Neutralizing B-Cell – Activating Factor Antibody Improves Survival and Inhibits Osteoclastogenesis in a Severe Combined Immunodeficient Human Multiple Myeloma Model

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Abstract

Purpose: B-cell – activating factor (BAFF) is a tumor necrosis factor superfamily member critical for the maintenance and homeostasis of normal B-cell development. It has been implicated in conferring a survival advantage to B-cell malignancies, including multiple myeloma (MM).

Experimental Design: Here, we validate the role of BAFF in the *in vivo* pathogenesis of MM examining BAFF and its receptors in the context of patient MM cells and show activity of anti-BAFF antibody in a severe combined immunodeficient model of human MM.

Results: Gene microarrays and flow cytometry studies showed increased transcripts and the presence of all three receptors for BAFF in $CD138^+$ patient MM cells, as well as an increase in plasma BAFF levels in 51 MM patients. Functional studies show that recombinant BAFF protects MM cells against dexamethasone-induced apoptosis accompanied by an increase in survival proteins belonging to the BCL family. These *in vitro* studies led to the evaluation of a clinical grade – neutralizing antibody to BAFF in a severe combined immunodeficient human MM model. Anti-BAFF – treated animals showed decreased soluble human interleukin 6 receptor levels, a surrogate marker of viable tumor, suggesting direct anti-MM activity. This translated into a survival advantage of 16 days ($P < 0.05$), a decrease in tartrate-resistant acid phosphatase – positive osteoclasts, and a reduction in radiologically evident lytic lesions in anti-BAFF – treated animals.

Conclusions: Our data show a role for BAFF as a survival factor in MM. Importantly, the *in vivo* antitumor activity of neutralizing anti-BAFF antibody provide the preclinical rationale for its evaluation in the treatment of MM.

Multiple myeloma (MM) is a B-cell malignancy characterized by clonal accumulation of malignant plasma cells in the bone marrow. Several autocrine and/or paracrine soluble factors, including interleukin (IL)-6, insulin-like growth factor-I, vascular endothelial growth factor, several TNF superfamily proteins, Wnt, and Notch family members play an important role in MM pathogenesis by mediating proliferation, survival, migration, and development of cell adhesion – mediated drug resistance (1 – 4). In MM, these studies have underscored the importance of targeting not only the tumor cell but also their bone marrow microenvironment (5, 6). Although the availability of new agents such as thalidomide (7), bortezomib (8), and lenalidomide (9) have led to improved responses and survival (10), MM remains incurable. Targeting growth and survival factors, such as the TNF superfamily member B-cell – activating factor (BAFF), therefore represents a promising therapeutic strategy in MM.

BAFF, also known as B lymphocyte stimulator, is an important survival factor for immature, naïve, and activated B cells (11, 12). It is expressed by monocytes, macrophages, dendritic cells, and some T cells (11, 12) and binds to three receptors of the TNF receptor family: transmembrane activator, calcium modulator, and cyclophilin ligand interactor (TACI; ref. 13); B-cell maturation antigen (BCMA; ref. 14); and BAFF receptor (BAFF-R; refs. 15, 16). Importantly, BAFF shares significant homology with a proliferation-inducing ligand (APRIL), which is expressed at a low level by normal lymphoid and myeloid cells and at a high level by a variety of human cancers (17) and binds to BCMA and TACI, but not to BAFF-R (18). Elevated serum BAFF levels have been detected in patients with autoimmune disease such as rheumatoid arthritis, systemic lupus erythematosus, and Sjogren's syndrome (19), as well as in many B-cell malignancies including B – chronic lymphocytic leukemia (20), non – Hodgkin's lymphoma (21),

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and MM. MM cell lines and primary cells express BAFF and its receptors, and serum levels of BAFF are increased in patients with MM compared with healthy donors (22, 23). Additionally, BAFF modulates the proliferation and survival of cytokine-stimulated MM cells (24) and activates nuclear factor-κB, phosphatidynositol-3 kinase, and mitogen-activated protein kinase pathways, as well as induces an up-regulation of Mcl-1 and Bcl-2 protein expression in MM cells (22). Recent studies have also indicated that the bone marrow environment is the main source of BAFF for MM cells, especially monocytes, neutrophils, and osteoclasts (25), and established its role in the localization and survival of MM cells in the bone marrow milieu (26).

To further explore the pathophysiologic role of BAFF in MM *in vivo*, we examined BAFF and its receptors in the context of patient MM cells. Importantly, we evaluated the *in vivo* activity of anti-BAFF antibody in the severe combined immunodeficient model of human MM (SCID-hu), providing the preclinical framework for its clinical evaluation in MM patients.

