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The Development of a Model for the Homing of Multiple Myeloma Cells to Human Bone Marrow

By Mitsuyoshi Urashima, Benjamin P. Chen, Shirley Chen, Geraldine S. Pinkus, Roderick T. Bronson, Douglas A. Dederer, Yasutaka Hoshi, Gerrard Teoh, Atsushi Ogata, Steve P. Treon, Dharminder Chauhan, and Kenneth C. Anderson

Prior *in vitro* studies have suggested a role of adhesion molecules, bone marrow stromal cells (BMSCs), and cytokines in the regulation of human multiple myeloma (MM) cell growth and survival. Although *in vivo* models have been developed in severe combined immunodeficient (SCID) mice that support the growth of human MM within the murine BM microenvironment, these xenograft models do not permit a study of the role of adhesion proteins in human MM cell-human BMSC interactions. We therefore established an *in vivo* model of human MM using SCID mice implanted with bilateral human fetal bone grafts (SCID-hu mice). For the initial tumor inoculum, human MM derived cell lines (1×10^4 or 5×10^4 ARH-77, OCI-My5, U-266, or RPMI-8226 cells) were injected directly into the BM cavity of the left bone implants in irradiated SCID-hu mice. MM cells engrafted and proliferated in the left human fetal bone implants within SCID-hu mice as early as 4 weeks after injection of as few as 1×10^4 MM cells. To determine whether homing of tumor cells occurred, animals were observed for up to 12 weeks after

injection and killed to examine for tumor in the right bone implants. Of great interest, metastases to the right bone implants were observed at 12 weeks after the injection of 5×10^4 MM cells, without spread of human MM cells to murine BM. Human MM cells were identified on the basis of characteristic histology and monoclonal human Ig. Importantly, monoclonal human Ig and human interleukin-6 (IL-6), but not human IL-1 β or tumor necrosis factor- α , were detectable in sera of SCID-hu mice injected with MM cells. In addition, specific monoclonal Ig light chain deposition was evident within renal tubules. This *in vivo* model of human MM provides for the first time a means for identifying adhesion molecules that are responsible for specific homing of human MM cells to the human, as opposed to murine, BM microenvironment. Moreover, induction of human IL-6 suggests the possibility that regulation of MM cell growth by this cytokine might also be investigated using this *in vivo* model.

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ADHESION MOLECULES on multiple myeloma (MM) cells play a role in the homing of malignant plasma cells to the bone marrow (BM) after Ig class switching within the lymph node; regulation of MM cell growth and survival in the BM microenvironment; tumor cell egress from the BM with the development of plasma cell leukemia (PCL); and, finally, metastatic seeding at extramedullary sites. However, to date, the majority of studies examining the role of adhesion molecules, BM stromal cells (BMSCs), and cytokines in regulating MM cell localization, growth, and apoptosis within the BM microenvironment have been performed *in vitro*¹⁻¹⁸ and may not accurately identify factors important for the pathophysiology of MM *in vivo*. For example, *in vitro* studies suggest that localization of tumor cells in the BM is related to MM cell binding to fibronectin and collagen I via very late antigen-4 (VLA-4) and syndecan, respectively,^{3,13} as well as tumor cell binding to BMSCs by VLA-4 to vascular cell adhesion molecule-1 (VCAM-1) and LFA-1 to intercellular adhesion molecule-1 (ICAM-1) interactions.^{1,4} Moreover,

changes in cell surface phenotype of tumor cells have been described with disease progression, ie, acquisition of CD11b and LFA-1 with loss of CD56, VLA-5, MPC-1, and syndecan-1 on PCL cells.^{12,17,18} However, *in vitro* adhesion assays are dependent on the ability of BMSCs to be grown to confluence under the influence of serum and growth factors, and it is therefore possible that their adhesion molecule profile may have been altered. In addition, cells may also be selected for outgrowth *in vitro* that do not reflect BMSCs *in vivo*. Delineation of the role of adhesion molecules in disease pathogenesis requires *in vivo* models. Available models in severe combined immunodeficient (SCID) mice have shown that intraperitoneal injection of MM cells permits tumor cell growth within the peritoneal cavity¹⁹ and that intravenous injection of ARH-77 MM cells results in disseminated tumor cell growth both in the murine BM and other organs.²⁰⁻²³ However, to date, an *in vivo* model to assess the role of adhesion molecules in homing of human MM cells to human BMSCs has not been described.

Once MM cells are localized in the BM microenvironment, BMSCs and cytokines regulate their growth and survival. Interleukin-6 (IL-6) is the major autocrine and paracrine growth factor for human MM²⁴ and can inhibit apoptosis of tumor cells induced by corticosteroids, serum starvation, and anti-Fas *in vitro*.²⁵⁻²⁸ Importantly, recent studies show that adhesion of MM cells to BMSCs, in addition to localizing tumor cells within the BM microenvironment, also upregulates IL-6 secretion by BMSCs.^{1,2,15} However, *in vitro* studies again may not accurately reflect the biologic significance of BMSCs and cytokines *in vivo*. For example, most freshly isolated MM patient cells do not produce IL-6 or demonstrate autocrine IL-6-mediated growth *in vitro*; however, triggering tumor cells via their cell surface CD40, as may occur *in vivo*, induces IL-6-mediated autocrine growth.²⁹ *In vitro* studies also show that MM cells produce transforming growth factor- β 1 (TGF- β 1), but lack TGF- β 1

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responsiveness.³⁰ However, TGF- β 1 stimulates IL-6 production by BMSCs and may therefore indirectly regulate paracrine IL-6-mediated MM cell growth in vivo. Previously described in vivo murine models have already shed considerable insight into the role of cytokines, ie, IL-6, in the pathogenesis of human MM. For example, either mice injected with pristane or IL-6 transgenic mice develop massive plasmacytosis and/or transplantable monoclonal plasmacytomas³¹⁻³³; in contrast, pristane injection in IL-6-deficient mice induces lymphocytosis without either plasmacytosis or the development of mouse plasmacytomas.^{34,35} However, these models do not allow for the characterization of the role of human BMSCs and cytokines in the regulation of human MM cell growth and survival in vivo.

The SCID-hu mouse, developed by surgical implantation of fetal hematolymphoid organs (eg, bone grafts, lymph node, and thymus) into SCID mice, has been used to study normal hematopoiesis and malignant cell growth as well as cytokine and gene therapies.³⁶⁻⁴² In this study, we have established an in vivo model for the study of MM localization and growth within the human BM microenvironment using SCID-hu mice implanted with bilateral human fetal bone grafts. Human MM-derived cell lines injected directly into the marrow cavity of the left bone implant of irradiated SCID-hu mice spread to the right bone implant, but did not home to murine BM. Immunoperoxidase staining confirmed monoclonal tumor cell growth, which was coupled with secretion of monoclonal human Ig and human IL-6 in sera of tumor-bearing mice. This in vivo model may therefore facilitate studies of homing of human MM cells to the human BM microenvironment as well as the role of BMSCs and cytokines in regulation of tumor cell growth and survival.

MATERIALS AND METHODS

MM-derived cell lines. The ARH-77, U-266, and RPMI-8226 human MM-derived cell lines were obtained from American Type Culture Collection (Rockville, MD) and cultured in RPMI-1640 medium with 10% fetal bovine serum.⁴³ The OCI-My5 MM cell line was kindly provided by Dr H.A. Messner (Ontario Cancer Institute, Toronto, Ontario, Canada), and cultured in Iscove's media (Sigma, St Louis, MO) with 10% fetal bovine serum.⁴⁴

SCID-hu mice and cell inoculation. Homozygous C.B-17 *scid* mice (SCID) were bred, treated with antibiotics, and used when 6 to 8 weeks old. The collection of fetal tissues and implantation of human fetal long bone grafts into SCID mice to produce SCID-hu mice has been previously described.³⁶⁻⁴² In brief, the femurs and tibias of 19 to 23 gestational week fetuses were cut into fragments (approximately 5 × 5 × 10 mm) and implanted subcutaneously into both sides of SCID mice. The human fetal tissues were obtained with appropriate informed consent and in compliance with regulations issued by the state and the federal governments. SCID-hu mice were exposed to 400 cGy γ irradiation from a ¹³⁷Cs γ irradiator at a dose rate of 75 cGy/min. Either 1 × 10⁴ or 5 × 10⁴ ARH-77, U-266, OCI-My5, or RPMI-8226 MM cells or phosphate-buffered saline (PBS) alone in 10 μ L was then injected directly into the marrow cavity of the left fetal bone implant in SCID-hu mice or was injected subcutaneously into SCID mice without human fetal bone implants.

