Anti-α4 integrin monoclonal antibody inhibits multiple myeloma growth in a murine model

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Abstract

In a syngeneic murine model of multiple myeloma with many of the characteristics of the human disease, a monoclonal antibody (mAb) to the integrin very late antigen-4 (VLA-4), given after the myeloma has already homed to and begun to establish itself within the bone marrow compartment, produces statistically significant effects on multiple disease variables. These include reductions in circulating levels of IgG2b; percentage of IgG2b-positive myeloma cells circulating in blood; spleen weight; and myeloma cell burden in spleen, bone marrow, and liver. mAb therapy had no effect on nonmalignant hematopoietic cells. An acute 6-day regimen of mAb treatment, initiated very late in disease to avoid mAb elimination in the immunocompetent animals, still significantly reduced spleen and blood myeloma cell burden. The ability of the VLA-4 mAb to affect multiple variables in this model, even as monotherapy, suggests this pathway plays a central role in disease progression. [Mol Cancer Ther 2005;4(1):91–9]

Introduction

Multiple myeloma is a B-cell malignancy characterized by the accumulation of monoclonal immunoglobulin-secreting plasma cells in the bone marrow compartment, accompanied by osteoclastic bone destruction and severe pain (1). A large body of evidence suggests that the support of stromal cells is indispensable for the retention, proliferation, and viability of cells of the B-cell lineage in the bone marrow compartment (reviewed in ref. 2). The adhesion molecule vascular cell adhesion molecule-1 (VCAM-1) expressed on hematopoietic cells is indispensable for the retention, proliferation, and viability of cells of the B-cell lineage in the bone marrow compartment (reviewed in ref. 2). The adhesion molecule vascular cell adhesion molecule-1 (VCAM-1) expressed on bone marrow stromal cells contributes significantly to this support through interaction with its counter-receptor, the integrin very late antigen-4 (VLA-4), expressed on B cells (3). Recent studies show that multiple B-cell malignancies, including myeloma, subvert this physiologic pathway to promote their own growth, survival, and resistance to chemotherapy (4–7). For example, adhesion of the human myeloma cell line 8226 to fibronectin via VLA-4 confers a survival advantage to these cells in response to doxorubicin or melphalan, whereas drug-resistant 8226 cell lines overexpress VLA-4 (4). In addition, the VLA-4/VCAM-1 interaction is important for myeloma-induced osteoclastogenesis, enhancing myeloma cell release of osteoclast differentiating factors (8).

To evaluate the possibility that blocking the VLA-4/VCAM-1 pathway might be of therapeutic benefit, we have used a recently developed murine model of myeloma (9). Vanderkerken et al. (10) first reported on the 5T myeloma arising spontaneously in C57BL/KaLwRij mice. We used a subclone of the 5T myeloma, designated 5TGM1, capable of reproducible aggressive induction of disease with many of the characteristics of human myeloma, including extensive tumor burden in bone marrow, monoclonal gammopathy, and osteolytic lesions, following injection (9). Bisphosphonates, established therapeutics in human myeloma for slowing the progression of bone disease (11), inhibit myeloma-induced bone destruction in these animals (12), validating their use as a model of human myeloma. In this study, we report that monoclonal antibody (mAb) PS/2, which binds to the α4 chain of murine integrin VLA-4, reduces tumor burden in bone marrow, spleen, liver, and in the blood compartment, as well as reduces circulating myeloma-specific IgG levels.

Materials and Methods

5TGM1 Myeloma Cell Line

5TGM1 myeloma cells (the gift of Dr. G. Mundy, University of Texas, San Antonio, TX) were grown in Iscove’s Modified Dulbecco’s Medium (GIBCO, Carlsbad, CA) supplemented with 20% fetal bovine serum (JRH BioScience, Lenexa KS), 2 mmol/L L-glutamine (Bio Whittaker, Walkersville, MD), and 1% penicillin streptomycin (Bio Whittaker) at 37°C in 5% CO2. Cells in log-phase growth were prepared for injection by precipitation in a centrifuge followed by a wash step with sterile endotoxin-free PBS. Finally, the cells were resuspended in endotoxin-free PBS at a concentration of 5 × 106 cells/mL.

