Featured Article

Transforming Growth Factor β Receptor I Kinase Inhibitor Down-Regulates Cytokine Secretion and Multiple Myeloma Cell Growth in the Bone Marrow Microenvironment

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ABSTRACT

Purpose: **Transforming growth factors (TGFs) have pleiotropic biological effects on tumor cells and their environment. In multiple myeloma (MM), we have reported that bone marrow stromal cells (BMSCs) from MM patients produce more TGF-β1 than BMSCs from healthy donors, which in turn induces interleukin (IL)-6 secretion. We show here that the TGF-β receptor I kinase inhibitor SD-208 significantly decreases secretion of both IL-6 and vascular endothelial growth factor (VEGF) from BMSCs, as well as tumor cell growth triggered by MM cell adhesion to BMSCs.**

Experimental Design: **Cytokine production and MM cell proliferation triggered by TGF-β1 or adhesion to BMSCs were examined in the presence or absence of SD-208. Effects of SD-208 on TGF-β1–induced signaling pathways triggering IL-6 and VEGF transcription in BMSCs were also delineated.**

Results: **SD-208 significantly inhibits not only transcription but also secretion of both IL-6 and VEGF from BMSCs triggered by either TGF-β1 or adhesion of MM cells to BMSCs. Moreover, SD-208 decreased tumor cell growth triggered by MM cell adhesion to BMSCs. SD-208 works, at least in part, by blocking TGF-β1–triggered nuclear accumulation of Smad2/3 and hypoxia-inducible factor 1α, as well as related production of IL-6 and VEGF, respectively.**

Conclusions: **These studies indicate that SD-208 inhibits production of cytokines mediating MM cell growth, survival, drug resistance, and migration in the BM milieu, thereby providing the preclinical rationale for clinical evaluation of SD-208 to improve patient outcome in MM.**

INTRODUCTION

The bone marrow (BM) microenvironment confers growth, survival, and drug resistance to multiple myeloma (MM) cells via both direct cell contact and soluble factors. Adhesion of MM cells to extracellular matrix proteins, such as fibronectin, inhibits apoptosis through up-regulation of both $p27^{kip1}$ and c-Fas–associated death domain-like interleukin (IL)-1-converting enzyme-like inhibitory protein-long (c-FLIP) (1, 2). Adhesion of MM cells to BM stromal cells (BMSCs) triggers secretion of cytokines, such as IL-6 and vascular endothelial growth factor (VEGF), which play a critical role in pathogenesis of MM. IL-6 induces growth, survival, and drug resistance in MM cells (3–6), and VEGF triggers growth and migration of MM cells (7), stimulates BM angiogenesis (8), and augments IL-6 production in BMSCs (9). In addition, IL-6 and VEGF contribute to immune deficits characteristic of MM (10). Conversely, abrogating adhesion of MM cells to BMSCs and associated IL-6 and VEGF secretion diminishes the growth advantage and drug resistance of MM cells in the BM milieu. For example, we have demonstrated previously that thalidomide and immunomodulatory derivatives, as well as the proteasome inhibitor bortezomib, which can overcome clinical drug resistance, act not only directly on MM cells but also in the BM microenvironment to inhibit the up-regulation of IL-6 and VEGF secretion triggered by the binding of MM cells to BMSCs (11–15).

Transforming growth factor (TGF)-β1 is a multifunctional cytokine that plays a major role in hematopoiesis, tumor development, and immune regulation (16–18). In MM, we have shown that adhesion of MM cells to patient BMSCs triggers more TGF-β1 secretion than adhesion of MM cells to BMSCs from healthy donors. TGF-β1, in turn, induces secretion of IL-6 and other cytokines (19). Moreover, TGF-β1 diminishes function of dendritic cells in MM by inhibiting up-regulation of CD80 expression (20). Inhibition of TGF-β1 may therefore overcome the growth advantages conferred by MM cell adhesion to BMSCs and the resultant cytokine production in the BM milieu as well as enhance host anti-MM immunity. SD-208 belongs to a family of potent, selective, and novel 2,4-disubstituted pteridine-derived TGF-β receptor type I (TβRI) kinase

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inhibitors (21). In this study, we demonstrate that SD-208 downregulates both cytokine secretion and proliferation of tumor cells induced by MM cell binding to BMSCs.

MATERIALS AND METHODS

Reagents. TGF-β receptor I kinase-specific inhibitor SD-208 (SCIOS Inc., Sunnyvale, CA; ref. 21) was dissolved in dimethyl sulfoxide (100 mmol/L) and stored at −20°C until use.