Materials and Methods

Cells and reagents

Dexamethasone-sensitive (MM.1S) human MM cell line was kindly provided by Dr. Steven Rosen (Northwestern University, Chicago, IL) and cultured in RPMI 1640 (Life Technologies) containing 10% fetal bovine serum, 2 mmol/L L-glutamine (Life Technologies), 100 units/mL penicillin, and 100 μg/mL streptomycin (Life Technologies).

For the *in vivo* experiments, INA-6 human MM cell line was kindly provided by Dr. Renate Burger (ref. 27; University of Erlangen-Nuernberg, Erlangen, Germany) and cultured in RPMI 1640 (Life Technologies) containing 10% fetal bovine serum, 2 mmol/L L-glutamine (Life Technologies), 100 units/mL penicillin, and 100 μg/mL streptomycin (Life Technologies), in the presence of 2.5 ng/mL of human recombinant IL-6 (R&D Systems, Inc.).

Bone marrow aspirates were obtained from either normal donors or MM patients following informed consent and subjected to Ficoll-Hypaque density gradient centrifugation. Mononuclear cells were separated and suspended in RPMI 1640 (Life Technologies) containing 20% fetal bovine serum, 2 mmol/L L-glutamine (Life Technologies), 100 units/mL penicillin, and 100 μg/mL streptomycin (Life Technologies). Positive selection for patient's $CD138^+$ MM cells was done as previously described (26). Recombinant BAFF was obtained from R&D Systems and used at the specified concentrations (200 nmol/L). Neutralizing anti-BAFF antibody and the specific isotype control were used at a final concentration of 5.95 and 8.23 mg/mL, respectively, and stored in aliquots at -20°C until use, to avoid multiple freeze-thaw cycles. The anti-BAFF antibody recognizes human BAFF with low pmol/L affinity and neutralizes BAFF with an IC_{50} of ~100 pmol/L. It recognizes both soluble and membrane expressed BAFF (Eli Lilly and Co.).

Gene expression profile

Transcriptional profiling of $CD138^+$ plasma cells using the Affymetrix U133A gene chip was done on 90 MM patients and compared with 11 healthy controls. These healthy controls were de-identified bone marrow donors. Total RNA was isolated from $CD138^+$ plasma cells using TRIzol Reagent (Invitrogen). Affymetrix U133Plus 2.0 arrays were hybridized with biotinylated *in vitro* transcription products (10 μg/chip), as per manufacturer's instructions. Fluidic station 400 (Affymetrix) was used for washing and staining the arrays. The DNA chips were then analyzed using a Gene Array Scanner (Affymetrix). CEL files were obtained using Affymetrix Microarray Suite 5.0 software, and a DNA Chip Analyzer (DChip; ref. 28) was used to normalize all CEL files to a baseline array with overall median intensity, using the model-based expression (perfect match only) to compute the expression values. Analysis identified signals varying by ≥2-fold (lower bound) with a 90% confidence interval.

Flow cytometry

Indirect immunofluorescence flow cytometry was done using the Coulter Epics XL (Coulter Corp.) to assay for expression of BAFF-Rs on BMMCs from MM patients. Antibodies against BAFF-R, TACI, CD138 (R&D Systems), and BCMA (Alexis Biochemicals) were used. Cells were washed in PBS and incubated in PBS with 20% human AB serum at room temperature for 20 min to eliminate nonspecific Fc receptor binding. After washing with PBS, cells were incubated with primary murine monoclonal antibodies reactive with the above antigens for 30 min on ice. After several washes, the cells were developed with secondary goat anti-murine antibody conjugated with FITC or PE. Cells were then washed, fixed with 2% paraformaldehyde, and evaluated by flow cytometry.

ELISA assays

BAFF secretion was measured using Duoset ELISA Development Kits (R&D Systems) in plasma from 55 MM patients and 11 healthy controls, according to manufacturer's guidelines.

Cell viability and survival assays

Colorimetric assays were done to assay recombinant human BAFF (rh-BAFF) activity (R&D Systems) on cell survival in the presence of