GFP Lentiviral Transduction of 5TGM1 Cells

A lentiviral vector encoding green fluorescent protein (GFP) driven by the cytomegalovirus promoter was constructed and packaged by a method described by Wu et al. (13) was the gift of C. Kaynor (Biogen Idec, Cambridge, MA). Lentiviral infection of 5TGM1 cells was done as described by Kaynor et al. (14). The highest GFP-expressing 5TGM1 cells were then sorted on a MoFlow cell sorter (Cytomation, Fort Collins, CO).
C57Bl/KaLwRij Mice

C57Bl/KaLwRij mice (gift of Dr. G. Mundy, University of Texas, San Antonio, TX) were bred at Biogen Idec. The mice were housed in ventilated cage racks and allowed food and water ad libitum. At approximately 8 to 12 weeks of age, female mice were injected with 1 × 10⁸ 5TGM1 cells in a 200 µL volume into the tail vein. The mice were then divided into three groups: an untreated control group, a rat anti-VA-4 mAb PS/2 (American Tissue Type Collection, Manassas, VA) group, and a rat IgG2b isotype control mAb (PharMingen, San Diego, CA) group. Both mAbs were supplied in a low endotoxin, no sodium azide form. The mAbs were diluted into sterile endotoxin-free PBS at a concentration of 1 mg/mL. The mAbs were injected i.p. at a dose of 10 mg/kg. Biogen Idec’s Institutional Animal Care and Use Committee approved all animal protocols.

Murine IgG2b ELISA

ELISA plates (Corning, Corning, NY) were coated overnight at 4°C in a solution of PBS with 2 µg/mL of monoclonal rat anti-mouse IgG2b antibody (Zymed, San Francisco, CA). The coating antibody was removed and the plates were blocked with PBS containing 3% bovine serum albumin for 1 hour at 37°C. The plates were then washed five times with PBS containing 0.05% Tween 20 (Fisher Scientific, Pittsburgh, PA). The assay standard was purified mouse IgG2b myeloma protein MOPC195 (Cappel, Irvine, CA). The mouse plasma and the assay standard were diluted in PBS with 3% bovine serum albumin and incubated on the plate for 1 hour at 37°C. The plates were washed thrice and horseradish peroxidase-conjugated rat anti-mouse IgG (Biodesign, Kennebunk, ME) was added at 1:5,000 dilution for 1 hour at 37°C. The plates were again washed thrice and developed with 0.1% hydrogen peroxide and 1:5000 diluted in PBS with 3% bovine serum albumin for 1 hour at 37°C. The plates were then stained for lineage markers and intracellular IgG2b using CellQuest software (Becton Dickinson). The mAbs were injected i.p. at a dose of 10 mg/kg Biogen Idec’s Institutional Animal Care and Use Committee approved all animal protocols.

Tumor Burden Determination

Blood was collected into tubes treated with 0.78 mg of dipotassium EDTA (Therumo Medical Corp., Somerset, NJ). The mice were then dissected and the spleen, liver, tibia, and fibula were collected. The myeloma tumor burden in whole blood was determined by first counting the blood cells using an Abbott CellDyn 3500 cell analyzer (Abbott Diagnostics, Abbott Park, IL). Twenty microliters of whole blood were stained for 30 minutes at 4°C with a cocktail of phycoerythrin-labeled antibodies against the lineage markers CD4, CD8, CD45R/B220, CD11b, Ly-6G, and NK1.1 (PharMingen). The antibodies were diluted 1:200 in PBS containing 0.01% sodium azide and 1% bovine serum albumin [fluorescence-activated cell sorting (FACS) buffer]. The cells were washed twice with FACS buffer. The cells were then fixed and prepared for intracellular staining following the instructions in the Cytofix/Cytoperm Kit (PharMingen). To identify the IgG2b-positive 5TGM1 myeloma cells, the permeabilized cells were stained for 30 minutes at 4°C with a Cy5 anti-mouse IgG2b specific antibody (PharMingen). The cells were washed twice and then fixed in 2% paraformaldehyde. The cells were read on a FACS Calibur (Becton Dickinson, San Jose, CA) and analyzed using CellQuest software (Becton Dickinson). Tumor burden was determined first as the percentage of cells that were positive for intracellular IgG2b and negative for lineage markers. The final tumor burden (cells/µL) was determined by multiplying the percentage of lineage-negative IgG2b-positive cells by the number of leukocytes per microliter in whole blood as determined by the CellDyn analyzer.