Cell Lines and Cell Culture. Human MM cell lines MM.1S (kindly provided by Dr. Steven Rosen, Northwestern University, Chicago, IL), RPMI8226, and U266 (American Type Culture Collection, Manassas, VA) were maintained as described previously (10).

Primary MM Cells, BMSCs from MM Patients, and Normal Lymphocytes. BM specimens were acquired from patients with MM after obtaining informed consent, and primary MM cells (>90% CD138+) were purified using the RosetteSep negative selection system (StemCell Technologies, Vancouver, British Columbia, Canada), as described previously (22). T cells (>95% CD3+) and B cells (>90% CD19+) were similarly purified from healthy donor peripheral blood. BM mononuclear cells separated by Ficoll-Paque (Pharmacia, Piscataway, NJ) were used to establish long-term BMSC cultures, as described previously (23).

Cytokine Array. BMSCs were cultured in serum-free Iscove's modified Dulbecco's medium (IMDM) alone or with TGF-β1 (2 ng/mL) for 48 hours, and then cytokine levels in culture supernatant were detected using the Human Cytokine Antibody Array V (RayBiotech, Norcross, GA), per the manufacturer's protocol. Briefly, supernatants were incubated for 2 hours with membranes arrayed with antibodies against 79 cytokines. After washing twice, membranes were incubated for 2 hours with biotin-conjugated primary anti-cytokine antibodies and then washed twice. Membranes were then incubated with horseradish peroxidase-conjugated streptavidin for 1 hour, washed twice, and placed in the detection buffer for a few minutes. Signals were detected by exposure to X-ray film (Kodak X-OMAT AR; Eastman Kodak, Rochester, NY). To quantitate relative cytokine level, densitometric analysis was performed using Scion Image analysis software (Scion Corp., Frederick, MD).

Measurement of Cytokine Levels. IL-6, VEGF, and TGF-β1 levels in culture supernatants and BM sera from MM patients were measured by enzyme-linked immunosorbent assay (ELISA; R&D Systems, Minneapolis, MN), per the manufacturer's protocol.

Growth Inhibition Assay. The inhibitory effect of SD-208 on growth of MM cells, BMSCs from MM patients, and normal lymphocytes was assessed by standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Chemicon International, Temecula, CA) assay, as described previously (23).

Cell Proliferation Assay. MM cell proliferation was measured by [^{3}H]thymidine (Perkin-Elmer Life Sciences, Boston, MA) incorporation for the last 8 hours of 48-hour cultures, as described previously (23).

Complementary DNA Synthesis and Quantitative Real-Time Polymerase Chain Reaction. Total RNA was extracted from BMSCs stimulated with TGF-β1 (10 ng/mL) for 12 and 24 hours in the presence or absence of SD-208 (300 nmol/L) by TRIzoL reagent (Invitrogen, Carlsbad, CA). RNA was used in a first-strand DNA (cDNA) synthesis using Invitrogen SuperScript III first-strand synthesis system for reverse transcription-polymerase chain reaction (PCR), according to the manufacturer's protocol. Real-time PCR was carried out in a 7500 real-time PCR system (Applied Biosystems, Foster City, CA) for real-time detection, per the manufacturer's protocol. This study was performed with the SYBR Green I nucleic acid gel stain (Cambrex Corp., East Rutherford, NJ). Oligonucleotide pri-mers for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were 5′-CCCTCCAAAATCAAGTGGGG-3′ (forward) and 5′-CGCCACAGTTCCCCGGAGGG-3′ (reverse). Oligonucleotide primers for IL-6 and VEGF were purchased from R&D Systems. The amplification for IL-6, VEGF, and GAPDH was carried out as follows: a 5-minute hot start at 95°C, followed by 50 cycles of denaturation at 94°C for 30 seconds, annealing at 58°C for 30 seconds, and extension at 72°C for 34 seconds. Quantitation of the amount of target in unknown samples is accomplished by measuring the threshold cycle (Ct). Quantitative data were analyzed using Sequence Detection System software (Applied Biosystems). This relative quantification is given by the ratio between the Ct value of the target gene and the Ct value of the GAPDH gene in each sample.

Immunoblotting. Total cell lysates and nuclear extracts were prepared from BMSCs incubated with TGF-β1 (10 ng/mL) in serum-free medium, as described previously (23). In some experiments, cells were preincubated with SD-208 (300 nmol/L) for 1 hour. Specific proteins were detected by Western blotting, as described previously (23).

Evaluation of Hypoxia-Inducible Factor 1α DNA Binding Activity. The DNA binding activity of hypoxia-inducible factor (HIF)-1α in BMSCs was quantified by *trans*-AM Transcription Factor ELISA Kit (Active Motif, Carlsbad, CA), according to the manufacturer's instructions.

