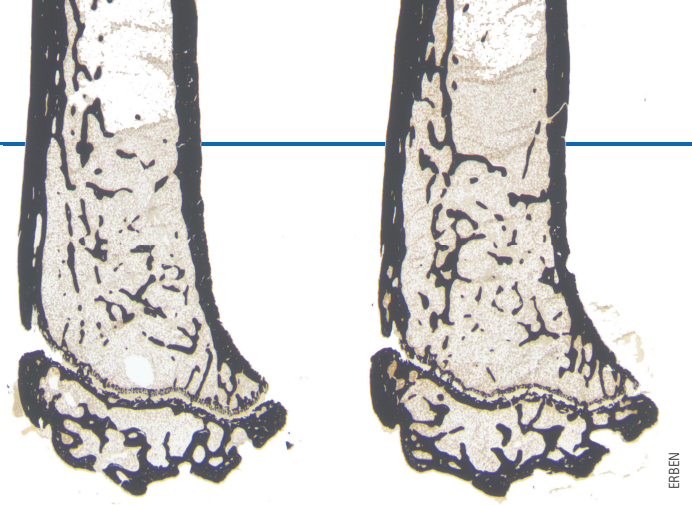
T cells to blame?



ERBEN

Bone loss after ovariectomy is comparable in the presence (left) or absence (right) of T cells.

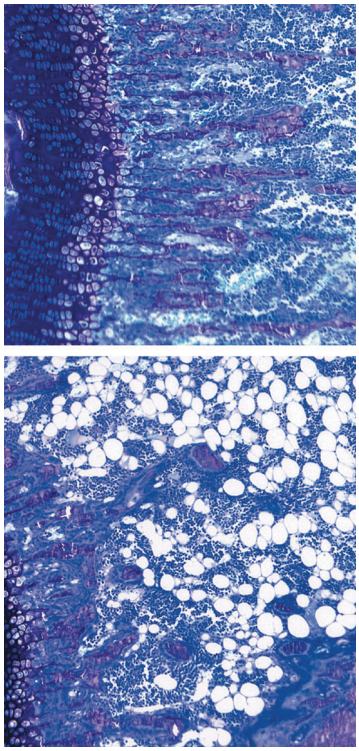
Low estrogen—during menopause in women or induced by ovary removal in rodents—causes osteoporosis. Roberto Pacifici (Emory University School of Medicine, Atlanta, GA) has put the blame for this bone loss squarely on T cells and, at the meeting, he provided corroborating evidence. But new data from Reinhold Erben (University of Veterinary Medicine, Vienna, Austria) seems to exonerate these cells.

Perhaps the strongest evidence that T cells cause bone loss when estrogen is in short supply comes from T cell–deficient ("nude") mice. In past studies, Pacifici and colleagues showed that nude mice—unlike wild-type mice—do not develop osteoporosis when their ovaries are removed. Bone loss kicks in when T cells are transferred into the mice. This effect depends on T cell production of the cytokine TNF, which causes stromal cells to produce growth factors that stimulate bone-resorbing osteoclasts.

Bone loss is also a symptom of T cell–driven autoimmune diseases, such as rheumatoid arthritis (RA). And the symptoms of RA worsen after childbirth and menopause, when estrogen levels are low, also hinting at a possible connection between estrogen, T cells and bone loss. Pacifici's group has now found that ovariectomized mice treated with a T cell–inhibiting drug (Abatacept) have lower levels of circulating TNF and are spared the bone loss normally induced by ovary loss. When the drug was discontinued, bone loss resumed.

Erben does not dispute the mouse data, but rather questions their relevance to other species. "This may be true for mice," says Erben. "But whether this model is predictive of human physiology is unclear." Erben's data showed that thymectomized rats depleted of circulating T cells still developed osteoporosis when their ovaries were removed. And the rat, suggests Erben, may be a better model of postmenopausal osteoporosis than the mouse. In the rat model, as in humans, bone loss is sustained, whereas in mice, bone loss is transient. JEM

Reference: Weitzmann, M.N. 2006. J. Clin. Invest. 116:1186–1194.



HOROWITZ/MUNKSGAARD

Fat cells (white) accumulate in the bone marrow of mice lacking EBF-1 (bottom).

Does this gene make my bone marrow look fat?

Bone marrow cavities fill up with fat in mice lacking the transcription factor EBF-1 (early B cell factor-1), according to data from Mark Horowitz (Yale University School of Medicine, New Haven, CT).