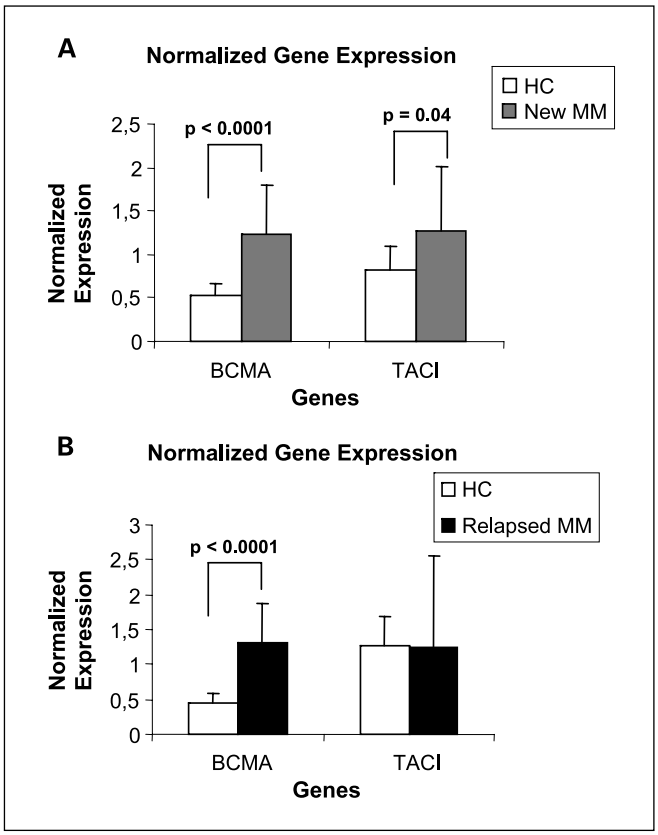

Fig. 1. MM patients show increased expression of TACI and BCMA. Gene expression profiling on $CD138^+$ plasma cells isolated from 90 MM patients (45 newly diagnosed and 45 relapsed) and 11 healthy controls (*HC*) was done using the Affymetrix U133A arrays. Expression of BCMA ($P < 0.0001$) and TACI ($P = 0.04$) were significantly increased in newly diagnosed MM when compared with healthy control (*A*). Similarly, BCMA ($P < 0.0001$) was increased in tumor cells from relapsed MM compared with healthy control, although no significant differences were noted with TACI expression (*B*).

