that the RANKL/OPG system may play an important role in this process (5–8). RANKL is a member of the TNF family, and is expressed by stromal cells and osteoblasts in the bone marrow microenvironment (9, 10). RANKL can bind to its signaling receptor RANK, on the surface of osteoclast precursors and mature

osteoclasts, to induce osteoclast recruitment and differentiation, and bone resorption (10). A soluble decoy receptor, OPG, a member of the TNF receptor family, has also been identified that can bind to RANKL and prevent its interaction with RANK, thus inhibiting osteoclastic bone resorption (11). Myeloma cells may express RANKL or increase expression of RANKL in cells of the local bone marrow microenvironment (5–7). In addition, OPG expression appears to be decreased in the bone marrow of patients with multiple myeloma, and serum concentrations of OPG are lower when compared with healthy individuals (5, 6, 12). This raises the possibility that dysregulation of RANKL and OPG may be important in the pathogenesis of myeloma bone disease; however, it is also possible that OPG may have additional functions in multiple myeloma.

TRAIL/Apo2L is also a member of the TNF family and has been shown to induce apoptosis in a range of tumor cells *in vitro* (13, 14). TRAIL/Apo2L has been shown to induce apoptosis of human myeloma cells *in vitro* and to have an antimyeloma activity *in vivo* (15, 16). TRAIL/Apo2L-induced apoptosis appears to be restricted to tumor cells; however, the mechanisms behind this selectivity are unclear (16). TRAIL/Apo2L mediates its effect on apoptosis through two membrane-bound receptors, DR4 and DR5, which contain cytoplasmic death domains (17). There are two additional cell surface receptors for TRAIL/Apo2L, DcR1 and DcR2, which do not induce apoptosis and function as decoy receptors (17). Recent studies have shown that TRAIL/Apo2L can bind to OPG and can prevent the antiosteoclastogenic activity of OPG *in vitro*, thus raising the possibility that OPG may also act as a soluble decoy receptor for TRAIL/Apo2L (18). Thus, OPG may be able to compete for the binding of TRAIL/Apo2L to its cell surface receptors and inhibit apoptosis. Therefore, the aim of the present study was to determine whether OPG could act as a survival factor in multiple myeloma by inhibiting TRAIL/Apo2L-induced apoptosis of myeloma cells and to determine whether cells of the bone marrow microenvironment could provide a source of such a signal.

Materials and Methods

Chemicals. Recombinant Fc.OPG was a kind gift from Dr. Colin Dunstan (Amgen plc, Thousand Oaks, CA). TRAIL/Apo2L and RANKL were purchased from R&D Systems (Abingdon, United Kingdom). Unless stated otherwise, all of the other chemicals were from Sigma Chemical Co. (Poole, United Kingdom).

Isolation and Maintenance of Cells *in Vitro*. The human myeloma cell lines NCI H929 and RPMI 8226 were obtained from the European Collection of Animal Cell Cultures (Salisbury, United Kingdom) and JJN-3 cells were kindly provided by Prof. Ian Franklin (University of Glasgow, Glasgow, Scotland). Myeloma cells were cultured in RPMI 1640 containing 10% FCS, 1 mM glutamine, 1 mM sodium pyruvate, 1× MEM nonessential amino acids, 100 units/ml penicillin, 100 μg/ml streptomycin (Life Technologies, Inc., Paisley, United Kingdom), and 50 μM 2-mercaptoethanol. The human osteoblast-like cell line, MG63, was obtained from American Type Culture Collection (Manassas, VA), and routinely cultured in DMEM containing 10% FCS,

1 mM glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, and 1 mM sodium pyruvate (Life Technologies, Inc.).

Human primary BMSCs were isolated from trabecular bone marrow samples obtained from subjects undergoing routine total hip replacement surgery. The samples were vortexed three times in α-MEM containing glutamax, 10% FCS, 100 units/ml penicillin, and 100 μg/ml streptomycin (Life Technologies, Inc.). The cell suspension was passed through a 70 μm cell strainer to remove remaining trabecular bone, and the cells were plated at $\sim10^6$/ml in 75 cm^2 tissue culture flasks. Cells were left undisturbed for 3–5 days before washing in PBS to remove nonadherent cells. Cells were maintained until confluent. The cells were then subcultured and used in subsequent experiments.

