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Commentary

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In this issue of the *JCI*, Mukherjee et al. report that bortezomib, a clinically available proteasome inhibitor active against myeloma, induces the differentiation of mesenchymal stem/progenitor cells (MSCs) — rather than mature osteoprogenitor cells — into osteoblasts, resulting in new bone formation (see the related article beginning on page 491). These results were observed when MSCs were implanted subcutaneously in mice or were used to treat a mouse model of postmenopausal bone loss. Others have reported that immunomodulatory drugs (e.g., thalidomide and lenalidomide), which are active against myeloma, also block the activity of bone-resorbing osteoclasts. These results reflect the utility of targeting endogenous MSCs for the purpose of tissue repair and suggest that combining different classes of agents that are antineoplastic and also inhibit bone destruction and increase bone formation should be very beneficial for myeloma patients suffering from severe bone disease.

Mesenchymal stem/progenitor cells (MSCs) can differentiate into adipocytes, muscle cells, osteoblasts, or cartilage and possess potential for tissue repair in patients with osteoporosis, diseased joints, and myocardial infarction. Many groups have investigated strategies involving the infusion of MSCs for the purpose of regenerative therapy; however, problems concerning MSC homing to diseased sites and the use of allogeneic MSCs have limited this approach. Therefore, the ability to use pharmacological agents to induce the differentiation of resident MSCs toward a certain lineage in vivo is an important therapeutic goal.

In the study by Mukherjee et al. (1) reported in this issue of the *JCI*, bortezomib (Bzb), a first-in-class proteasome inhibitor that is an active antineoplastic agent for patients with relapsed and refractory myeloma, is used to induce MSC differentiation into osteoblasts in mice. Bzb can increase serum levels of bone formation markers (alkaline phosphatase and osteocalcin) in myeloma patients (2, 3). This potential bone anabolic activity of Bzb suggests that in addition to its antineoplastic effects, it may also have

beneficial effects on the severe bone disease associated with myeloma.

Myeloma bone disease is characterized by markedly increased activity of bone-resorbing osteoclasts due to production or induction of osteoclast-activating factors (OAFs) by the myeloma cells, which stimulate osteoclast formation. These OAFs include RANKL, IL-3, macrophage inflammatory protein-1 α (MIP-1 α), and IL-6. In addition, the levels of the decoy receptor for RANKL, osteoprotegrin, are also decreased (4). Further, activity of bone-building osteoblasts in myeloma is markedly suppressed or absent due to production of osteoblast inhibitors by myeloma cells, such as dickkopf-1 (DKK1) and secreted frizzled-related protein 2, and IL-7 and IL-3, which further exacerbate the bone destruction process (5). Serum levels of RANKL and DKK1 have been reported to be elevated in patients with myeloma while osteoprotegrin levels are decreased (6), and the RANKL to osteoprotegrin ratio correlates with survival of patients with myeloma (7).

Bzb decreases RANKL and DKK1 levels in the serum of myeloma patients (8), and response to Bzb has been correlated with increased alkaline phosphatase activity in myeloma patients (2). However, increased levels of bone formation markers in patients receiving Bzb can occur independently of the antimyeloma response (8). The mechanism for Bzb's effects on osteoblast differentiation has not been clearly defined.

Bzb and osteoblast differentiation

In the current study, Mukherjee and coworkers (1) report that it is MSCs rather than the more differentiated osteoblast progenitors that are the target cells for Bzb's effect on bone formation. Bzb increased osteoblast growth and differentiation, suppressed adipocyte differentiation in vitro, and induced new bone formation in mice that had undergone oophorectomy or subcutaneous implantation of MSCs. However, the authors did not find any effects of Bzb on osteoclast differentiation at the low concentrations tested. Proteasome inhibitors have been shown to be toxic at doses required to achieve beneficial therapeutic effects; however, the low concentrations of Bzb used in the current study did not appear to be toxic in mice treated with the drug. The lack of effect on osteoclasts is surprising since Zavrski et al. reported that proteasome antagonists such as Bzb can inhibit osteoclast precursor differentiation and bone resorption at low concentrations (9). Since Bzb inhibits NF- κ B activity, which is critical for osteoclast formation and survival, inhibition of osteoclast formation should occur (10). It is possible that the concentrations used in the current study may not be sufficient to inhibit NF- κ B activity in osteoclast precursors. Mukherjee et al. also found that runt-related transcription factor 2 (Runx-2) activity was stabilized by Bzb. Runx-2 is a key transcription factor required for osteoblast differentiation. These results are consistent with previous studies, which showed that Runx-2 degradation is mediated by Smurf1, an E3 ubiquitin ligase (11, 12). Smurf1 interacts directly with Runx-2 to mediate Runx-2 degradation via a ubiquitin proteasome-dependent process. Thus, proteasome inhibitors should prevent Runx-2 degradation.