The evolutionarily conserved EBF transcription factors (EBF-1–4) are required for the development of olfactory neurons and B cells. EBF-1 also promotes fat cell (adipocyte) differentiation when overexpressed in preadipocyte cell lines, but the mechanism—and in vivo significance—of this finding was unclear.

To investigate the role of EBF-1 in fat formation, Horowitz and colleagues examined mice lacking the transcription factor. In addition to having no B cells, the mice had fat in all the wrong places. Subcutaneous fat was almost entirely missing, possibly explaining their previously described wasted appearance. The mice instead had fat where their bone marrow should be.

Excess fat wasn't their only problem. The EBF-1–deficient mice also had increased bone mass due to an overabundance of osteoblasts. The combined fat–bone phenotype prompted Horowitz to suggest that EBF-1 might regulate gene expression in a common adipocyte–osteoblast precursor cell, as these cells are known to arise from a common progenitor. But this hunch is difficult to test. Unlike in B cell development, few markers have been identified to distinguish osteoblast precursor cells at various stages of differentiation. In this respect, says Horowitz, "bone biologists are 15 years behind immunologists."

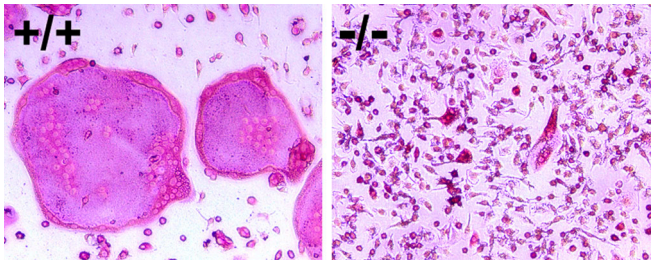
The only targets of EBF-1 identified thus far are those involved in B cell development. One of those targets, the B cell–specific transcription factor encoded by Pax5, is also required for bone maintenance. But in bone, the relationship between EBF-1 and Pax5 is more complicated, as the absence of Pax5 causes severe bone loss—the opposite of the defect caused by EBF-1 deficiency. JEM

Reference: Horowitz, M.C., et al. 2005. Immunol. Rev. 208:141–153.

Osteoclasts that can't unite

The formation of bone-resorbing osteoclasts requires cell–cell fusion between monocyte lineage precursor cells. But little is known about how this fusion is regulated and what proteins it requires. According to Yongwon Choi (University of Pennsylvania, Philiadelphia, PA), fusion among preosteoclasts requires a subunit of the vacuolar ATPase (v-ATPase) proton pump—but not the proton pumping action of the v-ATPase itself.

Choi and colleagues had previously identified a novel isoform of the v-ATPase d2 subunit (d2)—one of the five subunits that form the membrane-spanning proton channel—in a search for genes preferentially expressed in osteoclasts. Finding d2 was not surprising, as osteoclasts use plasma membrane v-ATPases to acidify the extracellular space between the cell and the bone surface—the first step in bone resorption.



CHOI

Fusion of preosteoclasts is faulty in the absence of d2 (right).

The surprise came, as presented by Choi, when the group eliminated d2 from mice. The d2-deficient mice had increased bone mass, not because their osteoclasts were defective in resorption, but simply because there were too few of them—at least of the multinucleated sort that digest bone the best. The paucity of multinucleated osteoclasts did not reflect a block in differentiation, as precursor cells from the d2-less mice still developed into mononuclear osteoclasts, which retained the normal low-level resorption activity. But without d2, the mononuclear cells couldn't fuse.

The normal mechanism of d2 action remains a mystery. In other cell types, v-ATPases are found on intracellular membranes where they promote pH-dependent membrane fusion. But, so far, a role for v-ATPase subunits in cell–cell fusion has been documented only in *C. elegans*. There, a v-ATPase subunit prevents the inappropriate fusion of embryonic cells by somehow blocking the expression of a fusion-promoting membrane protein. JEM

Reference: Rho, J., et al. 2002. DNA Cell Biol. 21:541–549.

