Close Encounters with an Osteoclast

Chemical factors produced at specific sites in bone—primarily by immune system cells in the marrow—regulate the resorption and formation processes required to maintain bone mass

"It would be like the scene from *Close Encounters of the Third Kind* when the spaceship finally descends to earth," says Gregory Mundy of the University of Texas Health Science Center at San Antonio. "If you were sitting in an area of bone that is about to be resorbed you would see a giant multinucleated osteoclast settling down like a spaceship. It would send out processes that look like seaweed and then pump acid across this border, leaching the minerals out of the adjacent bone. And in that acid environment, the osteoclast would degrade the remaining protein matrix, leaving a crater just like a crater on the moon."

Osteoclast-mediated breakdown of bone may sound malevolent, in contrast to the landing of the "Close Encounters" spaceship, but is instead part of the normal remodeling process of the skeleton. Identifying what local factors control this process and the events in bone formation that inevitably follow is, according to Mundy, perhaps the most exciting area in bone research today.

"If we can identify the endogenous regulatory processes for bone turnover, then we can develop useful treatments for bone diseases such as osteoporosis or even fracture repair in elderly people," Mundy predicts. About 1.2 million osteoporosis-related fractures occur in the elderly each year—about 538,000 new cases of spinal fractures per year and 227,000 hip fractures, according to a *New England Journal of Medicine* article by B. Lawrence Riggs and L. Joseph Melton of the Mayo Clinic in Rochester, Minnesota. Twelve to twenty percent of the hip fractures are fatal because they cause severe trauma and bleeding, and half of the surviving patients require nursing home care. Total costs related to osteoporosis now amount to at least $7 billion annually in the United States alone (*Science*, 20 February, p. 833).

Evidence gleaned within the past 2 or 3 years indicates that chemical factors secreted from immune system cells regulate the balance between bone resorption and formation, thus preserving bone mass and helping to impede the onset of osteoporosis. This group of substances—produced in discrete regions of bone by the blood-forming or hematopoietic cells of the marrow—interacts in complex ways at various stages of remodeling to control the two major processes of bone turnover, resorption and formation. These and other local factors apparently act in concert with factors that circulate in the bloodstream, particularly the hormones calcitonin and parathyroid hormone, and vitamin D.

Much of the new research has been made possible by the recent availability of purified immune system factors and growth factors that affect bone remodeling. Many researchers predict the effects of these agents on normal bone in vivo by evaluating their effects on fetal bone maintained in organ culture. Some of the substances that affect bone restructuring also enhance blood cell production by the bone marrow. Two of them, colony-stimulating factors G (for granulocyte) and GM (for granulocyte/macrophage), are being tested as potential treatments in patients with anemia, cancer, bone marrow transplants, and AIDS (*Science*, 1 May, p. 517).

"Over the years, all of the bone in a person's body will turnover," says Lawrence Raisz of the University of Connecticut Health Center in Farmington. "But the amount of bone surface that is being remodeled at any one time is quite small—probably about 5%." The turnover occurs at specific sites in trabecular bone, the light, honeycombed meshwork that also contains marrow and hematopoietic cells. Denser, cortical bone, about half of which is an organic matrix made of the protein collagen with the other half consisting of calcium-containing hydroxyapatite crystals imbedded in the matrix, undergoes remodeling at its borders with the marrow and along the numerous small canals that contain blood vessels and penetrate it.

A key to understanding what controls the first of the two remodeling processes, bone resorption, is identifying what activates osteoclasts, the mobile multinucleated cells that degrade existing bone. Osteoclasts themselves are somewhat of a mystery, at least in terms of their formation. The precursor cells originate in bone marrow and circulate in the blood, but researchers argue whether osteoclasts are part of the monocyte/macrophage line of cells.

Regardless of their origin, the processes related to bone resorption by osteoclasts include proliferation of the precursor cells, their migration to specific bone sites, fusion to form osteoclasts, activation of mature osteoclasts to cause bone resorption, and inactivation to stop resorption.

Recent data from Mundy, G. David Roodman, and Brian MacDonald, also of the University of Texas Health Science Center, indicate that colony-stimulating factors (CSF) probably contribute to early stages of the osteoclast production process. "Colony-stimulating factors are made by many different cell types," says Mundy. "They do not seem to stimulate an already-existing osteoclast to resorb bone, but they probably assist in the formation of osteoclasts from precursor cells." In particular, CSF-M (also called CSF-1), which controls macrophage production, and CSF-GM, which regulates the differentiation of the precursors that form white blood cells called granulocytes and macrophages, also stimulate the proliferation of osteoclast precursor cells.

A model formulated by Mundy and Roodman predicts that a collection of factors produced locally by lymphocytes, monocytes, and macrophages in the bone marrow promotes the next stage of osteoblast formation. Interleukin-1, transforming growth factor α, tumor necrosis factor α, and lymphotoxin (also called tumor necrosis factor β) stimulate the next round of cell proliferation of precursor monocyte-type cells to form an early-stage osteoclast, according to data from Mundy, Maxine Gowen, and David Bertolini, also of the University of Texas Health Science Center. Osteocalcin, also known as Gla protein, may attract osteoclast precursors to bone surfaces. Then 1,25-dihydroxyvitamin D and circulating parathyroid hormone may act synergistically, causing fusion of the precursors to form a multinucleated osteoclast.