Histopathological analysis. Mice were killed at 4, 8, and 12 weeks after MM cell injection. Excised organs included the left- and right-sided human fetal bone grafts, spleen, liver, thymus, kidney, lung, vertebrae, spinal cord, and brain. Tissues were fixed in Bouin's

solution, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E) to perform histopathological examination. Immunoperoxidase studies were performed on paraffin sections using an indirect technique as previously described, with modifications.⁴⁵ Rabbit antibodies (Abs) to human Ig light (κ and λ) and heavy (IgG, IgA, IgM, and IgE) chains, as well as swine antirabbit Ig conjugated to horseradish peroxidase, were obtained from Dako Corp (Carpenteria, CA). Antibody localization was effected using a peroxidase reaction with 3,3-diaminobenzidine tetrahydrochloride as chromogen.

Measurement of human IL-6, IL-1 β , and human tumor necrosis factor- α (TNF- α) secretion. IL-6 levels in the sera of SCID and SCID-hu mice, in sera of SCID-hu mice injected with human MM cells, and in the supernatants of human MM-derived cell lines cultured in media were measured using an enzyme-linked immunosorbent assay (ELISA), as previously described.^{29,46} Briefly, (1) 96-well plates (Costar, Cambridge, MA) were coated with anti-IL-6 MoAb (murine IgG1; Toray, Ohtsu-shi, Shiga, Japan); (2) wells were saturated with calfskin gelatin (BioRad, Melville, NY)-PBS for 1 hour; (3) serial dilutions (100 μ L) of test sample supernatants were added in duplicate to plates; and (4) biotinylated detector anti-IL-6 MoAb (Genetics Institute, Cambridge, MA) was added and developed with avidin-peroxidase (Amersham, Arlington Heights, IL), tetramethylbenzidine, and 30% peroxide (Sigma). The minimal detectable level of IL-6 (Kirin-Brewery Co Ltd, Minato-ku, Tokyo, Japan) was 10 pg/mL. Human IL-1 β and TNF- α levels in sera of mice were measured using ELISA kits (R&D Systems [Minneapolis, MN] and Endogen [Cambridge, MA], respectively). The minimal detection levels were 4 pg/mL IL-1 β and 5 pg/mL TNF- α . These ELISA systems do not detect mouse cytokines.

Solid-phase ELISA for IgG and IgA. Quantitative ELISA was used to measure levels of human IgG and IgA in the mouse sera in a manner similar to that described for IL-6 above.³⁰ Goat antihuman polyvalent Ig Ab (Sigma) was used as the coating Ab, and biotin-conjugated goat antihuman IgG Ab (Sigma) or antihuman IgA Ab (Sigma) was used as detection Ab. The levels of detection of both IgG and IgA were 1 ng/mL.

RESULTS

Human MM cells grow in fetal human bone implants within SCID-hu mice. To study homing of human MM cells to human BMSCs, we first attempted to establish MM cell growth in SCID-hu mice. After irradiation (400 cGy) of SCID-hu mice, 1 × 10⁴ or 5 × 10⁴ ARH-77, OCI-My5, U-266, or RPMI-8226 MM cells or PBS was injected directly into the left human bone implant. Mice were killed 4, 8, and 12 weeks later, and the left fetal bone implant was examined histologically by H&E staining and also for monoclonality by Ig heavy and light chain immunoperoxidase staining. As early as 4 weeks after injection of as few as 1 × 10⁴ MM cells, the left human bone implant specimens in all SCID-hu mice injected with MM cells showed infiltration of the human BM by tumor cells characteristic of the inoculated MM cell line, with only rare normal BM elements. By 12 weeks after injection of tumor cells, the surrounding tissue was largely replaced by neoplastic plasma cells. Histologic findings in left implants from ARH-77 injected animals (Fig 1A) are illustrative of findings in animals injected with OCI-My5, U-266, or RPMI-8226 MM cells. In contrast, BM within fetal bone implants from the SCID-hu mice injected with PBS alone (control) was hypoplastic with increased fatty deposits, without any signs of inflammation, granulation, or tumor either in the human BM or surrounding tissue

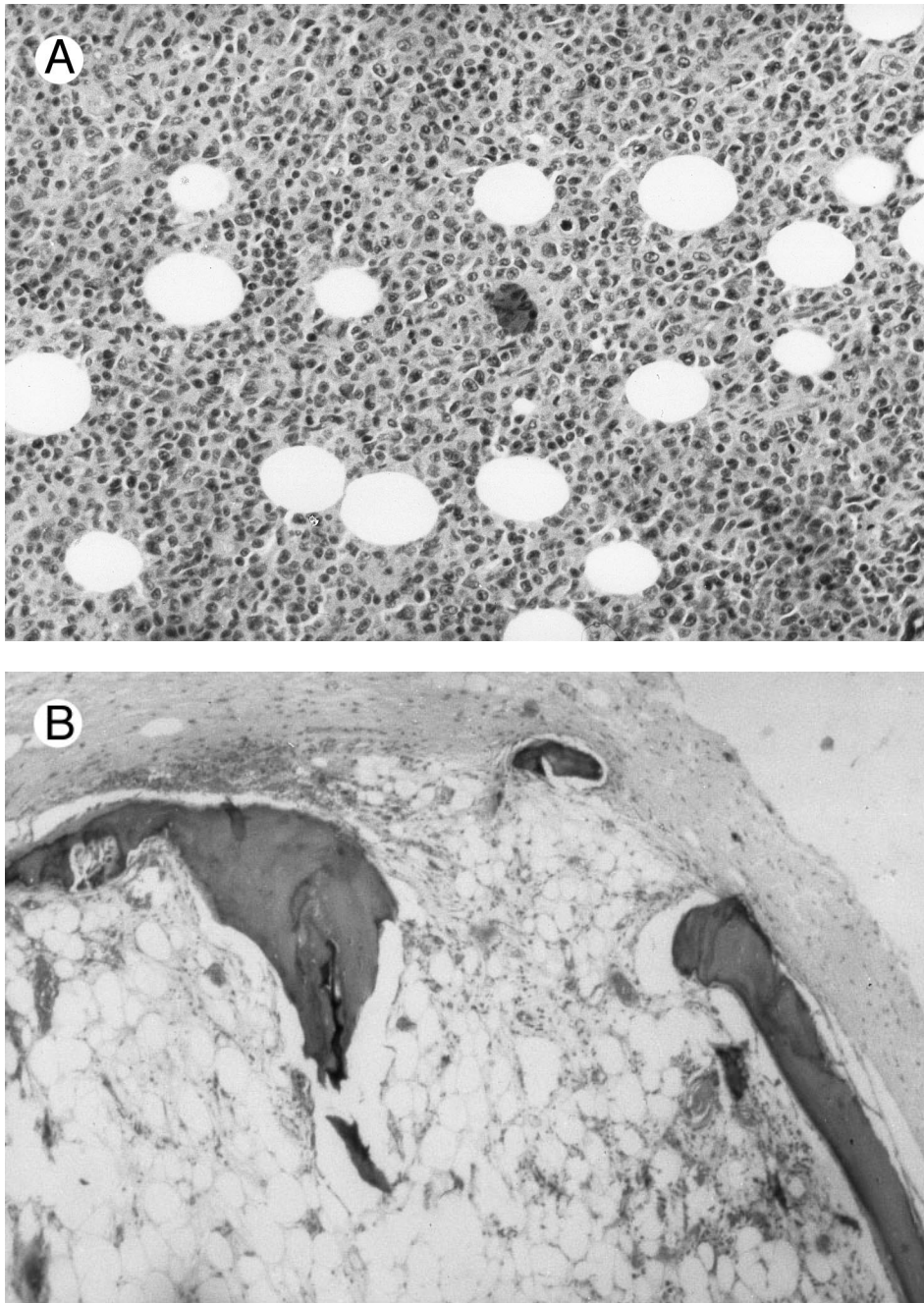


Fig 1. Human MM cells grow in fetal human bone implants within SCID-hu mice. SCID-hu mice were treated with irradiation (400 cGy), injected directly into the marrow cavity of the left fetal human bone implant with 5×10^4 ARH-77 MM cells or with PBS, and killed 12 weeks later. H&E-stained tissue sections of the left human fetal bone marrow implant from SCID-hu mice injected with ARH-77 MM cells (original magnification $\times 100$ [A]) or PBS (original magnification $\times 40$ [B]) are shown.

(Fig 1B). Finally, no tumor growth was observed in SCID mice without human bone implants that were similarly treated with irradiation (400 cGy), injected with the same MM cell inocula subcutaneously, and killed at these intervals (data not shown).

To confirm that the tumor cells infiltrating the left bone implant were monoclonal, immunoperoxidase staining was performed using antihuman κ or λ Ig light chain Abs and antihuman IgG, IgA, IgM, or IgE heavy chain Abs. For ARH-77 MM cell line, tumor cells infiltrating the bone implants stained with antihuman IgG heavy chain Ab and anti-

human κ Ig light chain Ab (Fig 1C), but not with antihuman IgA, IgM, or IgE heavy chain Abs or with antihuman λ Ig light chain Ab (Fig 1D), consistent with monoclonal ARH-77 cells that secrete IgG κ . These findings are illustrative of the characteristic monoclonal specificity observed in tumor cells within bone implants of SCID-hu mice injected with other MM cells: IgA λ , λ , and IgE λ for OCI-My5, RPMI-8226, and U-266 MM cells, respectively. Control murine BM and splenic cells were not stained with these Abs, confirming that they are not crossreactive with murine cells.