Garrett et al. reported that proteasome inhibitors induce bone morphogenetic protein-2 (BMP-2) expression by osteoblastic cells, which in turn induces osteoblastic differentiation in vivo and in vitro (13). This enhanced BMP-2 expression is due to

Nonstandard abbreviations used: BMP-2, bone morphogenetic protein-2; Bzb, bortezomib; DKK1, dickkopf-1; MSC, mesenchymal stem/progenitor cell; OAF, osteoclast-activating factor; PTH, parathyroid hormone; Runx-2, runt-related transcription factor 2.

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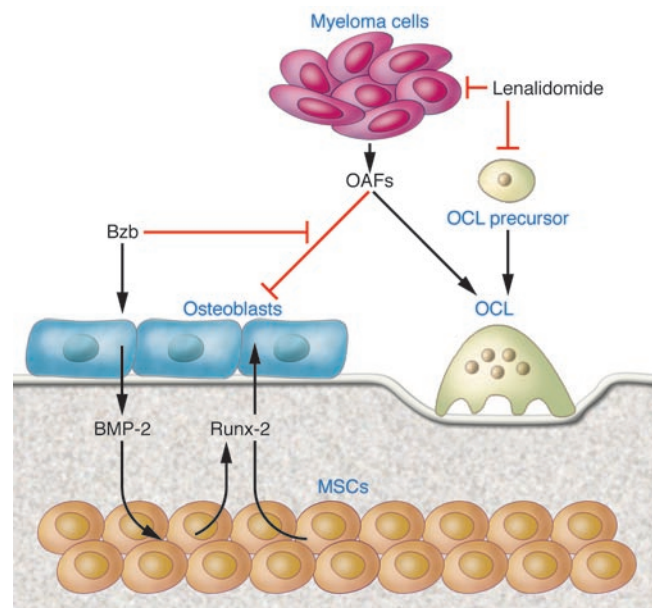


Figure 1

Combination treatment of myeloma bone disease. Myeloma cells produce or induce OAFs, which increase osteoclast formation as well as produce osteoblast-inhibiting factors, which block bone formation. In this issue of the *JCI*, Mukherjee et al. (1) show that the proteasome inhibitor Bzb can induce bone formation by increasing BMP-2 production by osteoblasts, which in turn increases Runx-2 levels, which induces MSCs to differentiate into osteoblasts and enhance bone regeneration in mice. In addition, other studies have shown that Bzb and lenalidomide can inhibit osteoclast (OCL) formation in addition to blocking the growth of myeloma cells (10, 16). These results suggest that combination therapy that includes Bzb with lenalidomide or thalidomide may both enhance the antineoplastic effects of either agent and increase bone formation by stimulating osteoblast activity and inhibiting osteoclastic bone destruction, respectively.

decreased proteolytic processing of Gli-3, which is normally degraded by the proteasome. Bzb's ability to increase BMP-2 expression has also been reported by Munemasa et al. in an abstract reported at the American Society of Hematology 49th Annual Meeting and Exposition (14).

Mukherjee et al. (1) also found that Bzb treatment of animals undergoing oophorectomy increased bone formation, although this did not reach statistical significance. Garrett et al. have shown that proteasome inhibitors can increase bone volume by 70% in mice after five days of treatment with these types of compounds (13). Further, Garrett et al. showed that this increased bone formation can be blocked by noggin, a BMP inhibitor. Possibly, Bzb may not be as potent an inducer of BMP-2 as the compounds used by Garrett et al.