Preparation of Conditioned Medium from MG63 Osteoblast-like Cells, Human BMSCs, and Cocultures of Myeloma Cells and MG63 Cells. MG63 cells were treated with 1 μg/ml mitomycin C for 3 h, washed three times in PBS, and plated at 10^5 cells/ml in 24-well plates and cultured overnight. Cells were then washed once with RPMI 1640 plus supplements, and $10^5 - 2 \times 10^6$ NCI H929, RPMI 8226, or JJN-3 myeloma cells were added (or vehicle control). The medium conditioned by these cells was collected, from MG63 cells alone, myeloma cells alone, and MG63 cells cocultured with myeloma cells, after 72 h. For fixation studies, MG63 cells were fixed with 1% paraformaldehyde for 8 min at 4°C and then washed three times with PBS before addition of myeloma cells. Human BMSCs were treated with mitomycin C as described previously, and then plated at 5×10^4 cells/ml in 24-well plates and cultured for 72 h. The medium was collected and stored at -80°C and OPG concentration measured as described below.

Treatment of Human Myeloma Cells with TRAIL/Apo2L. NCI H929 and RPMI 8226 myeloma cells were plated at a density of 1×10^6 cells/ml into 48-well plates (Costar), and treated with 5–100 ng/ml TRAIL/Apo2L or vehicle control (PBS + 0.1% BSA). In additional experiments, myeloma cells were treated with 50 ng/ml TRAIL/Apo2L in the presence or absence of OPG (10–1000 ng/ml) or vehicle control (PBS). Myeloma cells were also treated with 50 ng/ml TRAIL/Apo2L in the presence or absence of medium conditioned by MG63 cells (1–20%), or MG63 cells cultured with myeloma cells (20%) or vehicle control (RPMI 1640). In some experiments, cells were treated with a 5× molar excess of RANKL (R&D Systems) or vehicle control (PBS + 0.1% BSA) in addition to TRAIL/Apo2L and OPG or conditioned medium. In additional experiments, MG63 conditioned medium was incubated with 20 μg/ml goat antihuman OPG (R&D Systems) or goat IgG for 1 h at 37°C before being added to NCI H929 myeloma cells in the presence of 50 ng/ml TRAIL/Apo2L. In all of the experiments, myeloma cells were cultured for 24 h after treatment with TRAIL/Apo2L, and levels of apoptosis were measured as described below.

Measurement of OPG Expression. MG63, NCI H929, JJN-3, and RPMI 8226 cells were washed once in PBS and fixed in 4% formaldehyde for 20 min. Cells were then washed in PBS and stored in 100% ethanol at -20°C overnight to permeabilize the cells. Cells were then washed in wash buffer (PBS containing 1% FCS) and resuspended in 100 μl of wash buffer containing 20 μg/ml goat antihuman OPG antibody (R&D Systems) or goat IgG for 30 min on ice. Cells were then washed twice in wash buffer and resuspended in 100 μl of wash buffer containing 1 μg of antigoat antibody conjugated with FITC (Dako Ltd., Ely, United Kingdom) for 25 min on ice. Cells were then washed again in wash buffer, once in PBS, and finally resuspended in 300 μl PBS. The levels of FITC fluorescence were measured using a FACScalibur (Becton Dickinson, Oxford, United Kingdom).

Measurement of OPG Production. The concentration of OPG in medium conditioned by MG63 cells, human myeloma cell lines, human BMSCs, and cocultures of MG63 cells and myeloma cells was determined using an ELISA method. Briefly, 96-well plates were coated with a mouse monoclonal antihuman OPG antibody (2 μg/ml; R&D Systems). OPG was detected with a biotinylated antihuman OPG antibody (200 ng/ml; R&D Systems), which was detected with streptavidin-horseradish peroxidase (R&D Systems) and a 3′3′-5′5′-tetramethylbenzidine substrate. The reaction was stopped after ~15 min by addition of 2 M H_2SO_4. Absorbance was read at 450 nm on a Dynatech plate reader. An OPG standard curve was generated using either 31.25–2000 pg/ml recombinant human OPG (R&D Systems) or 10–1000 pg/ml recombinant Fc.OPG, and the concentration of OPG in the supernatant was determined by interpolation.