Time- and dose-dependent growth of tumor cells in fetal

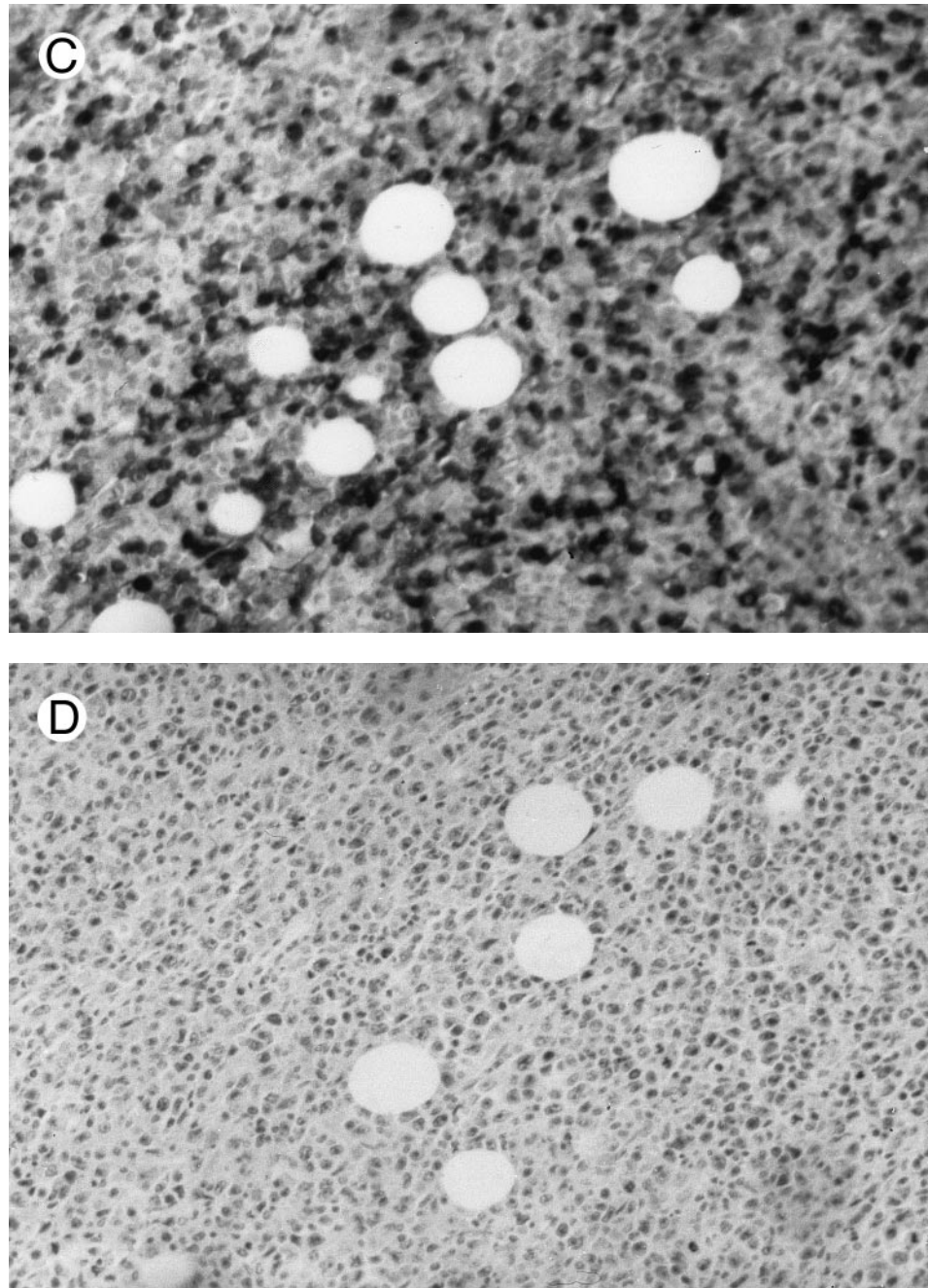


Fig 1. (cont'd). Immunoperoxidase staining of the same human fetal bone marrow implant from SCID-hu mice injected with ARH-77 MM cells was also performed using antihuman κ (original magnification $\times 100$ [C]) and antihuman λ (original magnification $\times 100$ [D]) Ig light chain Abs.

bone implants. The size of tumor within the left bone implants, as well as the extent of local infiltration, correlated with number of MM cells in the inoculum ($5 \times 10^4 > 1 \times 10^4$) and with time between injection of MM cells and killing of the animals (12 weeks $>$ 8 weeks $>$ 4 weeks; Fig 2).

Metastasis of MM cells to the right bone implant within SCID-hu mice. We next examined for homing of MM cells to the right bone implant in SCID-hu mice that had been treated with irradiation (400 cGy); injected directly into the left bone implant with ARH-77, OCI-My5, U-266, or RPMI-8226 MM cells (1×10^4 to 5×10^4) or PBS; and killed at

4, 8, and 12 weeks. Sites examined for tumor spread included not only the right bone implant, but also murine BM within vertebra and long bones, spleen, liver, thymus, kidney, lung, vertebrae, spinal cord, and brain. At intervals of 4 to 8 weeks after MM cell injection, no spread of tumor beyond the left bone implant was observed (data not shown). However, at 12 weeks after the injection of each of these MM cell lines (5×10^4 cells), characteristic tumor cell infiltration within the BM of the right bone implant was observed. Histologic findings in right-sided implants 12 weeks after inoculation of 5×10^4 ARH-77 cells into the left bone implants of SCID-

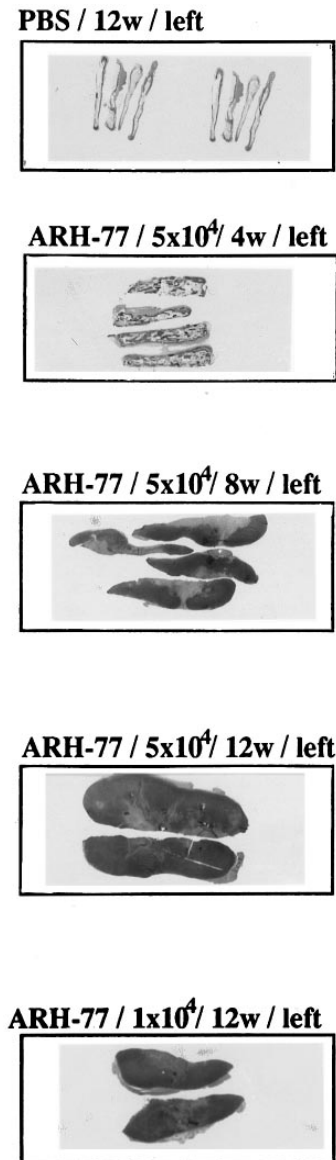


Fig 2. Time- and dose-dependent growth of tumor cells in fetal bone implants. ARH-77 MM cells (1×10^4 or 5×10^4) or PBS was injected directly into the marrow cavity of the left fetal human bone implant of previously irradiated (400 cGy) SCID-hu mice. Mice were killed 4, 8, and 12 weeks later, and macroscopic sections of left human bone implants were stained with H&E.

hu mice (Fig 3A and B) are illustrative of findings in animals injected with OCI-My5, U-266, or RPMI-8226 cells. Immunoperoxidase staining of the right bone implants with antihuman IgG, IgA, and IgE heavy chain Abs, as well as antihuman κ or λ Ig light chain Abs, confirmed monoclonality of tumor cells. For example, predominant IgG κ staining was noted in right bone implants from animals injected in the left implant with ARH-77 MM cells (Fig 3C). Tumor cells were rarely observed in blood vessels (Fig 3D). Additional spread of focal tumor formation was observed at 12 weeks: ARH-77 MM cells within the mediastinum (Fig 3E), perito-

neum, and epidural space; and OCI-My5 MM cells within the peritoneum. Importantly, no spread of ARH-77, U-266, and RPMI-8226 MM cells to murine BM was observed at any time (Fig 3F), except for focal spread of tumor cells to vertebral BM of SCID-hu mice 12 weeks after injection with 5×10^4 OCI-My5 MM cells. Although scattered isolated MM cells were present in lung, kidney, liver, and heart, there was no evidence of tumor formation at these sites. All metastatic lesions were shown to be monoclonal, based on pattern of immunoperoxidase staining using antihuman IgG, IgM, IgA, IgE, κ and λ Abs, and were identical to the tumor cells previously injected into the left bone implants.