Osteoclasts on steroids

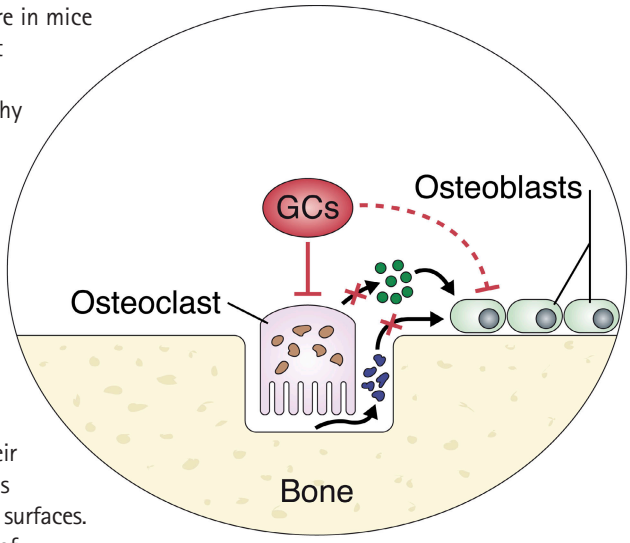
According to data from Steven Teitelbaum (Washington University, St. Louis, MO), glucocorticoids (GCs)—the most widely used class of antiinflammatory drug—prevent osteoclasts from rearranging their cytoskeletons and thus from digesting bone. And without proper osteoclast function, bone-building osteoblasts also shut down, which might help explain the crippling osteoporosis that often accompanies long-term steroid therapy.

Prolonged GC therapy impairs normal bone remodeling, an ongoing process in which osteoclasts dig small pits in bone and osteoblasts fill them up with new bone. This continual turnover is essential to maintain the structural integrity of the bone. In patients with GC-induced osteoporosis, these pits do not get fully filled. The mechanism behind this defect was controversial, particularly as treating osteoblasts with GCs in vitro actually enhances their activity.

GCs might also have an indirect effect on osteoblasts, reasoned Teitelbaum, as the activity of osteoblasts and osteoclasts are tightly linked—an increase or decrease in the activity of one cell type prompts a parallel change in the other. Teitelbaum found that mouse osteoclasts treated with the GC dexamethasone failed to spread out on bone surfaces and had decreased bone-resorbing capacity. This dampened osteoclast activity triggered a subsequent decrease in bone formation, as bone loss was less severe in mice whose osteoclasts (but not osteoblasts) lack the glucocorticoid receptor. Why the decrease in osteoblast activity ultimately outweighed the decrease in osteoclast activity is not yet clear.

The osteoclast defect was traced to a block in the activation of the GTPases RhoA and Rac, which are needed for osteoclasts to rearrange their actin cytoskeletons and thus form a tight seal with bone surfaces. GCs blocked the activation of GTPases by suppressing the phosphorylation of the osteoblast-specific guanine nucleotide exchange factor Vav3.

In the meantime, these data raise potential concerns about the use of antiresorptive agents (such as Fosamax) to treat osteoporosis. Although these agents curb bone loss, they have also been reported to decrease bone remodeling, which causes brittle, fracture-prone bones. "It is not only the amount of bone present [that is important]," says Teitelbaum, "but also the quality of the bone." JEM



Glucocorticoids inhibit osteoclast function, which in turn impairs osteoblast function.

Reference: Kim, H.J., et al. 2006. J. Clin. Invest. 116:2152–2160.

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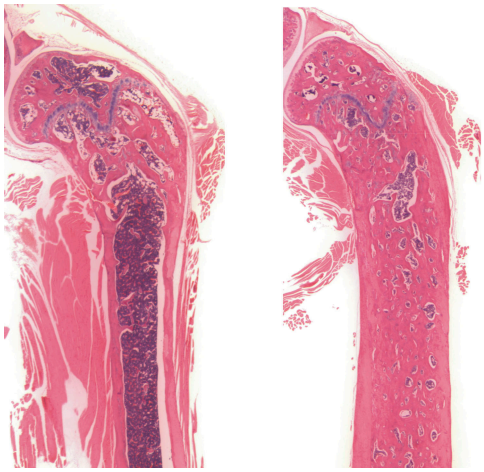

GLIMCHER/AAAS

Shn3-deficient mice (right) lack bone marrow.

Marrow-less mice

A give-and-take between the action of bone-building osteoblasts and bone-resorbing osteoclasts keeps bone mass constant. Both cell types are controlled by an array of regulatory proteins, only some of which have been identified. One of these regulators, described by Laurie Glimcher (Harvard Medical School, Boston, MA), suppresses bone-building activity by marking an osteoblast-specific transcription factor for destruction.

Glimcher's group had previously identified the regulator in question—the zinc finger protein schnurri-3 (Shn3)—as an adaptor protein expressed in $CD4^+$ T helper (Th)-1 cells that drives IL-2 expression. But when the group eliminated Shn3 from mice, they ran into an unexpected problem. "My postdoc, Dallas [Jones], was having trouble getting bone marrow from the deficient mice," says Glimcher, "so we took an x-ray [to find out why]." The x-ray revealed an age-related increase in bone mass so severe that, by seven months of age, the mice had virtually no marrow left.