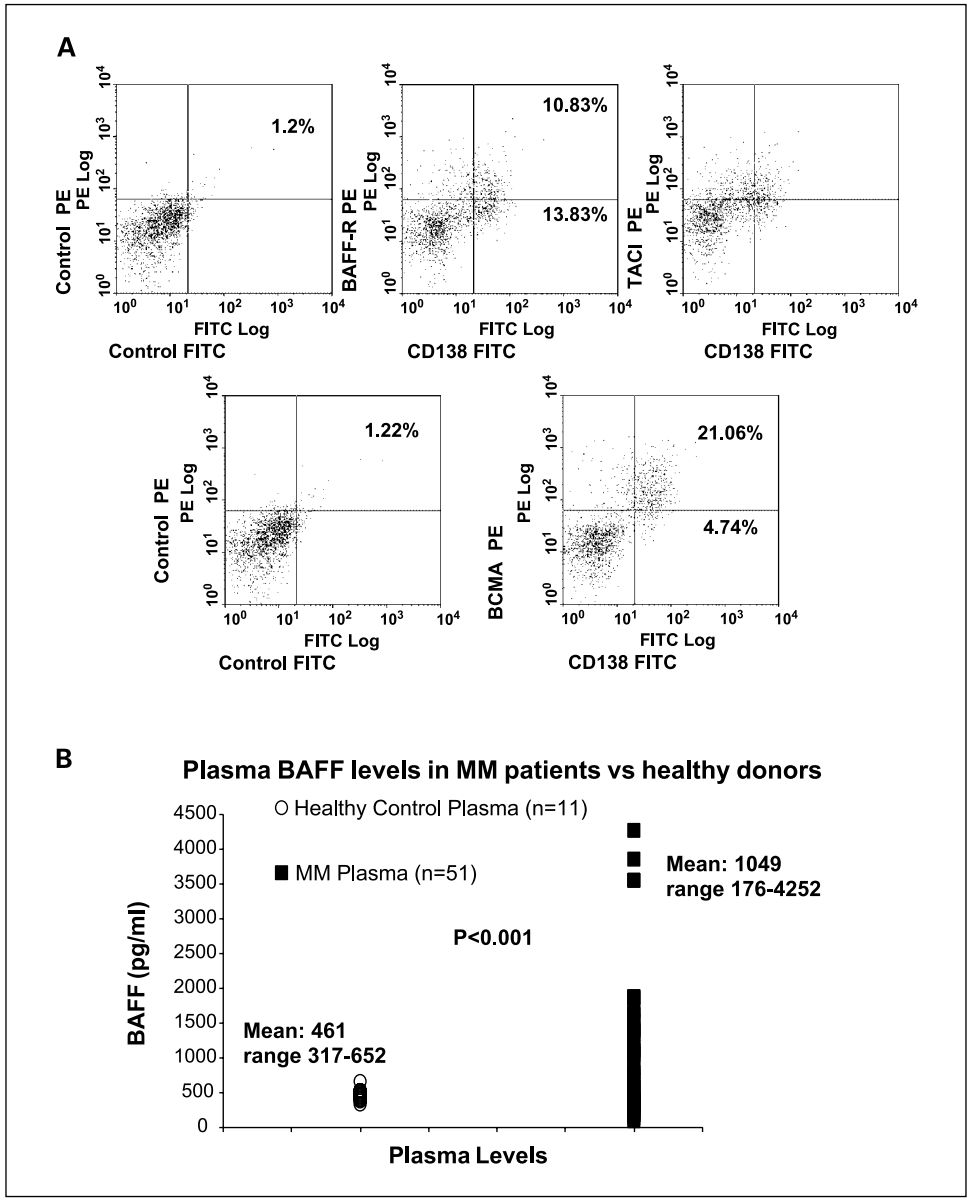

Fig. 2. BAFF-R, TACI, and BCMA are expressed on MM cells, and plasma BAFF levels are elevated in MM patients. Flow cytometry done on MM patient cells showed the presence of TACI, BCMA, and BAFF-R on $CD138^+$ cells (*A*). ELISA confirmed increased BAFF levels in plasma from 51 MM patients (mean 1,049 pg/mL; range 176-4,252 pg/mL) compared with 11 normal donors (mean 461 pg/mL; range: 317-652 pg/mL; $P < 0.001$; *B*). PE, phycoerythrin.

dexamethasone. Cells from 72-h cultures were pulsed with 10 μL of 5 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrasodium bromide (MTT; Chemicon International, Inc.) to each well; the 96-well plates were incubated at 37°C for 4 h, followed by 100 μL isopropanol containing 0.04 N HCl. Absorbance was read at a wavelength of 570 nm (with correction using readings at 630 nm) on a spectrophotometer (Molecular Devices Corp.). Human recombinant IL-6 (R&D Systems) was used as a positive control.

Western blotting

MM cells were cultured with rh-BAFF (200 nmol/L/mL) for the specified times, harvested, washed, and lysed using lysis buffer [50 mmol/L HEPES (pH 7.4), 150 mmol/L NaCl, 1% Triton-X 100, 30 mmol/L sodium PPi, 5 mmol/L EDTA, 2 mmol/L Na_3VO_4, 5 mmol/L NaF, 1 mmol/L phenylmethyl sulfonyl fluoride, 5 μg/mL leupeptin, and 5 μg/mL aprotinin]. For detection of apoptosis-related proteins, cell lysates were subjected to SDS-PAGE, transferred to nitrocellulose membrane, and immunoblotted with antibodies against antiapoptotic proteins including BCl_2 and XIAP (Santa Cruz Biotechnology). To characterize growth signaling, immunoblotting was also done with anti–phosphorylated mitogen-activated protein kinase antibody (Santa Cruz Biotechnologies), and anti–phosphorylated signal transducers and activators of transcription 3, anti–phosphorylated mitogen activated protein/extracellular signal-regulated kinase kinase, phosphorylated Akt, and anti–phosphorylated p65 nuclear factor-κB (Cell Signaling Technology). Antigen-antibody complexes were detected by using enhanced chemiluminescence (Amersham). Blots were stripped and reprobed with antitubulin antibody to ensure equal protein loading.

In vivo experiments

SCID-hu model of human MM. CB-17 SCID-mice were obtained from Taconic, maintained, and monitored in our Animal Research Facility. All animal studies were conducted according to protocols approved by the Institutional Animal Care and Use Committee. Human fetal long bone grafts were s.c. implanted into SCID mice (SCID-hu), as previously described (29, 30). Four weeks after this implantation, 2.5×10^6 INA-6 MM cells were injected directly into the human bone marrow cavity in the SCID-hu mice in a final volume of 100 μL of RPMI 1640. An increase in the levels of soluble human IL-6 receptor (shuIL-6R) released

by INA-6 cells was used as an indicator of MM cell growth and burden of disease in SCID-hu mice.

Mouse sera were serially examined for shuIL-6R levels by an ELISA (R&D Systems). Mice developed measurable serum shuIL-6R level ~4 weeks after INA-6 cell injection, and were then treated i.p. with anti-BAFF (10 mg/kg) or isotype control (10 mg/kg), respectively, for 4 weeks. Following treatment, blood samples were collected and analyzed.

Survival was evaluated by Kaplan-Meier from the first day of MM cell injections until death. Animals were sacrificed when paralysis or major compromise to their quality of life occurred.

Histologic analysis

Analysis was done on fetal bone chips retrieved from SCID-hu mice at the end of treatment. Samples were fixed in 3.7% formaldehyde/PBS decalcified in 14% EDTA. They were embedded in paraffin and cut in 5-μm sections along the longitudinal axis of the bone. Sections were then stained for the presence of tartrate-resistant acid phosphatase (TRAP), a marker of osteoclasts. Images of all sections were digitally captured with a color camera (AxioCam HR) linked to a Nikon microscope. The total number of $TRAP^+$ cells was counted and expressed per field of the bone trabecular surfaces.