To determine the tumor burden in the spleen the weight of the entire spleen was taken and divided in half. One half was preserved in 4% paraformaldehyde for histologic analysis. The remaining half was reweighed. The splenocytes were isolated from the later half of the spleen by grinding the tissue between two frosted glass slides (Fisher Scientific). The cells were put through a 100 µm nylon mesh cell strainer (Falcon/Becton Dickinson Labware, Bedford, MA) and then washed once with PBS. The cells were counted using a hemocytometer. The total number of cells in the spleen was then determined using the formula [number of isolated cells/(weight of spleen for cell isolation/total weight of spleen)]. Cells, 2 × 10⁵, were then stained for lineage markers and intracellular IgG2b as described above. Tumor burden was determined by multiplying the percentage of lineage-negative IgG2b-positive cells by the total number of cells calculated to be in the entire spleen.

To determine the tumor burden in the bone marrow, tibiae and fibulas were removed. Bone marrow cells were isolated from one tibia/fibula pair by flushing the marrow cavity with PBS using a 25-gauge needle and syringe (Becton Dickinson). The cells were collected, washed in PBS, and counted using a hemocytometer. Cells, 2 × 10⁵, were then stained for lineage markers and intracellular IgG2b as described above. Tumor burden was determined by multiplying the percentage of lineage-negative IgG2b-positive cells by the total number of cells in the tibia/fibula pair. One tibia/fibula pair was also preserved in 4% paraformaldehyde for histologic analysis.

Liver Histology and Blood Chemistry

To determine tumor burden, the livers were preserved in 4% paraformaldehyde, sectioned, and H&E stained. Liver sections were viewed at 200× magnification with a 10 × 10 objective grid on an Olympus BX41 microscope (Olympus America, Inc., Melville, NY). Grid squares containing nests of hematoxylin-positive myeloma cells were counted. Five to ten fields were counted per liver. Blood chemistry analysis of aspartate aminotransferase and lactic dehydrogenase was done by Ani Lytics, Inc. (Gaithersburg, MD).

Circulating PS/2 Levels

The concentration of free PS/2 in the blood was determined by FACS analysis. 5TGM1 cells (5 × 10⁶), grown in vitro, were incubated with plasma sample
Results

Intracellular IgG2b Staining Correlates with Tumor Burden

To measure myeloma cell burden in various tissues, we first evaluated the utility of intracellular IgG2b staining. The 5TGM1 myeloma cells secrete the IgG2b isotype, but above a background from endogenous plasma cells. We first generated GFP-expressing 5TGM1 cells using lentiviral transfection to allow us to follow the myeloma cells in vivo. Following injection of GFP-5TGM1 cells we found a direct correlation between GFP intensity and IgG2b staining (Fig. 1). These results indicate that myeloma cell burden can be followed using intracellular IgG2b staining, without the need to use luciferase, GFP, or other markers, which have the potential to alter the properties of the cells.

Effects of Long-term Treatment with mAb PS/2

Animals injected with $1 \times 10^6$ 5TGM1 cells on day zero were given mAb PS/2 at 10 mg/kg on days 4, 5, 6, 9, 12, 15, and 18, and tumor burden evaluated on day 21. A second independent experiment using identical dosing gave essentially the same results, and the data were combined. Four experimental groups were used: a disease-free control group ($N = 8$); an untreated myeloma control group ($N = 14$); a mAb PS/2-treated group ($N = 13$); and a rat IgG2b isotype control-treated group ($N = 10$). A third independent experiment in which the dose on days 9, 12, 15, and 18 was reduced from 10 to 5 mg/kg gave essentially identical results to those presented (data not shown).