Immunofluorescence Staining. BMSCs grown on plastic slides were stimulated with TGF-β1 (2 ng/mL) for 1 hour, with or without SD-208 (500 nmol/L) pretreatment for 30 minutes. Cells were fixed with 4% paraformaldehyde in PBS for 5 minutes, penetrated with 0.1% saponin plus 1% normal goat serum in PBS for 15 minutes, incubated with 5% normal goat serum plus 0.05% saponin in PBS for 15 minutes to block nonspecific binding, and then stained with anti-Smad2/3 antibody (BD Transduction Laboratories, San Diego, CA) at 4°C overnight. Slides were incubated with a biotinylated antimouse antibody (Vector Laboratories, Burlingame, CA) for 30 minutes, followed by fluorescein isothiocyanate-conjugated avidin (Vector Laboratories) for 30 minutes, and then analyzed by fluorescence microscopy.

Statistical Analysis. Statistical significance of differences observed in cytokine secretion from BMSCs and proliferation of MM cells was determined using the Mann-Whitney *U* test. The minimal level of significance was $P < 0.05$.

RESULTS

SD-208 Inhibits TGF-β1–Triggered IL-6 and VEGF Secretion in BMSCs. We first examined the effects of TGF-β1 on cytokine secretion from BMSCs because TGF-β1 did not alter growth of BMSCs in 48-hour MTT assays (data not shown), and MM cells did not respond to TGF-β1 due to low expression of TGF-β receptor type II (TβRII; data not shown). Densitometric analyses of the cytokine array revealed increased secretion of IL-6 (1.4-fold), VEGF (2.5-fold), and monocyte chemoattractant protein (MCP)-1 (1.7-fold) in supernatants of BMSCs cultured in the presence of TGF-β1 compared with control cultures. Among these cytokines, we next examined the dose-response effect of TGF-β1 on IL-6 and VEGF secretion, which mediates MM cell proliferation, drug resistance, and migration (7, 24, 25). As shown in Fig. 1*B*, increased IL-6 (3.6-fold) and VEGF (2.0-fold) secretion ($P < 0.02$) was triggered by TGF-β1 (5 ng/mL) at concentrations similar to the mean ± SD TGF-β1 concentration in BM sera from 25 MM patients (6.9 ± 3.6 ng/mL). We next examined the effects of TGF-β receptor 1 kinase inhibitor SD-208 on secretion of IL-6 and VEGF from BMSCs triggered by TGF-β1. BMSCs from MM patients were cultured in the medium with TGF-β1 (10 ng/mL), in the presence or absence of SD-208 (300 nmol/L); IL-6 and VEGF levels in culture supernatants were then measured using ELISA. SD-208 abrogated TGF-β1–induced increased secretion of IL-6 and VEGF from BMSCs in a dose-dependent manner (Fig. 1*B*). We also investigated the inhibitory effects of SD-208 on transcription of IL-6 and VEGF in BMSCs. BMSCs from MM patients were cultured for 12 or 24 hours with TGF-β1 (10 ng/mL) in the presence or absence of SD-208 (300 nmol/L), and then mRNA was extracted. Real-time PCR revealed that TGF-β1 triggered increased IL-6 and VEGF transcription; importantly, SD-208 abrogated transcription of both IL-6 and VEGF (Fig. 1*C*). These results indicate that SD-208 inhibits TGF-β1–induced transcription, as well as secretion of IL-6 and VEGF, cytokines known to mediate MM cell growth, survival, drug resistance, and migration in the BM milieu.

SD-208 Inhibits TGF-β1–Triggered Nuclear Translocation of Smad2 and HIF-1α in BMSCs. We next investigated the molecular mechanisms whereby SD-208 down-regulates TGF-β1–induced cytokine secretion in BMSCs. BMSCs from MM patients were stimulated with TGF-β1 (10 ng/mL) in the presence or absence of SD-208 (300 nmol/L), and then total cell