Bzb as a treatment for myeloma bone disease

These studies demonstrate that proteasome inhibitors can increase osteoblast activity and appear to achieve this effect by targeting cells early in the osteoblast

lineage. However, it is unclear whether treating myeloma patients with Bzb alone is sufficient to reverse their bone disease. Although Bzb can increase the expression of bone-formation markers and the number of osteoblasts in bone biopsies in patients with myeloma (15), to date, lytic lesions have not been reported to heal in patients receiving Bzb (7). This suggests that combinations of agents will be required to reverse myeloma bone disease. Immunomodulatory drugs such as lenalidomide or thalidomide can decrease osteoclast formation and activity (16) and do not affect osteoblasts. Thus, combination therapy that includes Bzb with lenalidomide or thalidomide may both enhance the antineoplastic effects of either agent and increase bone formation by stimulating osteoblast activity and inhibiting osteoclastic bone destruction, respectively (Figure 1). These treatment regimens may be preferable to treating myeloma patients with bone anabolic agents such as parathyroid hormone (PTH), which can increase bone formation (17) but could possibly affect tumor growth. PTH

can induce IL-6 production by cells of the osteoblast lineage (18), and IL-6 is an antiapoptotic factor as well as a growth factor for myeloma cells. Thus, PTH may not be an appropriate agent for use in patients with myeloma. However, combinations of agents that are antineoplastic and can block bone resorption and stimulate bone formation may be highly beneficial for myeloma patients with severe bone disease. The possibility of pharmacologic manipulation of resident MSCs in myeloma patients with agents such as Bzb offers the potential for regenerating bone in lytic lesions that previously did not heal and suggests that pharmacologic manipulation of MSCs should be a useful strategy for other conditions associated with loss of bone or muscle function or with cartilage damage.

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Colitis and cancer: a tale of inflammatory cells and their cytokines

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Chronic inflammatory disorders are often associated with an increased cancer risk. A particularly striking example of the chronic inflammation–cancer link is seen in inflammatory bowel disease, in which chronic colitis or persistent inflammation in the colon is associated with elevated risk of colorectal cancer. Animal models exploring the mechanisms by which inflammation increases the risk of colon cancer have shown that inflammatory cells, through the effects of the cytokines they produce, have a major role in promoting neoplastic transformation. In this issue of the *JCI*, Popivanova and colleagues demonstrate that TNF- α , through its effects on the immune system, plays a critical role in promoting neoplastic transformation in this setting (see the related article beginning on page 560). Importantly, the study also provides evidence that anti-TNF- α therapies, which are currently in clinical use, may interrupt the process.

Inflammatory bowel disease (IBD) affects approximately 1.4 million people in the United States, with an estimated annual cost exceeding \$2 billion (1). IBD mainly consists of two disorders, ulcerative colitis (UC) and Crohn disease (CD). UC is restricted to the colon and/or rectum and always involves a continuous segment of variable length starting from the rectum.

Nonstandard abbreviations used: AOM, azoxymethane; CAC, colitis-associated cancer; CD, Crohn disease; CRC, colorectal cancer; DSS, dextran sulfate sodium; IBD, inflammatory bowel disease; IKK, I κ B kinase.

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CD is a more varied disorder, which can affect essentially any segment of the gastrointestinal tract, with a preference for the terminal ileum (2). While UC causes inflammation restricted to the mucosa, CD is associated with granulomatous features and transmural inflammation that can be complicated by intestinal wall fibrosis and stenosis, internal and external fistulas, and intra-abdominal infections. The involvement of the colon and rectum, irrespective of the subtype of IBD, increases the risk for colorectal cancer (CRC), and the risk is more pronounced with early onset of the disease and greater severity and greater extent of the colitis (3). In fact, though patients

with colitis-associated cancer (CAC) represent only about 1% of CRC cases, colitis patients are among those in the population at greatest risk of CRC. In patients with prolonged (>20 years) and extensive colitis involving the entire organ, the risk of CRC approaches 20%. Certain subsets of patients, such as those that have concurrent inflammation in the biliary tract (termed “primary sclerosing cholangitis”), have an even greater lifetime risk of CRC, approaching 50% (3).

The abnormal inflammatory response observed in IBD is thought to require the interplay between host genetic factors and the intestinal microbiota (4). Indeed, some patients with IBD seem to improve upon antibiotic treatment, and multiple animal models of colitis are ameliorated by the administration of antibiotics or placement of animals in germ-free conditions (4). Recently, the demonstration that a subset of CD patients carries mutations in the nucleotide-binding oligomerization domain-containing 2 (*NOD2*) gene (5–7), which encodes an intracellular pattern-recognition receptor for bacterial muramyl dipeptides (4, 8), bolsters the notion that an abnormal balance in the immune response to gut bacteria may be a central and general feature in IBD.