Statistical Analysis

All data were represented as the mean ± SD and analyzed using the Student’s t test.

Effect of PS/2 Treatment in the Blood Compartment

Plasma IgG2b levels were reduced with PS/2 treatment by about 50% (Fig. 2A). In addition, the percentage of IgG2b-positive myeloma cells freely circulating in the blood was significantly reduced by about 70% (Fig. 2B).

Effect of PS/2 Treatment on Tumor Burden

Myeloma cell numbers in the spleen were reduced by about 70% only in the PS/2-treated group (Fig. 3A), as was total spleen weight (disease-free 89 ± 7 mg; untreated control 284 ± 77 mg; isotype-treated control 280 ± 39 mg; PS/2-treated 204 ± 55 mg; *, P < 0.01). Furthermore, tumor burden was also significantly reduced in the bone marrow, although by a more modest 15% (Fig 3B). Liver enzymes were elevated in myeloma-bearing animals, suggesting metastasis to the liver. In contrast, in the treated animals liver enzyme levels were significantly reduced (Table 1). Histologic examination showed a dramatic statistically significant reduction in tumor burden in this organ (Table 1; Fig. 4).

Hematopoietic Cell Numbers in Spleen and Bone Marrow

To examine the effects of treatment on normal hematopoietic cell numbers, we examined both spleen and bone marrow for the presence of nonmyeloma cells, as defined by IgG2b-negative FACS staining (Table 2). All groups with a myeloma cell burden had significantly higher nonmyeloma cell numbers in the spleen when compared with the disease-free control group, and PS/2 treatment did not significantly reduce the number of nonmyeloma cells in the spleen when compared with either the untreated group or the rIgG2b treatment group. In bone marrow all groups with myeloma burden had a significantly lower number of nonmyeloma cells, and PS/2 treatment had no statistically significant effect on cell numbers when compared with both untreated and
IgG2b-treated groups (Table 2). These results show that malignant cell numbers were selectively modified with PS/2 treatment (Figs. 3 and 4) whereas normal hematopoietic cells were spared.

**Circulating Levels of mAb PS/2**
We evaluated the levels of mAb PS/2 in circulation at day 21, and found that no mAb was detectable. Furthermore, although 84% of splenocytes were coated with PS/2, only 52% of bone marrow cells were coated (Table 3). These results show that in the long-term treatment protocol, saturation of VLA-4 on all tumor cells was not maintained despite the large quantities of mAb PS/2 injected into the treated animals.

**Effects of Short-term Treatment with mAb PS/2**
To evaluate conditions under which myeloma cell VLA-4 would remain saturated, we used a short-term treatment protocol. Animals injected with $1 \times 10^6$ 5TGM1 cells on day zero were given mAb PS/2 at 10 mg/kg on days 14 through 19, and tumor burden was again evaluated on day 20. The same four experimental groups were used as for the 21-day treatment protocol. In this protocol, circulating mAb PS/2 was still detectable on day 20, and 95% of splenocytes and bone marrow cells were coated with mAb, indicating receptor saturation (Table 3).
With this regimen, there was a 43% reduction in tumor burden in the blood and a significant 89% reduction in the spleen ($P < 0.01$). The mAb PS/2 significantly reduced bone marrow tumor burden compared with untreated control group ($P < 0.01$), however, the comparison to the isotype control mAb group was not statistically significant (Figs. 5A–C).

**Discussion**

Here we show that a mAb to integrin VLA-4 affects tumor burden in a highly aggressive murine model of multiple myeloma. MAb therapy, initiated after the myeloma has homed to and begun to establish itself within the bone marrow compartment, produces statistically significant reductions in (1) circulating levels of IgG2b; (2) percentage of IgG2b-positive myeloma cells circulating in blood; (3) spleen myeloma cell burden and spleen weight; and (4) bone marrow myeloma cell burden. MAb therapy also eliminates myeloma cell burden in the liver and reduces the increase in liver enzyme markers associated with disease, whereas it has no effect on nonmalignant hematopoietic cells. The immune response to the rat mAb PS/2 in the immunocompetent animals led to its rapid elimination, and prompted us to also evaluate an acute 6-day regimen