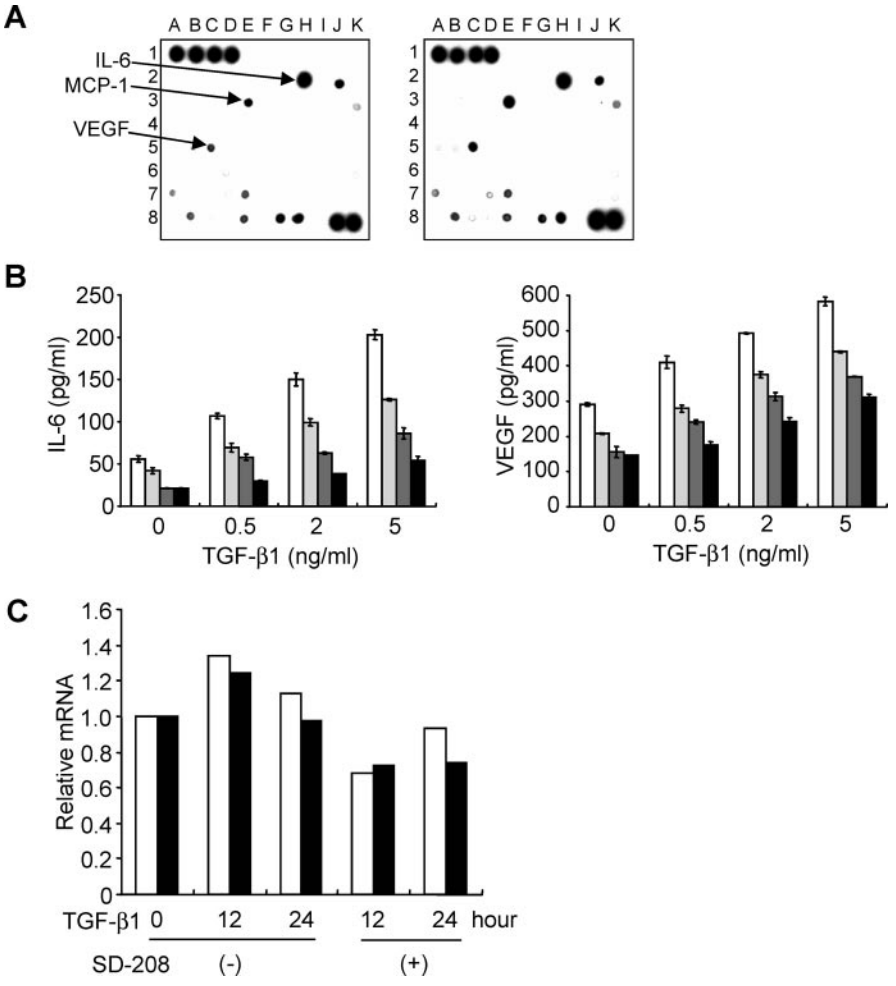

Fig. 1 SD-208 blocks TGF-β1–triggered cytokine secretion in BMSCs. *A*, BMSCs were cultured in serum-free IMDM alone (*left panel*) or with 2 ng/mL TGF-β1 (*right panel*) for 48 hours, and then cytokines in culture supernatant were detected using cytokine array. See Table 1 also. *B*, BMSCs from MM patients were cultured for 48 hours in serum-free IMDM with TGF-β1 (0, 0.5, 2, and 5 ng/mL) in the absence (□) or presence of 30 (▨), 100 (▩), and 300 nmol/L SD-208 (■). IL-6 (*left panel*) and VEGF (*right panel*) levels in culture supernatants were measured using ELISA. Results are representative of three experiments, and values indicate the mean ± SD of duplicate wells. *C*, BMSCs from patients with MM were stimulated with TGF-β1 (10 ng/mL) for 12 and 24 hours in the presence or absence of SD-208 (300 nmol/L). Levels of IL-6 (□) and VEGF (■) mRNA relative to GAPDH were detected using quantitative real-time PCR.

Table 1 TGF-β1 induces cytokine secretion in BMSCs

	A	B	C	D	E	F	G	H	I	J	K
1	Pos	Pos	Pos	Pos	Neg	Neg	ENA-78	G-CSF	GM-CSF	GRO	GRO-α
2	I-309	IL-1α	IL-1β	IL-2	IL-3	IL-4	IL-5	IL-6	IL-7	IL-8	IL-10
3	IL-12	IL-13	IL-15	IFN-γ	MCP-1	MCP-2	MCP-3	M-CSF	MDC	MIG	MIP-1β
4	MIP-1δ	RANTES	SCF	SDF-1	TARC	TGF-β1	TNF-α	TNF-β	EGF	IGF-1	Ang
5	OSM	Tpo	VEGF	PDGF-B	Leptin	BDNF	BLC	Ckb8-1	ETN	ETN-2	ETN-3
6	FGF-4	FGF-6	FGF-7	FGF-9	Flt-3 L	FKN	GCP-2	GDNF	HGF	IGFBP-1	IGFBP-2
7	IGFBP-3	IGFBP-4	IL-16	IP-10	LIF	LIGHT	MCP-4	MIF	MIP-3α	NAP-2	NT-3
8	NT-4	OPG	PARC	PIGF	TGF-β2	TGF-β3	TIMP-1	TIMP-2	Neg	Pos	Pos