Radiographic analysis

High-resolution whole-body radiographs of ketamine-anesthetized mice were obtained with the animals immobilized in a prone position on Kodak X-Omat AR radiographic film (Kodak), using a Cabinet X-ray System-Faxitron series (Faxitron Corp.). Radiographs were taken at the end of treatment; films were developed using a Konica film processor and analyzed for the number of osteolytic lesions in the different groups.

Statistical analysis

Statistical significances of differences were determined by using Student's t test. The minimal level of significance was $P < 0.05$.

Results

MM patients show increased expression of TACI and BCMA. Gene expression profiling done on $CD138^+$ plasma cells from 90 MM patients (45 newly diagnosed and 45 relapsed) versus 11 healthy controls shows increased expression of BCMA in MM patients either at diagnosis or relapse (Fig. 1A and B), whereas TACI is only increased relative to healthy controls in newly diagnosed MM patients. No significant differences in expression were noted when a comparison of relapsed and newly diagnosed patients was made (data not shown). Moreover, no significant differences between APRIL expression were noted in MM patients versus healthy controls. BAFF and BAFF-R could not be detected as the Affymetrix U133A gene chip lacks probes to these transcripts.

BAFF-R, TACI, and BCMA are differentially expressed on MM cells. To confirm gene expression data and study BAFF-R expression, we did flow cytometry on MM cells. MM cell lines and patient samples were stained with TACI, BCMA, or BAFF-R antibodies followed by analysis using the RXP cytomics software on an Epics flow cytometer (Coulter Immunology). Flow cytometry done on MM patient cells (three of five) showed all three receptors are expressed on $CD138^+$ cells. Representative data from one patient is shown in Fig. 2A. This expression is heterogeneous and was not noted in two of five patient samples.

Increased plasma BAFF levels are seen in MM patients. ELISA assays confirmed increased plasma BAFF levels in 51 MM plasma (mean 1,049 pg/mL; range 176-4,252 pg/mL) compared with 11 normal donors (mean 461 pg/mL; range 317-652 pg/mL; $P < 0.001$; Fig. 2B).

Recombinant BAFF confers a survival advantage to MM cells. We then studied the functional sequelae of BAFF in the context of MM. As for IL-6, rh-BAFF protected MM cells against dexamethasone-induced cytotoxicity (Fig. 3A). Importantly, rh-BAFF induced time-dependent up-regulation of antiapoptotic proteins Bcl2 and XIAP, (Fig. 3B) as well as Janus-activated kinase/signal transducers and activators of transcription, AKT, and mitogen-activated protein kinase pathways (data not shown), which mediate growth, survival, and dexamethasone resistance.

Neutralizing anti-BAFF antibody shows* in vivo *anti-MM activity in a SCID-hu model of human MM. Having identified a role for BAFF in MM patients, we next evaluated its *in vivo* activity on MM cells in the context of the human bone marrow microenvironment. We used the SCID-hu model of human MM, in which IL-6–dependent INA-6 MM cells are directly injected into previously implanted human fetal bone chips within SCID mice (31, 32). Although INA-6 alone does not produce BAFF, binding to human BMSCs results in an 8-fold increased BAFF level (data not shown). A cohort of 10 SCID-hu mice bearing INA-6 xenografts were treated i.p. weekly with anti-BAFF (10 mg/kg, $n = 5$) or isotype control (10 mg/kg, $n = 5$), respectively, for 4 weeks. In this model, the anti-MM activity was analyzed by measuring the serum levels of shuIL-6R secreted by INA-6 cells, as in previous studies (31, 32), as this is the surrogate marker of tumor burden. As shown in Fig. 4A, treatment with anti-BAFF antibody significantly decreased shuIL-6R levels in BAFF-treated compared with control mice ($P = 0.042$). Interestingly, there was also a significant ($P = 0.023$) prolonged survival in treated animals versus control. The median overall survival was 59 days in the control group and 75 days in the treated group (Fig. 4B). These data indicate a