Growth of human MM cells within SCID-hu mice triggers human monoclonal Ig secretion. To further confirm the growth of human MM cells within the SCID-hu mice, we next examined for human monoclonal proteins in the sera of mice at similar intervals after the injection of MM cells into the left bone implants. We therefore next measured levels of human IgG and IgA in sera of SCID-hu mice by ELISA (Fig 4). Although human IgG or IgA were undetectable in sera of SCID-hu mice injected with PBS, U-266 MM cells (IgE secreting), or RPMI-8226 MM cells (λ secreting), those mice injected with ARH-77 MM cells had low levels of human IgG but not IgA at 4 weeks, which increased at 8 and 12 weeks. In a parallel fashion, mice injected with OCI-My5 MM cells had detectable human IgA but not IgG in sera at 4 weeks, which increased at 8 and 12 weeks.

Growth of human MM cells within SCID-hu mice triggers human IL-6, but not human IL-1 β and TNF- α secretion. The levels of human IL-6, IL-1 β , and TNF- α in mouse serum were next examined by ELISA. Human IL-6 remained undetectable in SCID-hu mice before and at 4, 8, and 12 weeks after injection with PBS. In contrast, low levels of human IL-6 were present in sera of SCID-hu mice at 4 weeks after injection of RPMI-8226 MM cells and increased at 8 and 12 weeks after tumor cell injection, correlating with spread of disease beyond the left bone implant (Fig 5A). For each of the 4 MM cell lines studied, human IL-6 secretion into mouse sera was greater in animals injected with 5×10^4 than with 1×10^4 MM cells (Fig 5B). Neither human IL-1 β nor human TNF- α were detectable in the sera of mice under any conditions. Finally, IL-6 was undetectable in day-1, -2, -3, and -7 supernatants of these 4 cell lines (data not shown).

Deposition of human Ig light chain in kidney of SCID-hu mice. We next examined the kidneys of SCID-hu mice injected with MM cells (5×10^4 cells) and killed at 12 weeks. Although no MM cells were noted in the kidneys of these animals, characteristic specific renal tubular staining for human κ or λ light chain was observed in mice injected with ARH-77 or U-266, RPMI-8226, and OCI-My5 MM cells, respectively. For example, λ and κ tubular staining in animals injected with U-266 cells is shown in Fig 6A and B, respectively. Staining was predominant in the proximal tubules as reabsorption droplets, with less staining in collecting tubules and ducts. Glomerular staining or tumor formation within the renal parenchyma was not observed.

DISCUSSION

MM remains incurable, with a median survival at best of only 48 months regardless of whether a single agent or

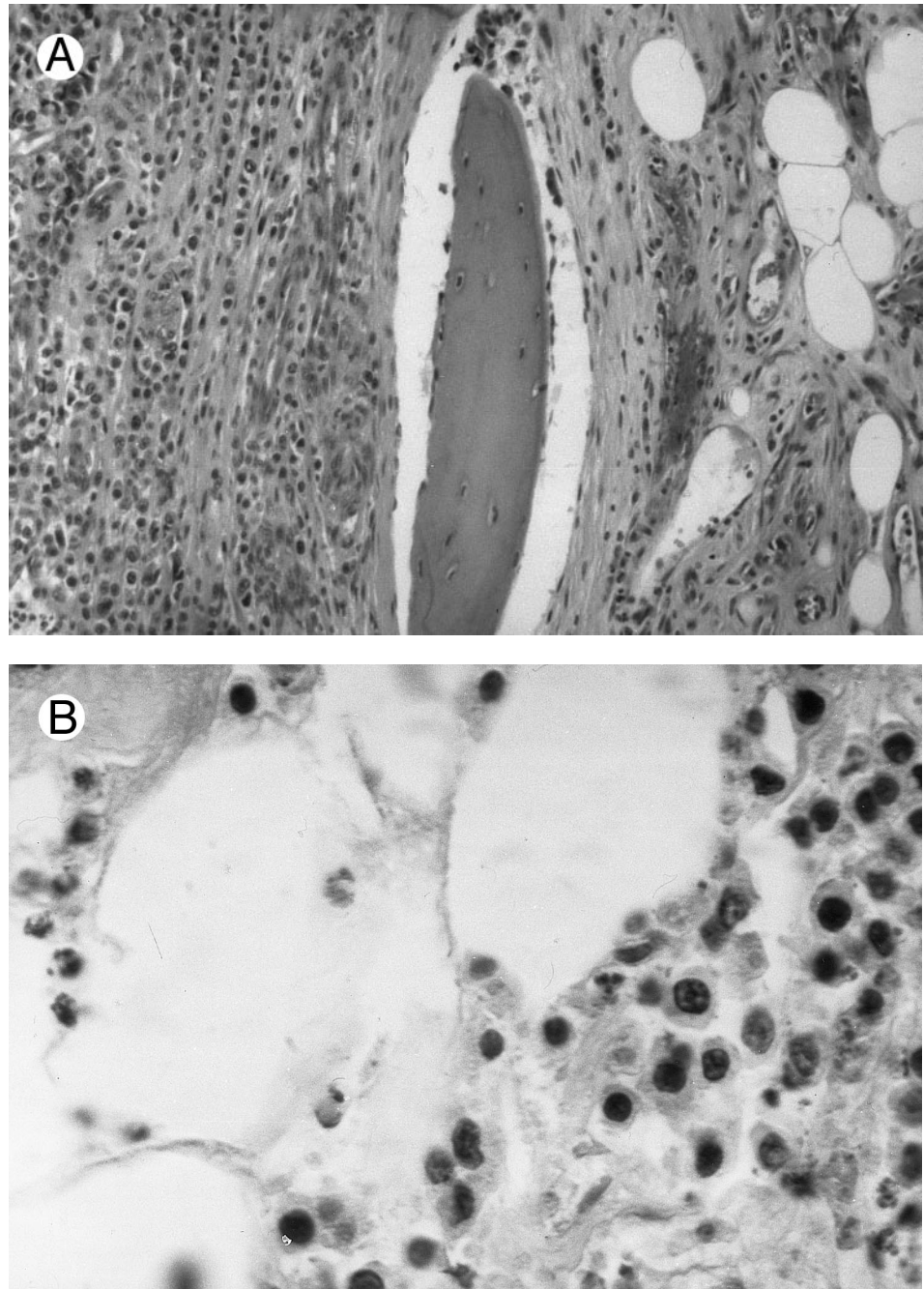


Fig 3. Metastasis of MM cells within SCID-hu mice. SCID-hu mice were treated with irradiation (400 cGy), injected directly into the marrow cavity of the left fetal human bone implant with 5×10^4 ARH-77 MM cells, and killed 12 weeks later. H&E (original magnification $\times 100$ [A] and $\times 400$ [B])

combination chemotherapy is used, and there is a great need for novel treatment approaches. The establishment of a murine model reflecting the pathophysiology of human MM *in vivo* is required not only for enhanced understanding of the mechanisms of tumor cell homing and localization to the BM and disease progression, but also for the development and testing of potential novel treatment approaches. To date, SCID mice have been used to establish a human MM mouse model. Feo-Zuppari et al¹⁹ first reported growth of human MM cells in SCID mice after intraperitoneal injection of 2 to 10×10^6 patient MM cells, evidenced morphologically

and by secretion of monoclonal Ig. In this model, most human plasmacytes were localized in the peritoneal cavity, without metastasis to murine BM. Others have shown sustained production of human antibodies by B cells after intraperitoneal transfer of normal human peripheral blood mononuclear cells into SCID mice.⁴⁷ Therefore, growth within the peritoneal cavity of SCID mice may not require a malignant phenotype. Huang et al²⁰ next showed the disseminated growth of ARH-77 MM cells after intravenous injection of 10^7 ARH-77 MM cells into irradiated SCID mice. Disease manifestations included hind limb paralysis due to infiltra-