The Shn3-deficient mice had bulked-up bones due to overly active osteoblasts. Osteoblasts from these mice had increased expression of Runx2, the preeminent transcription factor that drives osteoblast differentiation and function. In normal mice, Glimcher showed, Shn3 binds to the ubiquitin ligase WWP1. Shn3 and WWP1 then team up to ubiquitinate Runx2, tagging it for proteasomal degradation.

Normally, osteoclasts get revved up to compensate for increased osteoblast activity. Why this fails to occur in the absence of Shn3 is unclear, as Shn3-deficient osteoclasts appeared to function normally. The team is now investigating this question. JEM

Reference: Jones, D., et al. 2006. Science. 312:1223–1227.

Tackling TREM2

Data presented by Marco Colonna (Washington University, St. Louis, MO) and Mary Nakamura (UCSF, San Francisco, CA) raised more questions about the bone defects in a rare genetic disorder—Nasu-Hakola disease—than they answered.

Previous work by Colonna's group showed that monocytes from patients with Nasu-Hakola disease fail to form bone-resorbing osteoclasts in vitro, suggesting that TREM2 is required for osteoclast differentiation. Nakamura's new mouse data extended this finding, showing that blocking TREM2 with antibodies prevented both the differentiation of osteoclast precursors and the resorptive function of mature osteoclasts.

But Colonna's mouse studies suggest the opposite—precursor cells from mice lacking TREM2 differentiated into osteoclasts more efficiently than wild-type cells. "The problem [with the antibody data]," says Colonna, "is that we don't know whether the antibody is activating or inhibiting [TREM2 signaling]."

Indeed both inhibitory and activating functions for TREM2 have been reported. Ligation of TREM2 on brain cells, for example, increases phagocytosis. On macrophages, its ligation dampens cytokine production.

The avidity of a ligand for TREM2 might determine whether a positive or negative signal is transmitted. The hunt is on for these TREM2 ligands. JEM

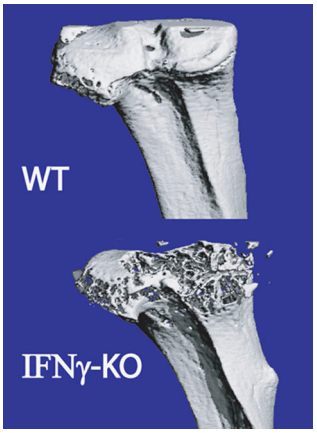
References: Cella, M., et al. 2003. J. Exp. Med. 198:645–651.
Humphrey, M.B., et al. 2006. J. Bone Miner. Res. 21:237–245.

T cells egg on osteoclasts

Inflammatory disease and infection often go hand-in-hand with bone loss. Mucosal infection with the parasite *Leishmania*, for example, can destroy bones in the face and palate. Bone destruction in a mouse model of this disease, according to Marlon Quinones (University of Texas, San Antonio, TX), is triggered by activated $CD4^+$ T cells, which spur on bone-digesting osteoclasts.

$CD4^+$ T cell differentiation is a known determinant of disease outcome in mouse models of leishmaniasis. A Th1 response—dominated by the $CD4^+$ T cell production of interferon (IFN)-γ—protects against the parasite, whereas a Th2 response—dominated by the production of interleukin (IL)-4, is associated with susceptibility.

This Th1–Th2 balance, according to Quinones, also contributes to infection-induced bone loss. Mice lacking IFN-γ developed local and systemic bone loss when infected intradermally with *Leishmania major*, due to a massive accumulation of osteoclasts. The overabundance of osteoclasts was initiated by T cells, as transferring IFN-γ–deficient $CD4^+$ T cells into lymphocyte-deficient mice triggered bone loss.



QUINONES

***Leishmania* infection triggers bone loss in IFN-γ–deficient mice.**

Bone destruction did not depend on T cell production of the osteoclast growth factor RANKL, which contributes to bone loss in other diseases, such as rheumatoid arthritis. The real culprit, suspects Quinones, is IL-17, which was produced in excess by the IFN-γ–deficient T cells and is required in other T cell–driven diseases. The group, co-led by Seema Ahuja, is now testing their suspicion by blocking IL-17 in the IFN-γ–deficient mice. JEM