Identification of Apoptotic Cells. After treatment with TRAIL/Apo2L as described above, myeloma cells were fixed in 4% formaldehyde, cytospun onto glass slides, stained with 1 μg/ml 4′,6-diamidino-2-phenylindole, and visualized as described previously (19). Apoptotic cells were defined as those with characteristic changes in nuclear morphology, and the proportion of apoptotic cells was determined for each slide as described previously (19).

Statistical Analysis. All of the experiments were performed in quadruplicate and repeated on at least two separate occasions, unless stated otherwise, in both NCI H929 and RPMI 8226 myeloma cells. Results are expressed as mean $\pm$ SE. One-way ANOVA was used to determine responses to TRAIL/Apo2L, OPG, MG63-conditioned medium, and myeloma cell number. Comparison between groups was performed using a Mann-Whitney U test.

Results

Myeloma Cells Do Not Express or Release the Antiosteoclastogenic Factor OPG. Flow cytometric analysis demonstrated strong expression of OPG in MG63 osteoblast-like cells when compared with the isotype control (Fig. 1A). In contrast, the human myeloma cells NCI H929 (Fig. 1B), RPMI 8226, and JJN-3 were not found to express OPG. Production of OPG by osteoblast-like cells, stromal cells, and myeloma cells was examined by ELISA. MG63 cells produced high levels of OPG, which were detected in the culture medium, whereas human myeloma cell lines did not produce detectable concentrations of OPG (Fig. 1C). Primary human BMSCs were also found to release OPG (Fig. 1D); however, the concentrations released were lower than those produced by MG63 cells.

TRAIL/Apo2L-induced Apoptosis of Human Myeloma Cells Is Prevented by OPG or Medium Conditioned by MG63 Cells. Since OPG may be able to bind to TRAIL/Apo2L, we investigated whether OPG could prevent TRAIL/Apo2L-induced apoptosis of human myeloma cells. TRAIL/Apo2L was able to induce changes in nuclear morphology characteristic of apoptosis in myeloma cells (Fig. 2A). TRAIL/Apo2L induced a concentration-dependent increase in apoptosis in NCI H929 and RPMI 8226 myeloma cells ($P < 0.001$; Fig. 2B). JJN-3 myeloma cells were resistant to the apoptotic effects of TRAIL/Apo2L (data not shown). Treatment of NCI H929 or RPMI 8226 myeloma cells with 50 ng/ml TRAIL/Apo2L for 24 h was found
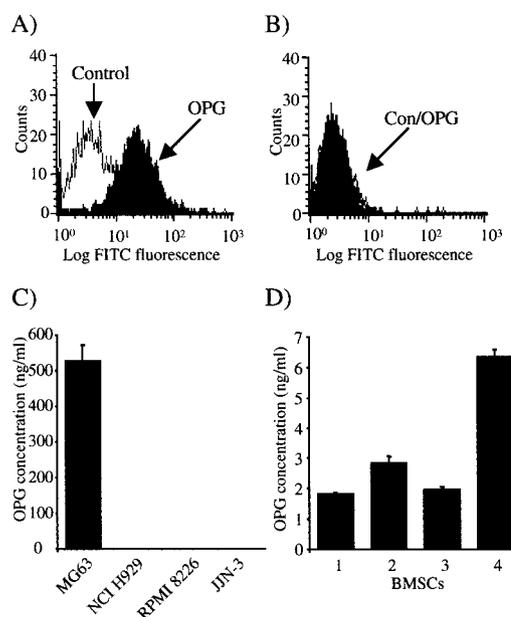