lysates or nuclear extracts were immunoblotted with antibodies directed against the TGF-β1 signaling cascade. TGF-β1 triggered phosphorylation of Smad2 in MM patient-derived BMSCs, which was abrogated by SD-208 (Fig. 2*A*). We next examined whether SD-208 blocks the nuclear translocation of Smad2 in response to TGF-β1 using immunofluorescence staining. Smad2/3 is predominantly intracytoplasmic in control media (Fig. 2*B*, *a*) and cultures with SD-208 only (Fig. 2*B*, *b*). Importantly, TGF-β1 (2 ng/mL) triggered nuclear translocation of Smad2/3 in BMSCs (Fig. 2*B*, *c*), which was blocked by SD-208 (Fig. 2*B*, *d*). In another system, TGF-β1 induces IL-6 transcription via activation of Smad2/3 (26), suggesting a similar role in BMSCs.

TGF-β1 also increased nuclear HIF-1α expression in BMSCs, which up-regulates VEGF (27); conversely, SD-208 blocks this HIF-1α induction (Fig. 2*C*). To confirm that SD-208 abrogates TGF-β1–triggered nuclear translocation of HIF-1α in BMSCs, we performed DNA binding assays on these nuclear extracts. As shown in Fig. 2*D*, TGF-β1 increased HIF-1α DNA binding activity, whereas SD-208 di-