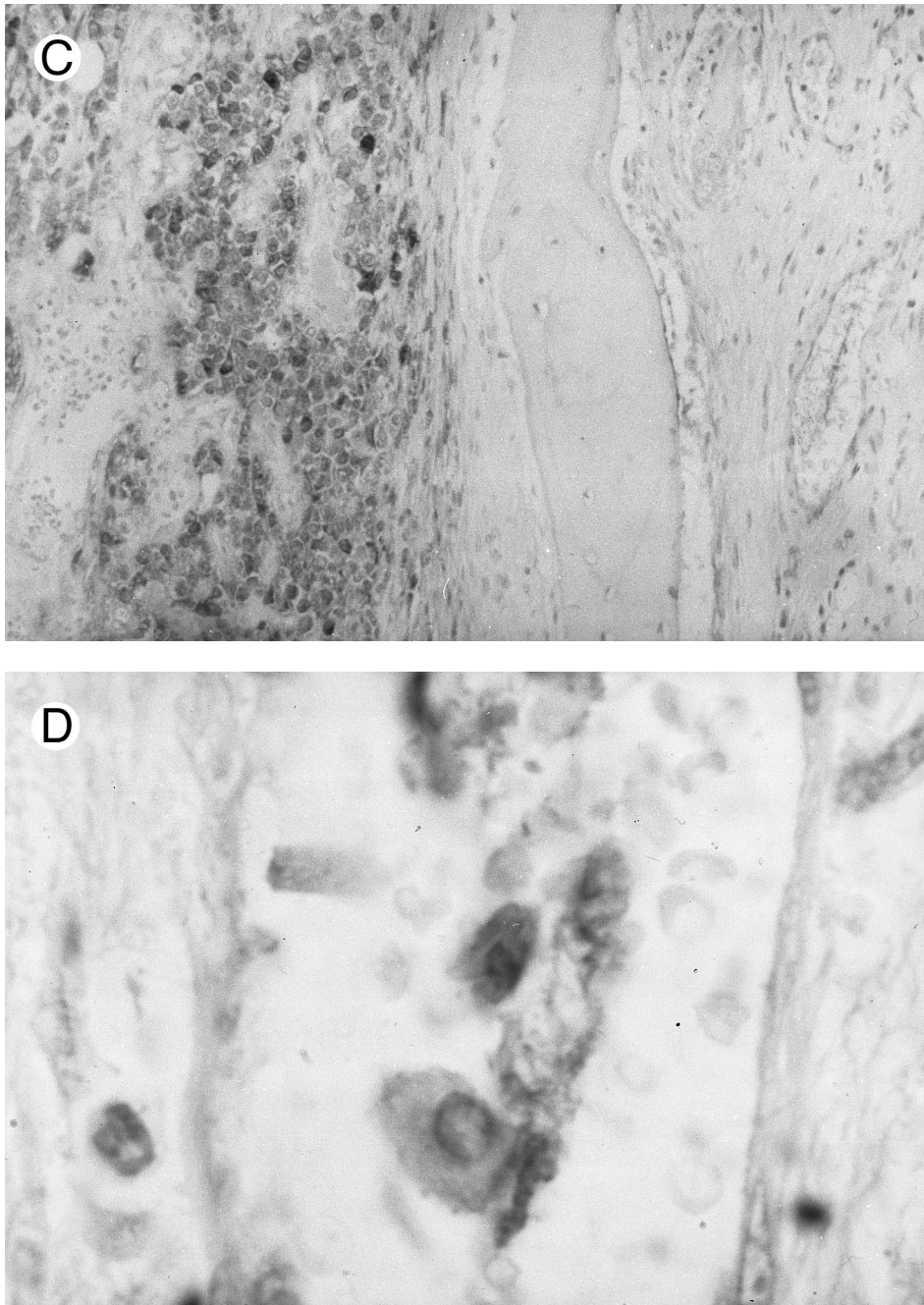


Fig 3. (cont'd). and immuno-
peroxidase staining with anti- κ
Ig Ab (original magnification $\times$
100 [C] and $\times$ 1,000 [D]) of the
right-sided human fetal bone
marrow implant is shown.

tion of tumor cells into the thoracolumbar vertebrae. Subsequently, these investigators evaluated the impact of Ab directed against intercellular adhesion molecule-1 (anti-CD54) on tumor cell growth in this model.²¹ More recently, the SCID mouse model of MM has proven useful for characterizing MM bone disease.²² After injection of 10^6 ARH-77 MM cells intravenously, hind limb paralysis was observed, as noted previously^{20,21}; however, of great interest was the development of bone disease manifest by lytic bone lesions and hypercalcemia. In this study,²² there was diffuse spread of tumor to murine BM and other organs, but no increase in

either murine or human cytokines, such as IL-6, IL-1, or TNF- α . Most recent reports suggest the utility of the MM SCID mouse model for evaluation of therapies for drug resistant MM⁴⁸ and of gene therapy approaches for MM.⁴⁹

Multiple prior studies have defined mechanisms of MM cell binding to BMSCs in vitro,¹⁻¹⁸ but these studies depend on the growth of BMSC cultures, which may not reflect the cellular composition or adhesion molecule profile of BMSCs in vivo. In the current study, we used the SCID-hu mouse model, which has been used to evaluate human infectious diseases, hematopoiesis, malignant cell growth, and gene

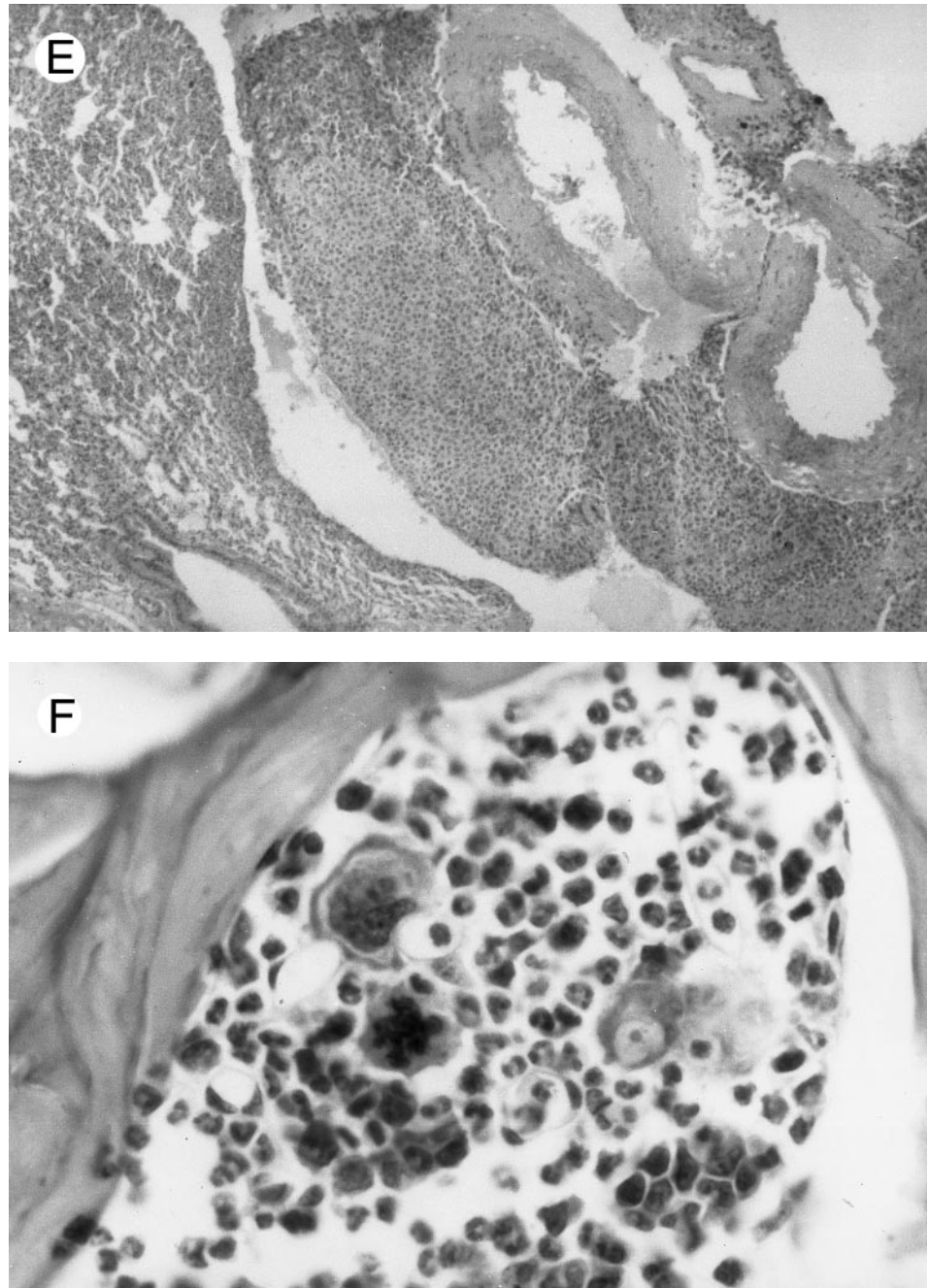


Fig 3. (cont'd). Mediastinum (original magnification $\times 40$ [E]) and murine vertebral BM (original magnification $\times 400$ [F]) were also stained with H&E.

therapies.³⁶⁻⁴² In contrast to all prior studies, the present model allows for the evaluation of the homing and adhesion of human MM cells to human BMSCs, because it is the only model with human fetal bone grafts in SCID mice. In our study, as few as 1×10^4 MM cells injected directly into the fetal bone implant grew in SCID-hu mice. Importantly, after MM cells were established in the left human bone implant, as evidenced by marked infiltration of neoplastic plasma cells, tumor cells subsequently homed and spread to human BM within the right-sided human bone implants. In our study, immunoperoxidase staining of tumor cells, growing

initially within left bone implants and subsequently in right bone implants, confirmed monoclonality consistent with tumor cells in the innoculum. Moreover, the level of serum monoclonal Ig, which was undetectable in control SCID-hu mice, correlated with tumor cell mass. In contrast to other studies, growth of human MM cells was rarely found in murine BM in SCID-hu mice, suggesting an affinity of human MM cells for human BM. Moreover, the presence of isolated MM cells without tumor formation in lung, kidney, liver, heart, and blood vessels further suggests that tumor cells circulate but grow only in specific environments in our