### Table 1. PS/2 treatment reduces tumor burden in the liver

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<tr>
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<th>Aspartate aminotransferase (units/L)</th>
<th>Lactic dehydrogenase (units/L)</th>
<th>Liver tumor colonies/field</th>
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<tr>
<td>Disease-free</td>
<td>124 ± 52</td>
<td>411 ± 73</td>
<td>None</td>
</tr>
<tr>
<td>Untreated control</td>
<td>573 ± 87</td>
<td>1349 ± 297</td>
<td>44 ± 22</td>
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<tr>
<td>PS/2 treatment</td>
<td>331 ± 50*</td>
<td>855 ± 129*</td>
<td>0.6 ± 0.3*</td>
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<tr>
<td>Rat IgG2b-treated control</td>
<td>641 ± 152</td>
<td>1542 ± 383</td>
<td>32 ± 23</td>
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NOTE: Analysis of plasma samples indicated that myeloma tumor burden resulted in a significant increase in aspartate aminotransferase and lactic dehydrogenase over untreated controls. Treatment with PS/2 mAb significantly reduced both aspartate aminotransferase and lactic dehydrogenase levels when compared with either the untreated group or the rat IgG2b isotype control group. Enzyme levels are expressed in units per liter. Histologic examination of H&E-stained liver sections revealed nests of myeloma cells in animals that received 5TGM1 cells. Tumor burden was measured as colonies per visual field. PS/2 mAb treatment, but not rat IgG2b control mAb, significantly reduced the number of myeloma cells in the liver. *$P < 0.01$.

With this regimen, there was a 43% reduction in tumor burden in the blood and a significant 89% reduction in the spleen ($P < 0.01$). The mAb PS/2 significantly reduced bone marrow tumor burden compared with untreated control group ($P < 0.01$), however, the comparison to the isotype control mAb group was not statistically significant (Figs. 5A–C).

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### Figure 4. PS/2 treatment reduced myeloma tumor burden in the liver.

H&E-stained liver sections are shown at ×40 magnification. Images of the liver sections were taken with a Leica DMR microscope. Untreated animals (B) have established myeloma colonies (arrows) in their livers when compared with disease-free animals (A). Animals treated with PS/2 (C) show a reduction in myeloma colonies whereas animals treated with rat IgG2b isotype control (D) show no effect.
Table 2. PS/2 treatment does not reduce endogenous splenocyte and bone marrow cell numbers

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<th>Spleen ($\times 10^7$)</th>
<th>Bone marrow ($\times 10^8$)</th>
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<tr>
<td>Disease-free</td>
<td>17.9 ± 2.57</td>
<td>38.5 ± 5.13</td>
</tr>
<tr>
<td>Untreated control</td>
<td>37.7 ± 10.5</td>
<td>2.9 ± 0.95</td>
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<tr>
<td>PS/2 treatment</td>
<td>35.2 ± 14.4</td>
<td>4.1 ± 1.68*</td>
</tr>
<tr>
<td>Rat IgG2b-treated control</td>
<td>29.2 ± 4.43</td>
<td>3.6 ± 0.9</td>
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NOTE: In all groups with 5TGM1 myeloma tumor burden, there was a significant increase ($P < 0.01$) in nonmyeloma (IgG2b-negative) cell numbers in the spleen. Conversely, in the bone marrow, all groups with myeloma burden had a significantly lower number ($P < 0.01$) of nonmyeloma cells (IgG2b-negative). In the spleen, PS/2 treatment did not significantly reduce the number of nonmyeloma cells when compared with either the untreated control group or the rat IgG2b isotype control group. In the bone marrow, PS/2 treatment group had a significant increase in IgG2b-negative cells when compared with the untreated control group. The difference between PS/2 treatment and IgG2b treatment groups was not significant.