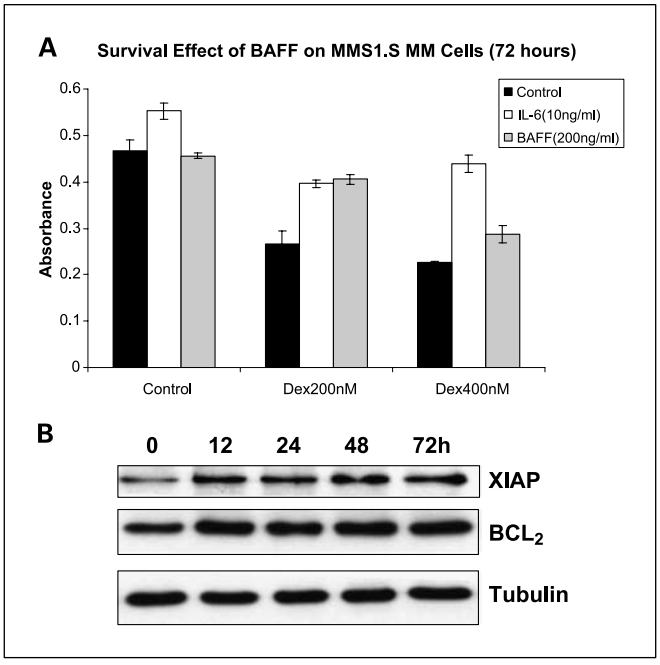

Fig. 3. Recombinant BAFF conferred a survival advantage to MM cells associated with an increase in antiapoptotic proteins, XIAP and BCL2. rh-BAFF conferred a survival advantage to MM 1.S MM cells in 72 h MTT assays and protected them against dexamethasone-induced cytotoxicity. IL-6 was used as the positive control (*A*). This was associated with a time-dependent increase in antiapoptotic proteins Bcl2 and XIAP as shown by Western blots (*B*).

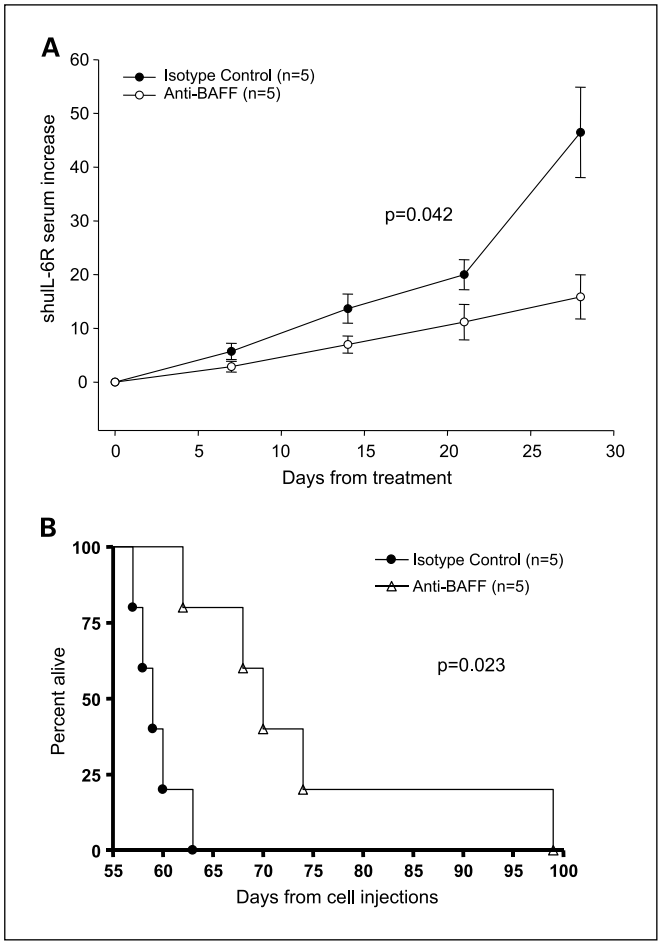

Fig. 4. Neutralizing anti-BAFF antibody has *in vivo* anti-MM activity. SCID-hu mice engrafted with INA-6 cells in a human bone chip were monitored by serial serum measurements of shuIL-6R as an indicator of MM cell growth. Mice were treated weekly with anti-BAFF (10 mg/kg, $n = 5$) or isotype control (10 mg/kg, $n = 5$) for 4 wk and the shuIL-6R levels were measured weekly. At the end of treatment, significant ($P = 0.042$) reduction was observed in shuIL-6R level in mice treated with anti-BAFF antibody versus control (*A*). This translated into a significant prolongation of 16 d in the median survival in the anti-BAFF – treated animals versus the control group ($P = 0.023$; *B*).

direct anti-MM activity of anti-BAFF neutralizing antibody in the context of human bone marrow microenvironment.

Neutralizing BAFF antibody inhibits osteoclastogenesis in a SCID-hu model of MM. To further understand the improved outcome of the antibody-treated mice, we evaluated *in vivo* effects of anti-BAFF on bone using both TRAP staining and radiographic analysis of human bone implants because osteoclasts have been previously shown to be a source of BAFF (25). As shown in Fig. 5A, a decrease in osteoclast recruitment and activation was observed in bone sections from treated mice compared with control mice, as detected by TRAP staining. The mean number of multinucleated TRAP-positive cells were 13.7 versus 6.5 per field ($P = 0.016$) in human bone rudiments from control mice versus treated mice, respectively (Fig. 5B). These data have been confirmed by radiographic analysis. Figure 5C shows bone radiographs from two representative chips in anti-BAFF–treated versus control SCID-hu mice. Multiple lytic lesions were detected radiologically in control mice, whereas radiologically lytic lesions were markedly decreased in mice treated with anti-BAFF.