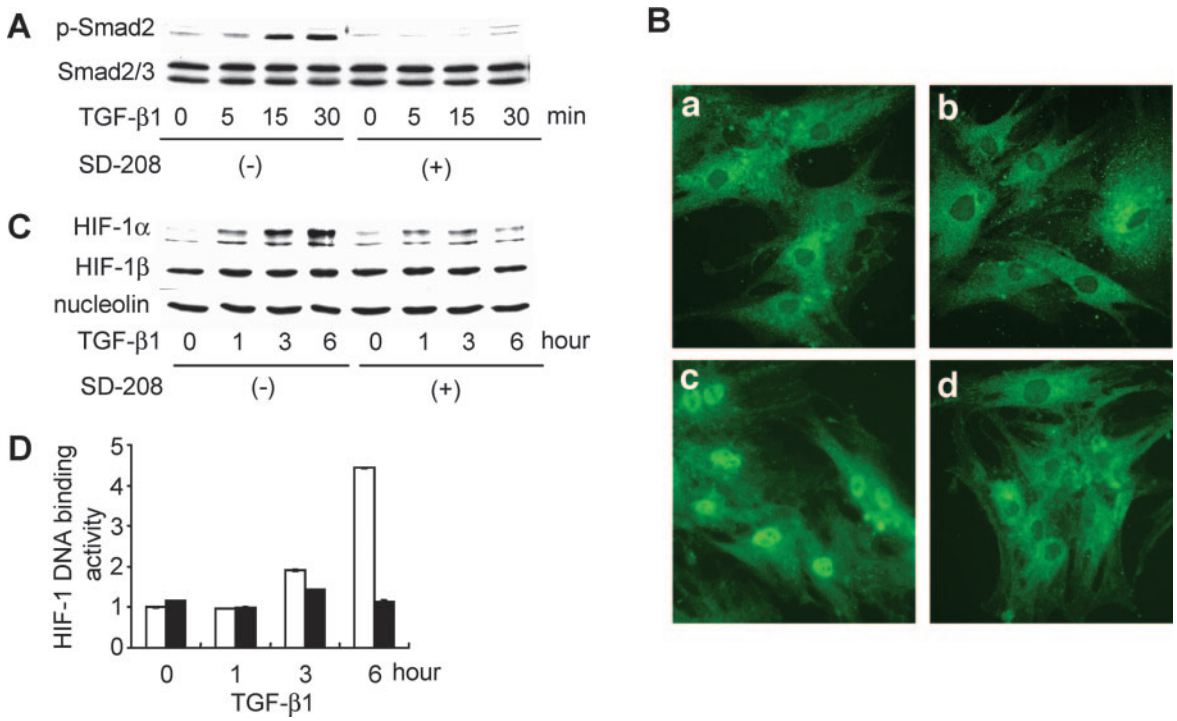

Fig. 2 SD-208 blocks TGF-β1–triggered Smad2/3 and HIF-1α nuclear translocation in BMSCs. *A*. BMSCs, with or without pretreatment with SD-208 (300 nmol/L, 1 hour), were incubated with TGF-β1 (10 ng/mL) for 5, 15, or 30 minutes, and then total cell lysates were immunoblotted with specific antibodies directed against phosphorylated Smad2. Blots were stripped and reprobed with antibodies against nonphosphorylated Smad2/3 to assure equal protein loading. *B*. BMSCs were incubated for 1 hour with dimethyl sulfoxide (*a*), SD-208 alone (500 nmol/L; *b*), TGF-β1 (2 ng/mL; *c*), or TGF-β1 (2 ng/mL) after SD-208 pretreatment (500 nmol/L, 30 minutes; *d*). Localization of Smad2/3 was detected by immunofluorescence staining using anti-Smad2/3 antibody. *C*. Nuclear extracts prepared from MM patient-derived BMSCs after incubation with TGF-β1 (10 ng/mL) for 1, 3, and 6 hours, with or without pretreatment with SD-208 (300 nmol/L, 1 hour), were electrophoresed and immunoblotted with anti–HIF-1α and HIF-1β antibody. Blots were stripped and reprobed with antinucleolin antibodies to assure equivalent loading. *D*. The DNA binding activity of HIF-1 in nuclear extracts of BMSCs induced by TGF-β1 (10 ng/mL) in the presence (300 nmol/L; ■) or absence (□) of SD-208 was quantified using transcription factor ELISA kits. Nuclear extracts were incubated in 96-well plates coated with immobilized oligonucleotide containing a hypoxia response element (5′-TACGTGCT-3′); HIF-1 binding to the target oligonucleotide was detected by incubation with primary antibody specific for HIF-1α, followed by visualization with horseradish peroxidase-conjugated anti-IgG and developing solution.