NOTE: Free PS/2 levels were measured in the plasma. Following 6 d of treatment there are substantial levels of PS/2 in the blood. After 21 d of PS/2 treatment there was no circulating PS/2 detected in the plasma. The percent of VLA-4 occupancy shows that the splenocytes were 84% coated after 21 d and 95% after 6 d of treatment. The isolated bone marrow cells were 95% coated following 6 d of PS/2 treatment, but after 21 d of treatment, only 52% of the VLA-4 receptors were occupied.

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<th>PS/2 circulating levels (µg/mL)</th>
<th>VLA-4 occupancy (%)</th>
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<tr>
<td></td>
<td>Splenocytes</td>
<td>Bone marrow cells</td>
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<tr>
<td>Acute 6-day treatment</td>
<td>90 ± 14.8</td>
<td>94.6 ± 3.1</td>
</tr>
<tr>
<td></td>
<td>Nondetected</td>
<td>96.0 ± 1.2</td>
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NOTE: PS/2 treatment does not reduce endogenous splenocyte and bone marrow cell numbers; following liver tumor burden via enzyme levels and histology; examining the effects of treatment on normal hematopoietic cell numbers; and measuring receptor saturation in spleen and bone marrow.

These data provide in vivo evidence to support a large body of in vitro data suggesting that B-cell malignancies subvert the physiologic function of α4 integrin pathways to promote their own growth and viability (4–7, 17). For example, adhesion of human B-CLL cells to fibronectin prevents apoptosis (5), and similar results have been observed with B-cell acute lymphoblastic leukemia (6), non-Hodgkin’s lymphoma (17), acute myelogenous leukemia (7), and multiple myeloma itself (4). Furthermore, cytokine activation can enhance these effects (18). These results parallel the synoviocyte-dependent survival of B cells seen in the joint in rheumatoid arthritis (19, 20). In addition, the same effects have been observed with T-cell malignancies (21). However, other mechanisms of action also need to be considered. For example, VLA-4 seems critical to the homing of malignant cells to bone marrow. Transfection of the α4 gene into Chinese hamster ovary cells changes their homing from lung to bone, where they cause osteolysis (22), whereas deletion of VLA-4 from Nalm-6 B-ALL cells reduces their ability to home to bone marrow (23). Thus, inhibition of homing of myeloma cells may also play a role in the observed therapeutic effects.

The ability of the VLA-4 mAb to affect multiple variables in this model, even as monotherapy, suggests this pathway plays an important role in disease progression.

In parallel studies, 5TGM1 cell-induced myeloma in the xenogeneic Nude/Beige/XID mouse system is also inhibited by VLA-4 mAb PS/2 (16). Decreased bone marrow tumor burden and circulating IgG2b levels were observed and, importantly, significant inhibition of bone loss, consistent with the suggested role of the VLA-4 pathway in osteoclast differentiation (8). Here we confirm and extend these results by using syngeneic mice to include the full innate and adaptive immune response to both myeloma cells and therapeutic agent; developing the use of intracellular IgG2b staining as a universal marker of 5TGM1 cells, thereby avoiding the need for marker genes to follow tumor burden; measuring circulating myeloma cell levels directly; following liver tumor burden via enzyme levels and histology; examining the effects of treatment on normal hematopoietic cell numbers; and measuring receptor saturation in spleen and bone marrow.

These data provide in vivo evidence to support a large body of in vitro data suggesting that B-cell malignancies subvert the physiologic function of α4 integrin pathways to promote their own growth and viability (4–7, 17). For example, adhesion of human B-CLL cells to fibronectin prevents apoptosis (5), and similar results have been observed with B-cell acute lymphoblastic leukemia (6), non-Hodgkin’s lymphoma (17), acute myelogenous leukemia (7), and multiple myeloma itself (4). Furthermore, cytokine activation can enhance these effects (18). These results parallel the synoviocyte-dependent survival of B cells seen in the joint in rheumatoid arthritis (19, 20). In addition, the same effects have been observed with T-cell malignancies (21). However, other mechanisms of action also need to be considered. For example, VLA-4 seems critical to the homing of malignant cells to bone marrow. Transfection of the α4 gene into Chinese hamster ovary cells changes their homing from lung to bone, where they cause osteolysis (22), whereas deletion of VLA-4 from Nalm-6 B-ALL cells reduces their ability to home to bone marrow (23). Thus, inhibition of homing of myeloma cells may also play a role in the observed therapeutic effects.