Fig. 1. Human osteoblast-like cells but not human myeloma cells produce OPG. Flow cytometric analysis demonstrated strong expression of OPG (*solid profiles*) in MG63 cells (A) but not in NCI H929 myeloma cells (B) when compared with isotype controls (*clear profiles*). ELISA analysis of supernatants from MG63 cells, primary BMSCs, and human myeloma cell lines demonstrated high levels of OPG produced from MG63 cells (C) and BMSCs derived from four separate donors (D), with no detectable OPG produced by myeloma cells (C). Data are the mean of a representative experiment; *bars,* $\pm$SE.
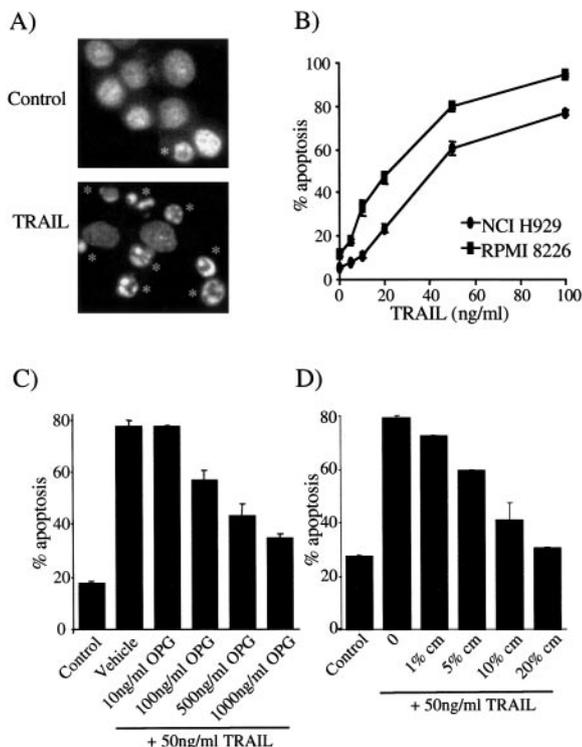
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Fig. 2. OPG protects against TRAIL/Apo2L-induced apoptosis in human myeloma cells. *A,* TRAIL/Apo2L-induced apoptosis in NCI H929 myeloma cells was determined by examination of characteristic changes in nuclear morphology (×40 original magnification; *, cells with nuclear morphology characteristic of apoptosis. *B,* NCI H929 (●) and RPMI 8226 (■) myeloma cells were treated with 5–100 ng/ml TRAIL/Apo2L or control (0.1% BSA in PBS) for 24 h and the proportion of apoptotic cells determined. NCI H929 myeloma cells were treated with 50 ng/ml TRAIL/Apo2L or control, and/or 10–1000 ng/ml OPG (*C*), or 1–20% MG63 conditioned medium (*cm*) or control (*D*) for 24 h, and the proportion of apoptotic cells determined. Data are the mean ($n = 4$) of a representative experiment; *bars,* ±SE.

to induce at least a 3-fold increase in apoptosis, and this concentration of TRAIL/Apo2L was used in all of the subsequent experiments. TRAIL/Apo2L-induced apoptosis in NCI H929 and RPMI 8226 myeloma cells was inhibited by the addition of recombinant OPG in a concentration dependent-manner ($P < 0.001$; Fig. 2*C*). Similarly, TRAIL/Apo2L-induced apoptosis was also blocked by the addition of medium conditioned by MG63 osteoblast-like cells in a concentration-dependent manner ($P < 0.05$; Fig. 2*D*). Medium from these cells was shown to contain OPG at a concentration of ∼600 ng/ml. Recombinant OPG had no effect on constitutive levels of apoptosis of myeloma cells (data not shown).

Conditioned Medium from the Coculture of Myeloma Cells and MG63 Cells Has a Reduced Concentration of OPG and Is Less Able to Inhibit TRAIL/Apo2L-induced Apoptosis. In the local bone marrow microenvironment, myeloma cells are found in close contact with stromal cells and osteoblasts; therefore, we have studied the influence of myeloma cells on OPG release by these cells and the consequent effects on TRAIL/Apo2L-induced apoptosis. The addition of human myeloma cells to cultures of MG63 osteoblast-like cells resulted in a significant decrease in OPG in the culture supernatant when compared with MG63 cells cultured alone ($P < 0.05$; Fig. 3*A*). The magnitude of this decrease was related to the number of myeloma cells added to the cultures of MG63 cells ($P < 0.001$; Fig. 3*B*). When MG63 cells were fixed before addition of myeloma cells OPG was undetectable in the culture medium, suggesting that the OPG was released from MG63 cells and not from myeloma cells (data not shown). A significant difference was found between the antiapoptotic effects of 20% conditioned medium from MG63 cells and 20%