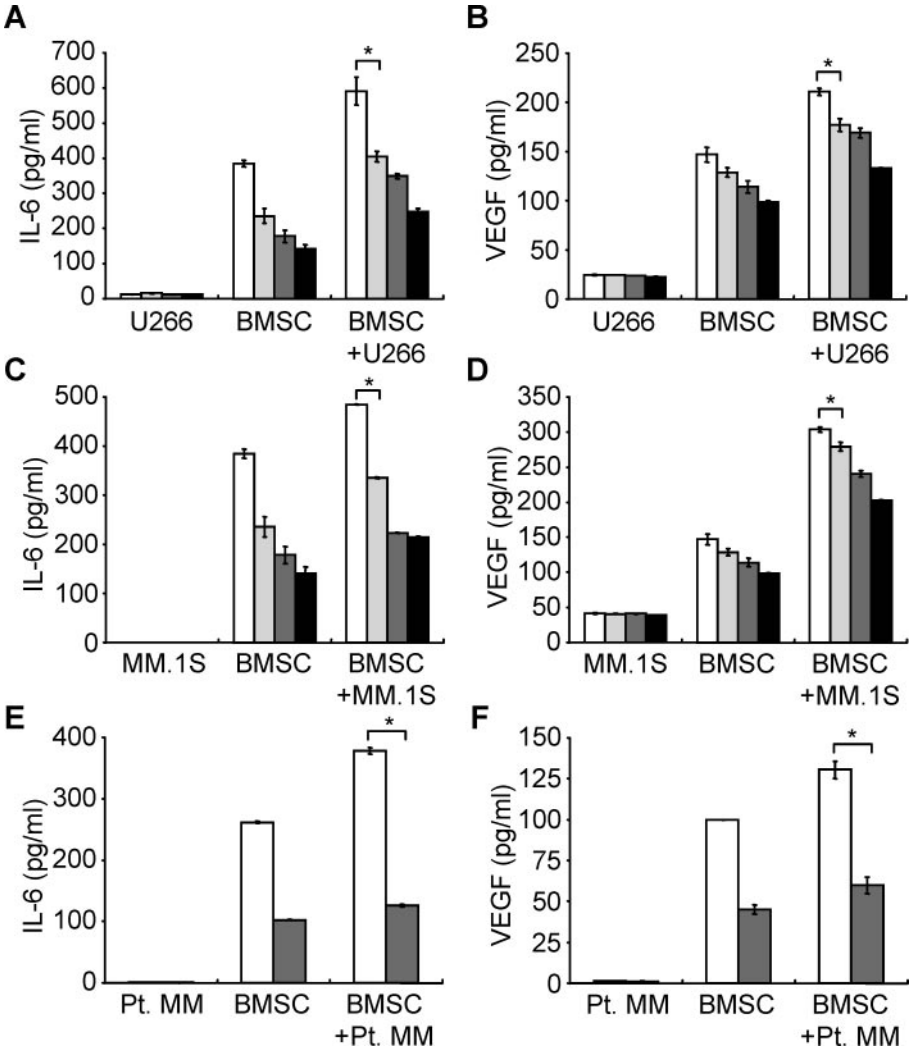

Fig. 3 SD-208 abrogates IL-6 and VEGF secretion from BMSCs cultured with MM cells. U266 (*A* and *B*), MM.1S cells (*C* and *D*), and patient MM cells (*E* and *F*) were cultured alone or together with BMSCs in the absence (□) or presence of 30 (▨), 100 (▩), and 300 nmol/L SD-208 (■). IL-6 (*A*, C, and *E*) and VEGF (*B*, D, and *F*) levels in 48-hour culture supernatants were measured by ELISA. Statistically significant (*, $P = 0.02$) decreased cytokine levels were observed in supernatants from cultures with SD-208.

minished this response. These results indicate that SD-208 neutralizes TGF-β1–induced secretion of VEGF in BMSCs by blocking nuclear translocation of HIF-1α, a downstream target of TGF-β receptor (28).

SD-208 Inhibits IL-6 and VEGF Secretion in BM Microenvironment. Because our previous data have shown that adhesion of MM cells to BMSCs triggers TGF-β1 and IL-6 secretion (19), we next investigated the effect of SD-208 on secretion of IL-6 and VEGF induced by MM cell adhesion to BMSCs. MM cell lines or patient MM cells were cocultured for 48 hours with BMSCs from MM patients, in the presence or absence of SD-208; IL-6 and VEGF concentrations in supernatants were then examined by ELISA. As shown in Fig. 3, coculture of MM cells with BMSCs augmented IL-6 and VEGF secretion; conversely, SD-208 significantly abrogated this response in a dose-dependent fashion. These results indicate that SD-208 blocks IL-6 and VEGF secretion in the BM microenvironment.

SD-208 Inhibits MM Cell Proliferation in the BM Microenvironment. To define the functional sequelae of SD-208, we next examined whether this agent inhibits MM cell proliferation in the BM microenvironment. MM cells, BMSCs from MM patients, and lymphocytes from healthy donors were cultured in the presence of SD-208 (3–300 nmol/L) for 48 hours, and cell viability was assessed by MTT assay. SD-208 did not directly inhibit the growth of MM cells, BMSCs, or B and T cells (Fig. 4*A*). Importantly, SD-208 significantly inhibited proliferation of U266 and MM.1S cells induced by coculture with BMSCs in a dose-dependent manner (Fig. 4*B*). These results indicate that SD-208 overcomes the growth advantage conferred by IL-6 and VEGF in the BM milieu.

DISCUSSION

TGF-β1 regulates the proliferation and differentiation of hematopoietic progenitor cells (16). In MM, we have shown that adhesion of MM cells to BMSCs triggers secretion of TGF-β1 (19), associated with immunosuppression observed in MM patients, as well as MM cell growth or resistance to apoptosis. Therefore, inhibition of TGF-β1 has the potential to both enhance tumor cell cytotoxicity and abrogate immunosuppression. In the present study, we show that the TGF-β receptor I kinase