Discussion

In the past few years, considerable progress has been made in the development of novel drugs to treat MM. The identification of key molecular mechanisms that play a role in the growth and survival of MM cells, both alone and in the context of the bone marrow microenvironment, have been critical to our understanding of mechanisms of resistance to conventional chemotherapeutic agents. A complex network of cytokines, chemokines, and cell surface molecules modulate the interaction between MM cells and the bone marrow microenvironment thereby regulating MM cell proliferation, growth,

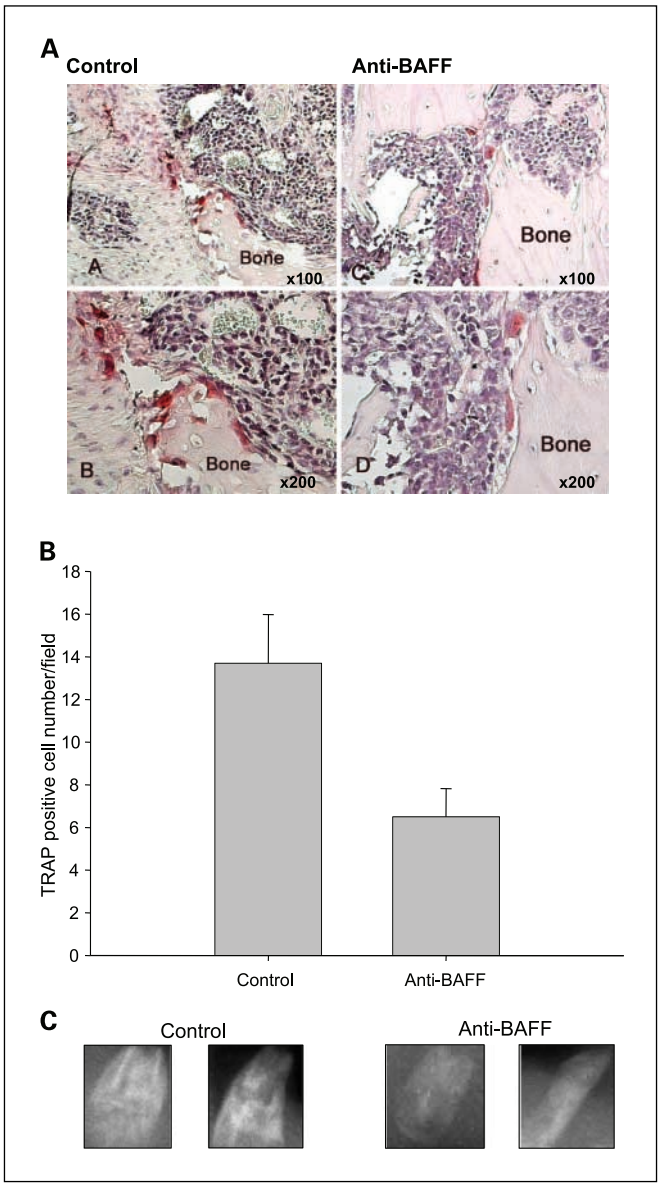

Fig. 5. Neutralizing anti-BAFF antibody inhibits osteoclastogenesis *in vivo*. To evaluate *in vivo* effects of anti-BAFF on bone formation and resorption, SCID-hu mice were sacrificed after four weekly treatments with anti-BAFF (10 mg/kg) and isotype control (10 mg/kg) antibodies. Human bone radiographs were obtained at the end of treatment. Histologic analysis of human fetal bone sections revealed decreased TRAP-positive cells (i.e., osteoclasts) in anti-BAFF – treated specimens versus controls (*A* and *B*). Fewer lytic lesions were detected radiographically in anti-BAFF antibody treated versus control mice (*C*).

survival, antiapoptosis, and development of drug resistance (33–35). This increased understanding of pathogenesis has led to development of novel agents that target not only MM cells, but also the bone marrow milieu, thereby overcoming resistance to conventional therapies (36).

In this report, we have characterized the role of BAFF on patient MM cells *in vitro*, and evaluated the *in vivo* activity of anti-BAFF antibody, against human MM cells. Previous studies have shown that BAFF is a TNF superfamily member critical for the maintenance and homeostasis of normal B-cell development (12, 37–39). BAFF-deficient mice, as well as mice exposed to neutralizing agents to BAFF, have severe impairment in B-cell development. Conversely, BAFF transgenic mice develop autoimmunity resembling systemic lupus and Sjogren's syndrome. It is hypothesized that increased levels of serum BAFF promote the survival and proliferation of autoreactive B cells in the periphery resulting in autoimmunity. More importantly, with regards to MM, BAFF is noted to promote the generation of rapidly dividing immunoglobulin-secreting cells or plasmablasts from activated memory B cells by enhancing their survival (38).