conditioned medium from the coculture of MG63 cells and myeloma cells ($P < 0.05$; Fig. 3, *C* and *D*). Medium from cocultures of myeloma cells and MG63 cells had a significantly reduced ability to block the apoptotic effect of TRAIL/Apo2L in myeloma cells ($P < 0.05$), when compared with medium conditioned by MG63 cells alone, in both NCI H929 myeloma cells (Fig. 3*C*) and RPMI 8226 myeloma cells (Fig. 3*D*). Myeloma cell-conditioned medium had no significant effect on TRAIL/Apo2L-induced apoptosis.

The Antiapoptotic Effect of MG63 Culture Medium Can Be Inhibited by RANKL or a Neutralizing Antibody to OPG. We have demonstrated that there is a soluble factor produced by MG63 osteoblast-like cells that can protect myeloma cells against TRAIL/Apo2L-induced apoptosis. Soluble RANKL was used to determine whether this factor could be OPG. When NCI H929 or RPMI 8226 myeloma cells were treated with 50 ng/ml TRAIL/Apo2L in the presence of 500 ng/ml OPG, the addition of a 5× molar excess of RANKL completely prevented the antiapoptotic effect of OPG ($P < 0.05$; Fig. 4*A*). Similarly, when myeloma cells were treated with TRAIL/Apo2L in the presence of 20% conditioned medium from MG63 cells or MG63/myeloma cell cocultures, the addition of RANKL significantly increased the proportion of apoptotic cells ($P < 0.05$; Fig. 4*B*). RANKL had no significant effect on constitutive myeloma cell apoptosis or on TRAIL/Apo2L-induced apoptosis (Fig. 4, *A* and *B*). To confirm that the antiapoptotic factor produced by MG63 cells was OPG, medium conditioned by MG63 cells was
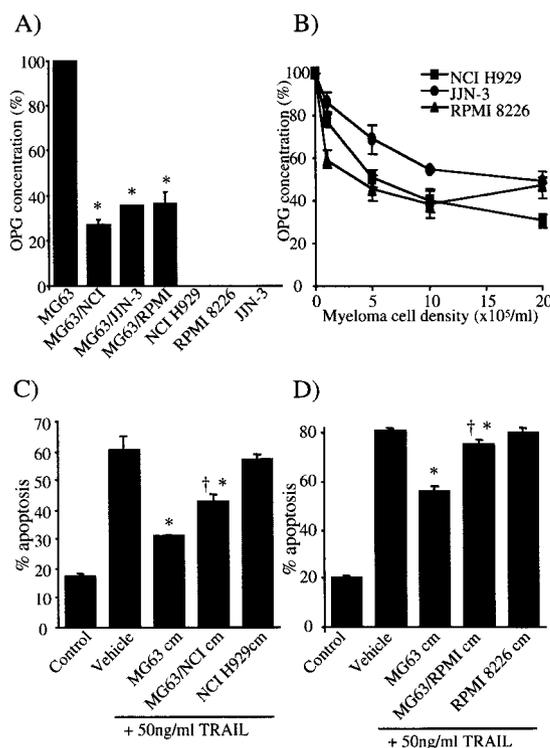