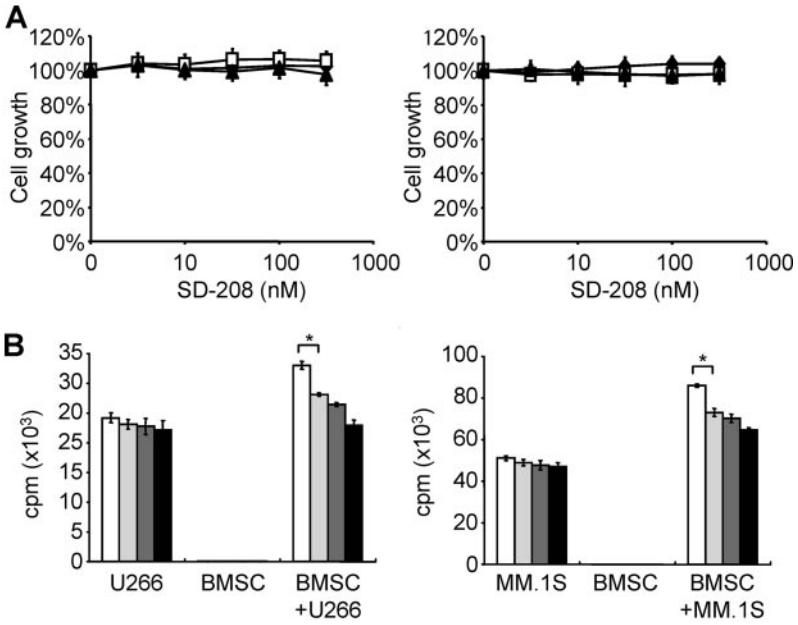

Fig. 4 SD-208 abrogates MM cell proliferation induced by culture with BMSCs. *A*, Cells were cultured in serum-free AIM-V medium (MM cells and lymphocytes) or in serum-free IMDM (BMSCs), alone or in the presence of SD-208 (3, 10, 30, 100, and 300 nmol/L) for 48 hours; cell viability relative to control culture was assessed by MTT assay. *Left panel*, RPMI8226 (♦), U266 (□), and MM.1S cells (▲). *Right panel*, BMSCs from MM patients (♦), as well as B cells (□) and T cells (▲) from healthy donors. Results shown are the mean ± SD of four experiments in triplicate wells. *B*, U266 (*left panel*) and MM.1S cells (*right panel*) were cultured in 96-well plates with or without BMSCs, in the absence (□) or presence of 30 (▨), 100 (▩), and 300 nmol/L (■) SD-208. DNA synthesis was measured for the last 8 hours of 48-hour cultures. Statistically significant (*, $P = 0.02$) decreased proliferation of MM cells was observed in the presence of SD-208.

inhibitor SD-208 significantly inhibits transcription and secretion of both IL-6 and VEGF from BMSCs triggered by either TGF-β1 or adhesion of MM cells to BMSCs. Importantly, SD-208 decreased tumor cell growth triggered by MM cell adhesion to BMSCs. These studies provide the preclinical rationale for clinical evaluation of SD-208 to improve patient outcome in MM.

We first demonstrate that 5 ng/mL TGF-β1, equivalent to mean concentrations of TGF-β1 in BM sera from MM patients, induced secretion of cytokines including IL-6, VEGF, and MCP-1 from BMSCs (Fig. 1*A*; Table 1). IL-6 induces growth, survival, and drug resistance in MM cells (3–6); VEGF triggers growth and migration of MM cells (7), stimulates BM angiogenesis (8), and augments IL-6 production in BMSCs (9). In addition to these effects on MM cells and BMSCs in the BM microenvironment, we have shown that both cytokines contribute to immunosuppression characteristic of MM (10). In this study, we show that SD-208 decreased IL-6 and VEGF secretion triggered by TGF-β1 in a dose-dependent manner. SD-208 also inhibited TGF-β1–triggered secretion of MCP-1, which binds to CCR2 on MM cells and mediates their migration to BMSCs (29), further suggesting that SD-208 decreases adhesion of MM cells to BMSCs and associated cytokine secretion.

We have shown that SD-208 blocks TGF-β1–triggered nuclear translocation of Smad2/3 and HIF-1α, which regulate IL-6 and VEGF transcription, respectively (26, 28). TGF-β1 binds to a cell surface receptor complex consisting of TβRI and TβRII; TβRII then activates TβRI, which in turn phosphorylates Smad2/3. Activated Smad2/3 bound to Smad4 translocates into the nucleus and regulates transcription of IL-6 and other genes (30, 31). TβRI kinase inhibitor SD-208 blocks this pathway and resultant IL-6 secretion (21). TGF-β1 can also activate Smad-independent pathways including extracellular signal-regulated kinase, c-Jun NH_2-terminal kinase, and p38 mitogen-activated protein kinase (31). In BMSCs, we showed that TGF-β1 triggered minor phosphorylation of p38 mitogen-activated protein kinase and did not activate extracellular signal-regulated kinase and c-Jun NH_2-terminal kinase. Although signaling between TβRI and HIF-1α in BMSCs remains to be fully delineated, inhibition of upstream TβR1 with SD-208 confers, at least in part, reduction of VEGF secretion.

SD-208 blocks not only cytokine production, but also MM cell proliferation, triggered by adhesion to BMSCs. Inhibitory effects of SD-208 on MM cell growth in the BM milieu suggest that it may overcome resistance to conventional therapeutic agents, such as dexamethasone and doxorubicin (12, 32). Novel biologically based therapeutics targeting the MM cell in its BM milieu, including thalidomide and immunomodulatory derivatives as well as bortezomib, can overcome drug resistance and achieve response even in refractory and relapsed myeloma (12, 14, 15). Therefore, SD-208, by overcoming the growth advantage conferred by IL-6 and VEGF in the BM milieu, has the potential to similarly overcome clinical drug response.

In summary, in this study we demonstrate that SD-208 inhibits production of cytokines (IL-6 and VEGF) mediating MM cell growth, survival, drug resistance, and migration in the BM microenvironment, providing the rationale for its clinical evaluation to improve patient outcome in MM.

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