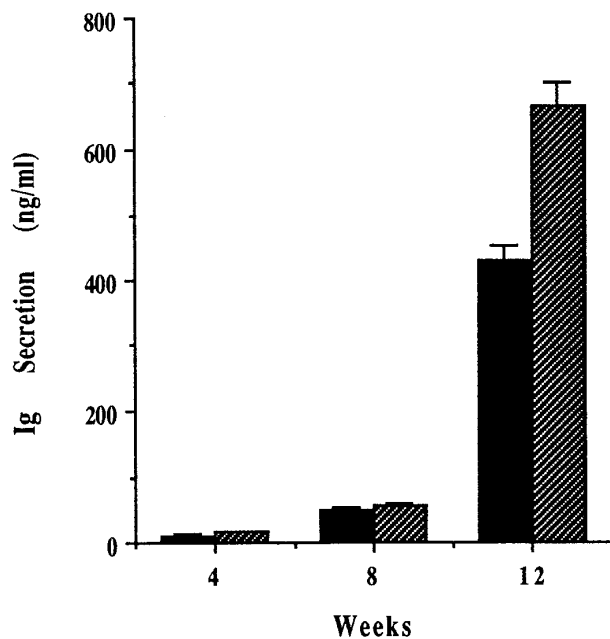


Fig 4. Growth of human MM cells within SCID-hu mice triggers human monoclonal Ig secretion. ARH-77 and OCI-My5 MM cells were injected directly into the left human fetal bone implant in SCID-hu mice, and sera samples were taken from these mice 4, 8, and 12 weeks later. Human IgG (■) in sera from mice injected with ARH-77 MM cells and human IgA (▨) in sera from mice injected with OCI-My5 MM cells were quantitated by ELISA.

SCID-hu model. We also confirmed MM cell growth and related limb paralysis in SCID mice injected intravenously with higher numbers (10^7) of MM cells, as noted in prior reports.²⁰⁻²² However, in the present study, the subcutaneous injection of fewer (10^4) MM cells did not result in tumor growth, suggesting that both cell doses and mode of inoculation may affect MM cell growth in SCID mice. These observations suggest that species-specific interactions between BMSCs and MM cells may exist allowing for the homing of human MM cells to human BMSCs and further highlight the importance of our model to understand migration and localization of human MM cells within the human BM microenvironment.

We and others have shown that adherence of MM cells to BMSCs *in vitro* can not only localize tumor cells in the BM microenvironment, but also trigger IL-6 secretion.^{1,2,15} This is related, at least in part, to induction of IL-6 transcription in BMSCs conferred through NF- κ B.² In the current study, metastasis of MM cells was observed only at 12 weeks, after extensive localized tumor cell growth in the left implant, and was accompanied by elevation of human IL-6 in mouse serum. The current SCID-hu MM mouse model therefore may allow for isolation and characterization of those human MM cells responsive to IL-6, as well as study of the mechanisms regulating IL-6 production in the marrow environment *in vivo*. Prior MM models in SCID mice that lack human BMSCs cannot be used for these studies, because murine IL-6 does not act on human cells. In an initial attempt to define triggers of IL-6 secretion in our model, we assayed

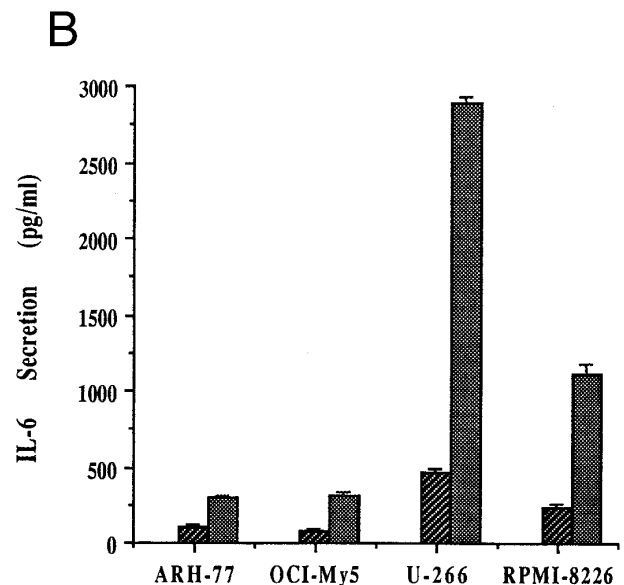
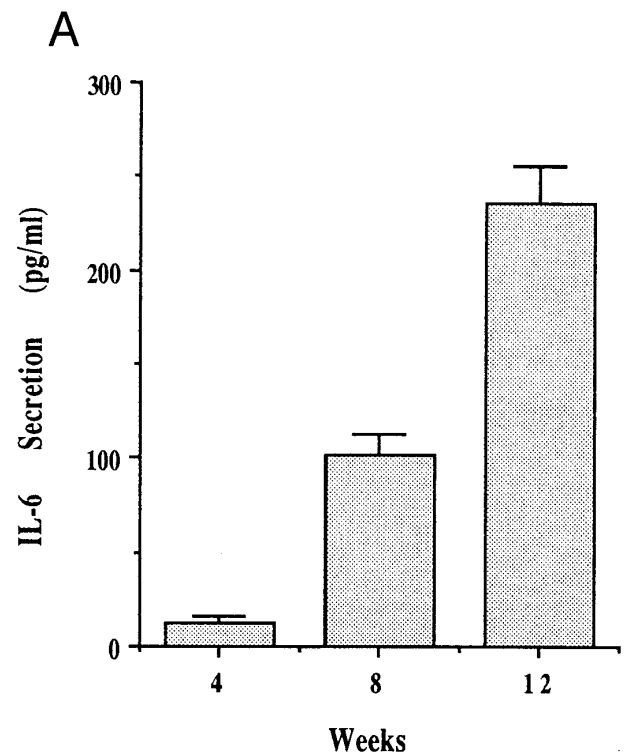


Fig 5. Growth of human MM cells within SCID-hu mice triggers human IL-6 secretion. RPMI-8226 MM cells (1×10^4) were injected directly into the marrow cavity of the left human fetal bone implant in SCID-hu mice, and secretion of human IL-6 in mouse sera at 4, 8, and 12 weeks was determined by ELISA (A). ARH-77, OCI-My5, U-266, and RPMI-8226 MM cells (1×10^4 MM cells; and 5×10^4 MM cells) were also injected directly into the marrow cavity of the left human fetal bone implant in SCID-hu mice, and the levels of human IL-6 in sera samples taken from these mice 12 weeks later were measured by ELISA (B).