Fig. 3. Myeloma cells down-regulate OPG release from human osteoblast-like cells. *A,* the addition of human myeloma cells to cultures of MG63 cells down-regulated release of OPG from MG63 cells. *B,* the decrease in OPG released from MG63 cells was dependent on the density of NCI H929 (■), JJN-3 (●), and RPMI 8226 (▲) myeloma cells. OPG concentration in the cell supernatant after coculture was measured by ELISA. Results are expressed as a percentage of OPG concentration in control MG63 cells ($n = 4$), *, $P < 0.05$, as compared with control MG63 cells. NCI H929 (*C*) and RPMI 8226 (*D*) myeloma cells were treated with 50 ng/ml TRAIL/Apo2L or control in the presence or absence of 20% medium conditioned by MG63 cells (*MG63 cm*), myeloma cells (*NCI H929 cm* and *RPMI 8226 cm*), and MG63/myeloma cell cocultures (*MG63/NCI cm* or *MG63/RPMI cm*) for 24 h. Apoptotic cells were defined by characteristic changes in nuclear morphology. Data are the mean ($n = 4$) of a representative experiment; *bars,* ±SE. *, $P < 0.05$ as compared with TRAIL + vehicle, †, $P < 0.05$, as compared with TRAIL + MG63 conditioned medium.
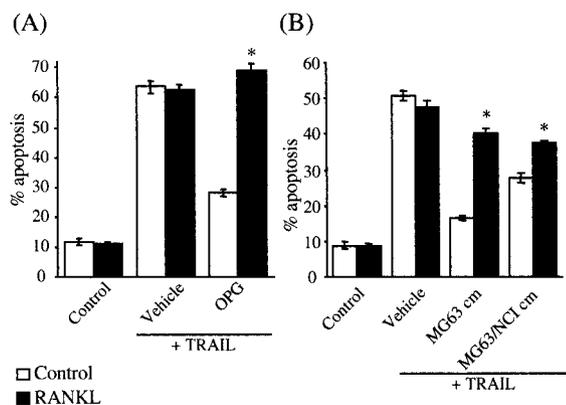
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Fig. 4. OPG is produced by human osteoblast-like cells and can prevent apoptosis of human myeloma cells. *A,* NCI H929 myeloma cells were treated with TRAIL/Apo2L in the presence or absence of 500 ng/ml OPG and/or a 5× molar excess of RANKL (■) or control (□). *B,* NCI H929 myeloma cells were treated with TRAIL/Apo2L in the presence or absence of 20% medium conditioned by MG63 cells or MG63/NCI H929 cells grown in coculture and/or a 5× molar excess of RANKL (■) or control (□). Apoptosis was determined by fluorescence microscopy and visualization of characteristic changes in nuclear morphology. Data are the mean ($n = 4$) of a representative experiment; *bars,* ±SE. ∗, $P < 0.05$, as compared with RANKL control.

incubated with a neutralizing antibody to OPG. The treatment of NCI H929 myeloma cells with 50 ng/ml TRAIL/Apo2L induced an increase in apoptosis from 14.0% ± 1.1% to 67.2% ± 4.2% ($P < 0.05$), which was reduced to 19.9% ± 1.0% ($P < 0.05$) in the presence of 20% medium conditioned by MG63 cells which was preincubated with 20 μg/ml goat IgG. When NCI H929 myeloma cells were treated with TRAIL/Apo2L in the presence of 20% conditioned medium from MG63 cells, which was preincubated with 20 μg/ml neutralizing anti-OPG, the level of apoptosis was increased significantly from 19.9% ± 1.0% to 56.6% ± 2.4% ($P < 0.05$).

Discussion

The interactions between human myeloma cells and other cells found in the local bone marrow environment, including stromal cells, osteoblasts, and osteoclasts, play an important role in the growth and survival of the tumor cells. However, the signals responsible for mediating these effects are unclear. The present study has demonstrated that TRAIL/Apo2L is able to induce apoptosis in NCI H929 and RPMI 8226 myeloma cells. This is consistent with recent reports that TRAIL/Apo2L can selectively induce apoptosis in human myeloma cells *in vitro* and can prevent tumor growth in xenografted nu/xid/bg mice (15, 16). Because TRAIL/Apo2L has been shown to block the antiosteoclastogenic activity of OPG *in vitro* (18), we examined whether OPG could prevent TRAIL/Apo2L-induced apoptosis of myeloma cells. Recombinant OPG was shown to dose-dependently inhibit TRAIL/Apo2L-induced apoptosis of NCI H929 and RPMI 8226 myeloma cells. This is consistent with the recent demonstration that the autocrine production of OPG can prevent TRAIL/Apo2L-induced apoptosis of prostate cancer cells *in vitro* (20). These data demonstrate that OPG is a soluble decoy receptor for TRAIL/Apo2L and support our suggestion that OPG may function as a survival factor for myeloma cells.