BAFF exerts its effects via three identified receptors: TACI, BCMA, and BAFF-R (38–40). Nuclear factor-κB–dependent signaling is activated after receptor ligand interaction of BAFF and APRIL, which regulates B-cell survival (41, 42). Additionally, death of resting B cells is regulated by nuclear localization of serine/threonine protein kinase Cδ: BAFF stimulation of resting B cells prevents this nuclear accumulation of protein kinase Cδ, thereby affecting B-cell survival (43). BAFF has also been shown to play a significant role in cell cycle homeostasis by enhancing cyclin D2 synthesis via nuclear factor-κB activation in murine B cells and inducing cell cycle entry and G_1 progression (44). In B-cell non–Hodgkin's lymphoma cells, increased expression of BAFF and APRIL has been observed (21, 24) and neutralization of endogenous BAFF and APRIL by soluble TACI and BCMA decoy receptors both down-regulates antiapoptotic proteins Bcl-2 and Bcl-xl and decreases survival of non–Hodgkin's lymphoma B cells (21, 24). TACI and BAFF-R mRNA is also detected in B–chronic lymphocytic leukemia cells (20). BAFF and APRIL is expressed at the membrane of B–chronic lymphocytic leukemia cells, and soluble BAFF is detected in sera of chronic lymphocytic leukemia patients (20). Prior studies have also suggested that BAFF may be a growth factor for human MM cells and that serum levels of BAFF are increased in these patients (22, 23); however, its exact role in pathogenesis is undefined. Using Affymetrix microarrays, we here show that BCMA and TACI are expressed on both newly diagnosed and relapsed MM patients. In addition, by flow cytometric analysis and ELISA, we provide evidence that $CD138^+$ human MM cells express all receptors for BAFF and confirm increased plasma BAFF levels in MM patients. Furthermore, we have confirmed that rh-BAFF plays a role in MM cell survival and protects against dexamethasone-induced cytotoxicity. Taken together, our data extends previous reports indicating that BAFF and its receptors are involved in the growth and survival of MM cells and provides strong preclinical rationale for targeting BAFF.

We therefore next evaluated the efficacy of a clinical grade–neutralizing antibody to BAFF in SCID-hu mice, implanted with fetal bone chips, and engrafted with the MM cell line: INA-6. This model recapitulates the human microenvironment and the huIL-6/BMSCs dependence of INA-6 cells, similar to that of primary MM cells in the context of human disease. It is a predictable and reproducible *in vivo* system for the preclinical evaluation of new anti-MM agents active in the context of human bone marrow milieu. We observed in this system a significant tumor inhibition *in vivo* as shown by decreased shuIL-6R levels, a surrogate marker of viable tumor, suggesting direct anti-MM activity. Importantly, this translated into a significant survival advantage in mice treated with the BAFF-neutralizing antibody versus control. Our *in vivo* data therefore shows anti-MM activity of anti-BAFF–neutralizing antibody for the first time, and confirms its ability to overcome the protective effects of the bone marrow milieu (BMSCs, IL-6, vascular endothelial growth factor, insulin-like growth factor-I) that normally promote MM cell growth, survival, and drug resistance (6).

Lytic bone lesions and their associated complications are a major cause of morbidity in human MM (45). A predominant feature of this complication is increased osteoclast number and activity, leading to increased bone resorption along with decreased osteoblast activity (46). Previous studies have shown enhanced growth and survival of MM cells triggered by interaction between MM cells and osteoclasts, associated with increased MM growth and bone destruction (24, 47). Studies have also suggested that osteoclasts may be the predominant cell source of BAFF in the MM bone microenvironment (25) and that osteoclast-derived BAFF is an important MM survival factor (48). Interestingly, after anti-BAFF treatment in our SCID-hu model of human MM, we observed a significant reduction in osteoclast number and lytic lesions as shown by TRAP assays and radiology.

In summary, the present report shows the biological role of BAFF on MM cell survival both directly and via effects on the bone marrow microenvironment by affecting bone resorption. This is the first study showing *in vivo* evidence of anti-MM activity of neutralizing anti-BAFF antibody. One of the obvious limitations is targeting BAFF alone with the neutralizing antibody, without any significant effects on APRIL. The role of APRIL, however, remains less clear in the context of MM and is the subject of ongoing studies. Our data thus far provide the preclinical rationale for testing anti-BAFF antibody in clinical studies of relapsed/refractory MM with an emphasis on MM bone disease.

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