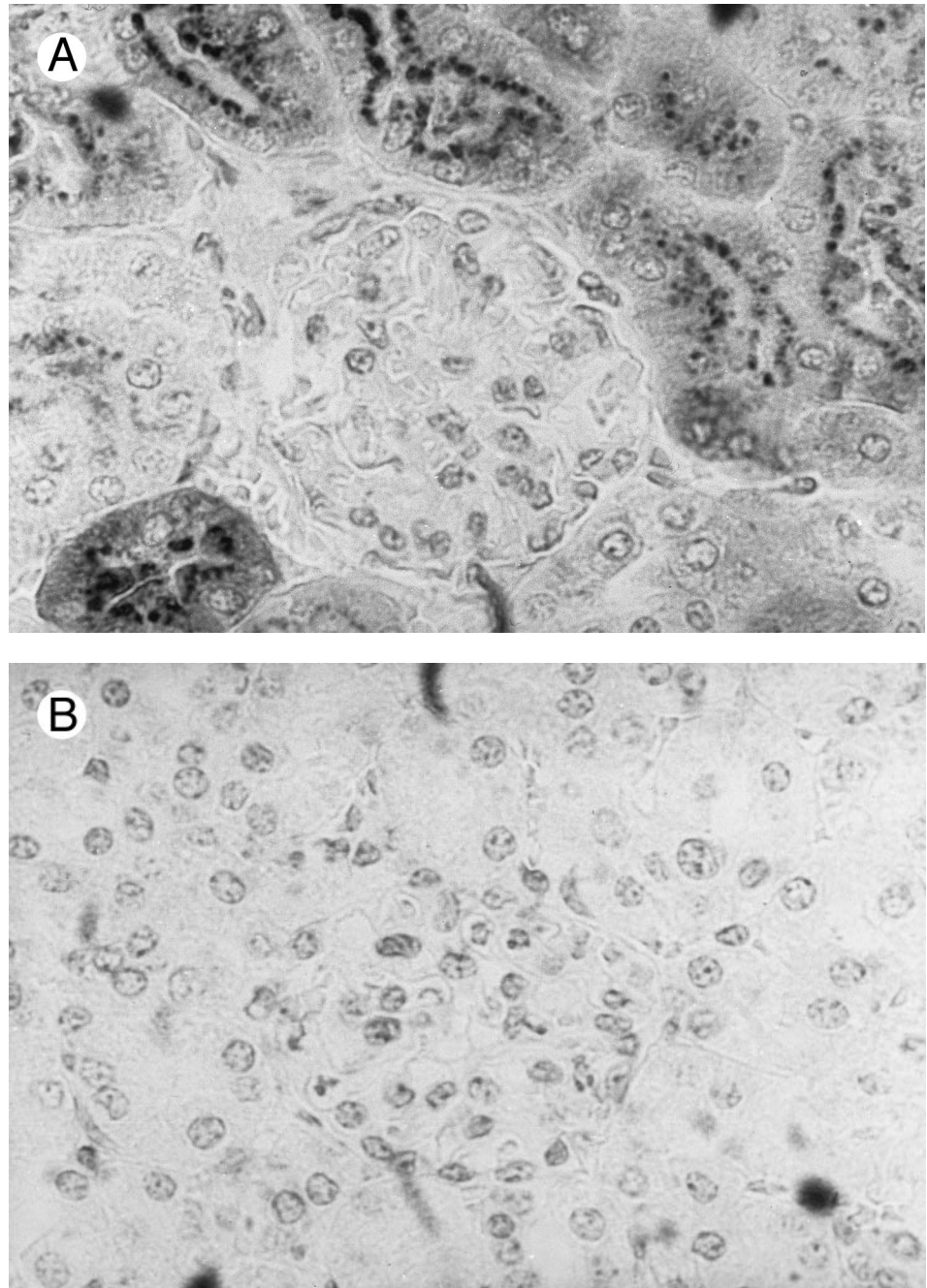


Fig 6. Growth of human MM cells within SCID-hu mice triggers monoclonal Ig light chain renal tubular staining. U-266 MM cells (5×10^4) were injected directly into the marrow cavity of the left human fetal bone implant in SCID-hu mice. Mice were killed 12 weeks later and renal tissue was stained with rabbit antihuman λ (original magnification $\times 100$ [A]) and κ (original magnification $\times 100$ [B]) immunoperoxidase Abs.

for human IL-1 β and TNF- α within the sera of SCID-hu mice bearing human MM cells, because both can be produced by MM cells and/or BMSCs and can upregulate IL-6 gene transcription. In addition, IL-1 β and TNF- α increase inflammation and stimulate IL-6 production.⁵⁰ Our data show that the observed elevations of human IL-6 in sera of SCID-hu mice were not accompanied either by increases in human IL-1 β and TNF- α or by inflammation around bone implants and other organs. Our in vitro studies suggest that these MM cells do not secrete IL-6 and that adherence of MM cells to BMSCs triggers IL-6 secretion primarily from BMSCs.¹

Future studies using this model will delineate in vivo mechanisms regulating IL-6 production in the marrow microenvironment and specifically define whether human tumor cells adhering to human BMSCs activate NF- κ B and IL-6 transcription in BMSCs in vivo, as is noted in vitro.² Finally, IL-6 is also a survival factor for MM cells because it can prevent tumor cell apoptosis triggered by dexamethasone, serum starvation, and Fas.²⁵⁻²⁸ It is possible that the elevated levels of serum human IL-6 seen in the SCID-hu mouse model may prevent apoptosis of MM cells within the BM as well as at distant sites. This model may therefore be useful

for defining the critical role of the human BM microenvironment not only in supporting localized growth and survival of MM cells in BM, but also in the progression and dissemination of disease.

In a recent study, Ahsmann et al²³ have suggested that the simultaneous introduction of tumor and accessory cells from peripheral blood or BM facilitates MM cell engraftment in SCID mice. In our study, the lack of tumor cell growth in SCID mice, coupled with growth of tumor cells in SCID-hu mice, also highlights the importance of the human BM microenvironment to facilitate human MM cell growth. Taken together, these studies further suggest that factors may exist in SCID mice that inhibit MM cell growth but that can be overcome by human BM cells. In SCID mice lacking a human bone microenvironment, for example, it is possible that most MM cells in the inoculum are rapidly cleared by the mouse reticuloendothelial system.⁵¹ In this case, the human bone implant within SCID-hu mice may counteract tumor cell clearance by providing a supportive microenvironment to which human MM cells home by specific interactions mediated by adhesion molecules. Alternatively, it is possible that the human BM microenvironment within SCID-hu mice may be overcoming MM growth-suppressive factors present in SCID mice, because the MM cells used in this study grow rapidly *in vitro* without the need for specific growth factors.⁴³ Ongoing studies will define the advantages of the SCID-hu model for tumor cell growth, using IL-6–dependent and IL-6–independent cell lines as well as freshly isolated patient tumor cell samples.

In our study, characteristic monoclonal tubular staining for κ or λ Ig light chains was observed in renal tubules, without involvement of glomerulae, at 12 weeks after MM cell injection into SCID-hu mice. Although we did not monitor renal function in these animals, myeloma nephropathy is characterized by the tubular deposition of monoclonal light chains. Prior animal models of renal disease in MM have injected Bence Jones proteins intraperitoneally into mice and shown that particular proteins produce distinctive types of protein deposition in renal tissue.⁵² In addition, IL-6 transgenic mice develop progressive renal pathology characterized initially by glomerulonephritis, followed by focal glomerulosclerosis, and finally by extensive tubular damage, reflecting changes observed in patients at terminal stages of MM.⁵³ Ongoing studies will determine whether this SCID-hu human MM cell model, if observed at longer intervals after tumor cell injection, may mimic pathological changes and renal dysfunction characteristic of MM kidney.

REFERENCES

1. Uchiyama H, Barut BA, Mohrbacher AF, Chauhan D, Anderson KC: Adhesion of human myeloma-derived cell lines to bone marrow stromal cells stimulates interleukin-6 secretion. *Blood* 82:3712, 1993
2. Chauhan D, Uchiyama H, Akbarali Y, Urashima M, Yamamoto KI, Libermann TA, Anderson KC: Multiple myeloma cell adhesion-induced interleukin-6 expression in bone marrow stromal cells involves activation of NF- κ B. *Blood* 87:1104, 1996
3. Uchiyama H, Barut BA, Chauhan D, Cannistra SA, Anderson KC: Characterization of adhesion on human myeloma cell lines. *Blood* 80:2306, 1992
4. Ahsmann EJM, Lokhorst HM, Dekker AW, Bloem AC: Lymphocyte function-associated antigen-1 expression on plasma cells correlates with tumor growth in multiple myeloma. *Blood* 79:2068, 1992
5. Barille S, Collette M, Bataille R, Amiot M: Myeloma cells upregulate IL-6 but downregulate osteocalcin production by osteoblastic cells through cell contact. *Blood* 86:3141, 1995
6. Huang N, Kawano MM, Harada H, Harada Y, Sakai A, Kuramoto A, Niwa O: Heterogeneous expression of a novel MPC-1 antigen on myeloma cells: Possible involvement of MPC-1 antigen in the adhesion of mature myeloma cells to bone marrow stromal cells. *Blood* 82:3721, 1993
7. Hamilton MS, Ball J, Bromidge E, Franklin IM: Surface antigen expression of human neoplastic plasma cells includes molecules associated with lymphocyte recirculation and adhesion. *Br J Haematol* 78:60, 1991
8. Kalasz V, Pap T, Csanaky G, Kelenyi G: Endothelial cell receptors on leukemic plasma cells. *Leuk Res* 13:863, 1989
9. Kawano MM, Huang N, Harada H, Harada Y, Sakai A, Tanaka H, Iwato K, Kuramoto A: Identification of immature and mature myeloma cells in the bone marrow of human myelomas. *Blood* 82:564, 1993
10. Kim I, Uchiyama H, Chauhan D, Anderson KC: Cell surface expression and functional significance of adhesion molecules on human myeloma-derived cell lines. *Br J Haematol* 87:483, 1994
11. Masellis-Smith A, Belch AR, Mant MJ, Turley EA, Pilarski LM: Hyaluron-dependent motility of B cells and leukemic plasma cells in blood, but not of bone marrow plasma cells, in multiple myeloma: Alternate use of receptor for hyaluronan-mediated motility (RHAMM) and CD44. *Blood* 87:1891, 1996
12. Pellet-Deceunynck C, Barille S, Puthier D, Rapp MJ, Harousseau JL, Bataille R, Amiot M: Adhesion molecules on human myeloma cells: Significant changes in expression related to malignancy, tumor spreading and immortalization. *Cancer Res* 55:3647, 1995
13. Ridley RC, Xiao H, Hata H, Woodliff J, Epstein J, Sanderson RD: Expression of syndecan regulates human myeloma plasma cell adhesion to type I collagen. *Blood* 81:767, 1993
14. Smith SR, Auerbach B, Morgan L: Serum neural cell adhesion molecule in multiple myeloma and other plasma cell disorders. *Br J Haematol* 92:67, 1996
15. Lokhorst HM, Lamme T, de Smet M, Klein S, de Weger RA, van Oers R, Bloem AC: Primary tumor cells of myeloma patients induce interleukin-6 secretion in long-term bone marrow cultures. *Blood* 84:2269, 1994
16. van Riet I, Bakkus M, De Greef C, Faide L, van Camp B: Homing mechanisms in the etiopathogenesis of multiple myeloma. *Stem Cells* 13:22, 1995 (suppl 2)
17. Vidrales MB, Anderson KC: Adhesion of multiple myeloma cells to the bone marrow microenvironment: Implications for future therapeutic strategies. *Mol Med Today* 2:405, 1996
18. Teoh G, Anderson KC: Interaction of tumor and host cells with adhesion and extracellular matrix molecules in the development of multiple myeloma. *Hematol Oncol Clin North Am* 11:27, 1997
19. Feo-Zuppari FJ, Taylor CW, Lopez MHA, Grogan TM, Odeleye AO, Hersh EM, Salmon SE: Long-term engraftment of fresh human myeloma cells in SCID mice. *Blood* 80:2843, 1992
20. Huang Y-W, Richardson JA, Tong AW, Zhang B-Q, Stone MJ, Vitetta ES: Disseminated growth of a human multiple myeloma cell line in mice with severe combined immunodeficiency disease. *Cancer Res* 53:1392, 1993
21. Huang Y-W, Richardson JA, Vitetta ES: Anti-CD54 (ICAM-1) has antitumor activity in SCID mice with human myeloma cells. *Cancer Res* 55:610, 1995
22. Alsina M, Boyce B, Devlin RD, Anderson JL, Craig F, Mundy