Flow cytometric analysis demonstrated expression of OPG by MG63 osteoblast-like cells. Furthermore, MG63 cells and primary human BMSCs released OPG into the culture supernatant. In contrast, myeloma cells did not produce OPG, suggesting that autocrine production of OPG is unlikely to account for any survival effect in multiple myeloma. Medium conditioned by MG63 osteoblast-like cells was shown to significantly inhibit TRAIL/Apo2L-induced apoptosis of myeloma cells in a concentration-dependent manner. In

contrast to OPG, medium conditioned by MG63 cells could completely rescue TRAIL/Apo2L-induced apoptosis, raising the possibility that osteoblast-like cells may release additional factors that can modulate TRAIL/Apo2L-induced apoptosis. Soluble RANKL, which is also able to bind OPG, was able to significantly block the effects of the medium conditioned by MG63 cells. The protective effect of medium conditioned by MG63 cells could also be blocked by a neutralizing antibody to OPG. These data demonstrate that OPG released from MG63 cells can protect against TRAIL/Apo2L-induced apoptosis.

Whereas human myeloma cells were not found to express OPG or to release OPG into the culture supernatant, these cells were able to regulate release of OPG from MG63 osteoblast-like cells. The presence of myeloma cells in culture with MG63 cells was shown to down-regulate production of OPG. The down-regulation of OPG was dependent on the number of myeloma cells present in the coculture, with an increase in myeloma cell number corresponding to a greater decrease in OPG concentration. MG63 cells were treated with mitomycin C before culture with myeloma cells, suggesting that the effect of the coculture was not because of inhibition of MG63 cell growth by the myeloma cells. To confirm that the OPG was released from MG63 cells in the coculture and that myeloma cells were not induced to release OPG, MG63 cells were fixed before coculture. No OPG was detected in the conditioned medium from cocultures of fixed MG63 cells and myeloma cells. These observations are consistent with a recent study that demonstrated that XG-6 and XG-1 myeloma cells decreased OPG mRNA expression and OPG production in primary bone cells (5). The addition of medium conditioned by myeloma cells cultured with MG63 cells had a reduced survival effect against TRAIL/Apo2L-induced apoptosis in myeloma cells when compared with medium conditioned by MG63 cells alone. The antiapoptotic effect of this medium was inhibited significantly by soluble RANKL, confirming that the soluble survival factor in this medium was OPG.

In vivo, OPG has been shown to play a key role in inhibiting osteoclast formation and bone resorption (11). The ability of myeloma cells to down-regulate OPG production by osteoblasts and stromal cells is consistent with the hypothesis that decreased OPG may play a role in the pathogenesis of myeloma bone disease (5, 6). This is supported by the demonstration that recombinant Fc.OPG can prevent myeloma bone disease in a murine model of multiple myeloma (21). The present study suggests that OPG may also act as a soluble decoy receptor for TRAIL/Apo2L, which raises the possibility that this molecule may have additional functions. It is intriguing that myeloma cells can down-regulate the production of a tumor-survival factor, and the consequences of this are unclear, but may play a role in the development of myeloma bone disease. However, the nature of the role of OPG as a survival factor, *in vivo*, will depend on the relative concentrations, timing, and location of expression of OPG, TRAIL/Apo2L, and their ligands in the local bone marrow microenvironment. Equally, the binding affinity of OPG to TRAIL/Apo2L compared with RANKL must also be considered, because TRAIL/Apo2L has been reported to have a low affinity for OPG at physiological temperatures (22). Taken together, our data demonstrate that OPG can prevent TRAIL/Apo2L-induced apoptosis of myeloma cells *in vitro*. Cells of the local bone marrow microenvironment, including osteoblast-like cells and stromal cells, but not myeloma cells, produce OPG, suggesting that OPG may have the capacity to function as a paracrine survival factor in this environment.

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