The ability of the VLA-4 mAb to affect multiple variables in this model, even as monotherapy, suggests this pathway is important in disease. Nevertheless, the statistically significant but modest effect on the tumor burden in the marrow compartment shows that other pathways must also play a major role in the recruitment and retention of myeloma cells in bone marrow. For example, the CD44 pathway has been implicated in normal and malignant cell adhesion to bone marrow stroma (24, 25), and the SDF1/CXCR4 chemokine pathway is central to both normal and malignant B-cell recruitment (26, 27). Nevertheless, despite a modest effect on tumor burden, a significant effect was observed on circulating antibody levels. It is possible that mAb treatment inhibits antibody production from viable myeloma cells through blockade of VLA-4. Consistent
with this possibility, recent studies show that IL-6-mediated antibody secretion by plasma cells is dependent on their interaction with bone marrow stroma via VLA-4 (28, 29).

The almost complete elimination of myeloma cell burden in the liver was also evident, as seen histologically (Fig. 4) and as reflected in reduced liver enzyme values (Table 1). Whereas there is rarely liver involvement in human multiple myeloma, hepatic metastases are a common occurrence with several other malignancies, including breast, lung, and particularly colon carcinomas, as well as melanoma. Multiple publications suggest that the VCAM-1/VLA-4 pathway is important in liver metastasis. Both melanoma and colon carcinoma metastases are dependent on this pathway in mouse models (30–32), and increased levels of VCAM-1 are found on vessels adjacent to colon metastases in clinical samples (33).

Although the significant reduction in tumor burden in liver in this model may be relevant only to the very late stages of human myeloma, it is entirely consistent with the proposed role of the pathway in other metastatic settings. Cell adhesion-mediated chemotherapy resistance is a rapidly emerging concept in oncology, and integrin ligation by extracellular matrix is central to this process (34, 35). Whereas VLA-4-dependent ligation of both normal and malignant B cells inhibits apoptosis, as discussed earlier, it is now clear that such ligation can also result in striking resistance to chemotherapeutic agents. This has been clearly shown in vitro with B-cell acute lymphoblastic leukemia resistance to cytarabine or etoposide (6), lymphoma resistance to etoposide (36), B-cell chronic lymphocytic leukemia resistance to fludarabine (37), and multiple myeloma resistance to doxorubicin or melphalan (4, 38).

Recently, in vivo data were published showing strong synergy between VLA-4 mAbs and chemotherapy in eliminating minimal residual disease in acute myelogenous leukemia, which was also found to be resistant to cytarabine in vitro on ligation via VLA-4 (7). Interestingly, the VLA-4 mAb alone had no effect on survival in this model, despite striking synergy with cytarabine in vitro (7). These data argue that the combination of a VLA-4 mAb with the standard chemotherapeutic regimens in myeloma, which use agents such as melphalan or vincristine, might be particularly effective. Indeed, in initial experiments combination therapy with VLA-4 mAbs and melphalan seems to be efficacious (16).

Bisphosphonates, which inhibit bone loss, have recently become an established therapy for myeloma (11). Ibradonate inhibits bone loss in the 5TGM1 murine model but does not affect disease progression (12). Bisphosphonates
may also prove highly effective in combination with VLA-4 mAbs. Other therapeutic options for myeloma include thalidomide (39), proteasome inhibitors (39), erythropoietin (40), and blockade of the IL-15 or Rank ligand pathways (41, 42). Further exploration of combination therapies of VLA-4 mAbs with these and other modalities will be required to define synergies and optimal regimens.

Whereas VLA-4 blockade is being studied extensively for autoimmune and allergic diseases (43), the potential role of this pathway in oncologic applications has not been widely pursued. Our data, combined with others (7, 16), suggest that blockade of VLA-4, alone or in combination with other modalities, may have a significant effect in multiple myeloma and other B-cell malignancies.

Acknowledgments

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References

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