GR, Roodman GD: Development of an in vivo model of human multiple myeloma bone disease. *Blood* 87:1495, 1996

23. Ahsmann EJM, van Tol MJD, Oudemans-Gruber J, Lokhorst H, Uytendaele FGCM, Schuurman H-J, Bloem AC: The SCID mouse as a model for multiple myeloma. *Br J Haematol* 89:319, 1995

24. Klein B, Zhang XG, Lu ZY, Bataille R: Interleukin-6 in human multiple myeloma *Blood* 85:863, 1995

25. Lichtenstein A, Tu Y, Fady C, Vescio R, Berenson J: Interleukin-6 inhibits apoptosis of malignant plasma cells. *Cell Immunol* 162:248, 1995

26. Kawano MM, Mihara K, Huang N, Tsujimoto T, Kuramoto A: Differentiation of early plasma cells on bone marrow stromal cells requires interleukin-6 for escaping from apoptosis. *Blood* 85:487, 1995

27. Hata H, Matsuzaki H, Takeya M, Yoshida M, Sonoki T, Nagasaki A, Kuribayashi N, Kawano F, Takatsuki K: Expression of Fas/Apo-1 (CD95) and apoptosis in tumor cells from patients with plasma cell disorders. *Blood* 86:1939, 1995

28. Chauhan D, Kharbanda S, Ogata A, Urashima M, Teoh G, Robertson M, Kufe DW, Anderson KC: Interleukin-6 inhibits Fas-induced apoptosis and SAP kinase activation in multiple myeloma cells. *Blood* 89:227, 1997

29. Urashima M, Chauhan D, Uchiyama H, Freeman GJ, Anderson KC: CD40 ligand triggered interleukin-6 secretion in multiple myeloma. *Blood* 85:1903, 1995

30. Urashima M, Ogata A, Chauhan D, Hatzianthi M, Vidriales MB, Dederda DA, Schlossman RL, Anderson KC: Transforming growth factor β 1: Differential effects on multiple myeloma versus normal B cells. *Blood* 87:1928, 1996

31. Ohno S, Babonits M, Wiener F, Spira J, Klein G, Potter M: Nonrandom chromosome changes involving the Ig gene-carrying chromosome 12 and 6 in pristane-induced mouse. *Cell* 18:1001, 1979

32. Suematsu S, Matsuda T, Aozasa K, Akira S, Nakao N, Ohno S, Miyazaki J, Yamamura K, Hirano T, Kishimoto T: IgG1 plasmacytosis in interleukin 6 transgenic mice. *Proc Natl Acad Sci USA* 86:7547, 1989

33. Suematsu S, Matsusaka T, Matsuda T, Ohno S, Miyazaki J, Yamamura K, Hirano T, Kishimoto T: Generation of plasmacytomas with the chromosomal translocation t(12;15) in interleukin-6 transgenic mice. *Proc Natl Acad Sci USA* 89:232, 1994

34. Dederda DA, Urashima M, Chauhan D, LeBrun DP, Bronson R, Anderson KC: Interleukin-6 is required for pristane-induced plasma cell hyperplasia in mice. *Br J Haematol* 94:53, 1996

35. Hilbert DM, Kopf M, Mock BA, Kohler G, Rudikoff S: Interleukin-6 is essential for in vivo development of B lineage neoplasms. *J Exp Med* 182:243, 1995

36. McCune JM, Namikawa R, Kaneshima H, Shultz LD, Lieberman M, Weissman IL: The SCID-hu mouse; a model for the analysis of human hematolymphoid differentiation and function. *Science* 241:1632, 1988

37. Namikawa R, Ueda R, Kyoizumi S: Growth of human myeloid leukemias in the human marrow environment of SCID-hu mice. *Blood* 82:2526, 1993

38. Kyoizumi S, Murray LJ, Namikawa R: Preclinical analysis of cytokine therapy in the SCID-hu mouse. *Blood* 81:1479, 1993

39. Akkina RK, Rosenblatt JD, Cambell AG, Chen ISY, Zack JA: Modeling human lymphoid precursor cell gene therapy in the SCID-hu mouse. *Blood* 84:1393, 1994

40. Namikawa R, Ueda R, Kyoizumi S: Growth of human myeloid leukemias in the human marrow environment of SCID-hu mice. *Blood* 82:2526, 1993

41. Chen BP, Galy A, Kyoizumi S, Namikawa R, Scarborough J, Webb S, Ford B, Cen DZ, Chen S: Engraftment of human hematopoietic precursor cells with secondary transfer potential in SCID-hu mice. *Blood* 84:2497, 1994

42. Sandhu JS, Clark BR, Boynton EL, Atkins H, Messner H, Keating A, Hozumi N: Human hematopoiesis in SCID mice implanted with human adult cancellous bone. *Blood* 88:1973, 1996

43. Barut B, Zon LI, Cochran MK, Paul SR, Chauhan D, Mohrbacher A, Fingerhuth J, Anderson KC: Role of interleukin 6 in the growth of myeloma-derived cell lines. *Leuk Res* 16:951, 1992

44. Hitzler J, Martinez-Valdez H, Bergsagel D, Minden MD: Role of interleukin-6 in the proliferation of human multiple myeloma cell lines OCI-MY1-7 established from patients with advanced stage of the disease. *Blood* 78:1996, 1991

45. Pinkus GS, Said JW: Specific identification of intracellular immunoglobulin in paraffin sections of multiple myeloma and macroglobulinemia using an immunoperoxidase technique. *Am J Pathol* 87:47, 1977

46. Urashima M, Chauhan D, Hatzianthi M, Ogata A, Hollenbaugh D, Aruffo A, Anderson KC: CD40 ligand triggers interleukin-6-mediated B cell differentiation. *Leuk Res* 20:507, 1996

47. Hesselton RM, Koup RA, Cromwell MA, Graham BS, Johns M, Sullivan JL: Human peripheral blood xenografts in the SCID mouse: Characterization of immunologic reconstitution. *J Infect Dis* 168:630, 1993

48. Bellamy WT, Odeleye A, Finley P, Hizenga B, Dalton WS, Weinstein RS, Hersh EM, Grogan TM: An in vivo model of human multidrug-resistant multiple myeloma in SCID mice. *Am J Pathol* 142:691, 1993

49. Dilber MS, Abedi MR, Bjorkstrand B, Christensson B, Gahrton G, Xanthopoulos KG, Smith CIE: Suicide gene therapy for plasma cell tumors. *Blood* 88:2192, 1996

50. Guerne PA, Carson DA, Lotz M: IL-6 production by human articular chondrocytes. Modulation of its synthesis by cytokines, growth factors, and hormones in vitro. *J Immunol* 144:499, 1990

51. Fraser C, Chen BP, Webb S, van Rooijen N, Kraal G: Circulation of human hematopoietic cells in severe combined immunodeficient mice after CL₂MDP-liposome-mediated macrophage depletion. *Blood* 86:183, 1995

52. Solomon A, Weiss DT, Kattine AA: Nephrotoxic potential of Bence Jones proteins. *N Engl J Med* 324:1845, 1991

53. Fattori E, Della Rocca C, Costa P, Giorgio M, Dente B, Pozzi L, Ciliberto G: Development of progressive kidney damage and myeloma kidney in interleukin-6 transgenic mice. *Blood* 83:2570, 1994