As bone marrow lymphocytes and cells of the monocyte/macrophage line interact to form osteoclasts, the factors they secrete, termed lymphokines and cytokines, respectively, also appear to activate mature osteoclasts. These factors are aided by prostaglandin E_2, one of a family of compounds whose cellular origin in bone is unknown but may include either cells of the osteoblast or monocyte/macrophage lineages.

But rather than acting directly on osteoclasts, bone-resorbing factors—cytokines and systemic factors such as parathyroid hormone and 1,25-dihydroxyvitamin D—act on an intermediate cell type. Researchers do not know the exact identity of the cellular target, but it may be a cell in the osteoblast

lineage, according to recent evidence from Timothy Chambers of St. George's Hospital in London and his colleagues. Mundy and Roodman's model suggests that this intermediate cell then secretes its own activating factor(s)—as yet unidentified—to stimulate the osteoclast directly.

Following its activation, the osteoclast is ready to conduct its bone-dissolving business. "The osteoclast needs to digest the extracellular matrix of bone, which is calcified," says Roland Baron of Yale University School of Medicine. In a deviation from Mundy's osteoclast spaceship analogy, Baron likens the apposition of the osteoclast and bone to a cup set upside down on a table. "The cup (or osteoclast) seals off a compartment underneath it and into this compartment it secretes protons and lysosomal enzymes," he says. The hydrogen ions or protons create the acidic environment in which the calcium dissolves and the enzymes digest the organic matrix made of collagen and other proteins.

Researchers are also exploring the means by which the osteoclast establishes its external acidic environment. Several years ago, Carol Gay of Pennsylvania State University in State College and her colleagues showed that carbonic anhydrase, the enzyme that forms bicarbonate from carbon dioxide and water, may provide the protons.

Recently, Baron and his colleagues described two different membrane pumping mechanisms for moving these protons across the osteoclast membrane. The first, identified by Baron, Lynn Neff of Yale, Daniel Louvard of the Pasteur Institute in Paris, and Pierre Courtoy of the International Institute of Cellular and Molecular Pathology in Brussels, Belgium, requires an ATPase, an enzyme that degrades the energy-containing molecules of adenosine triphosphate. Perhaps as it does in cells that line the stomach, the ATPase in osteoclasts exchanges protons for potassium ions. Or, as in the kidney, it may simply pump the protons out. The second pump, reported by Baron, Neff, Christine Roy, Angela Boisvert, and Michael Caplan, also of Yale, is an ATPase that exchanges sodium ions for potassium ions. It may set up a gradient that allows the influx of calcium from bone into the extracellular space.

Osteoclast-mediated bone resorption occurs over a prolonged period of about 10 days in vivo and then terminates, probably by the activity of several factors. For instance, the overall effect of the circulating hormone calcitonin, both in vitro and in vivo, is to inhibit bone resorption transiently, although its precise mechanism of action is unknown. Glucocorticoids (hormones secreted from the adrenal cortex) also inhibit osteoclast formation and activity. Acting more locally at the site of resorption, gamma interferon—produced by stimulated lymphocytes—inhibits bone formation in vitro. Roodman and Chantel Chenu, also of the University of Texas Health Science Center have new evidence indicating that transforming growth factor β also has the same effect.

The substances that regulate the second major process of bone remodeling—bone formation—are even less well understood, according to Ernesto Canalis of St. Francis Hospital Medical Center in Hartford and the University of Connecticut School of Medicine in Farmington. Over a period of 3 or 4 months in vivo, bone-forming osteoblasts undergo processes that parallel those of their bone-resorbing counterparts. Osteoblast precursors proliferate, migrate to the site at which osteoclasts have resorbed bone, differentiate into mature osteoblasts, become activated to produce collagen, and may also play a role in bone mineralization.

Several factors probably work in concert to regulate the early processes. "One group includes a bone-derived growth factor, somatomedin C (also called insulin-like growth factor 1), and transforming growth factor β, none of which are unique to bone," says Canalis. All stimulate collagen synthesis, which precedes bone formation, and the proliferation of osteoblast precursors, which are cells of unknown origin.

A second set of formation factors found in bone matrix, but not made in vitro like the first group, includes fibroblast growth factor and platelet-derived growth factor. Cartilage-derived growth factor, which is similar to bone-derived growth factor, Canalis says, also stimulates bone formation.

In addition, "prostaglandin E_2 seems to

New Leads in Osteoporosis

Osteoporosis occurs most frequently in women 50 years of age and older and is attributed, at least in part, to the postmenopausal decline in estrogen levels. The disease, often characterized by spontaneous fractures of the spinal vertebrae, hip, and forearm, is due to bone loss. But what role estrogen plays in maintaining normal bone mass—and thereby decreasing the severity of osteoporosis—has remained a mystery. New evidence from B. Lawrence Riggs of the Mayo Clinic in Rochester, Minnesota, and his colleagues indicates that estrogen may act directly on bone, specifically on the bone-forming cells called osteoblasts.

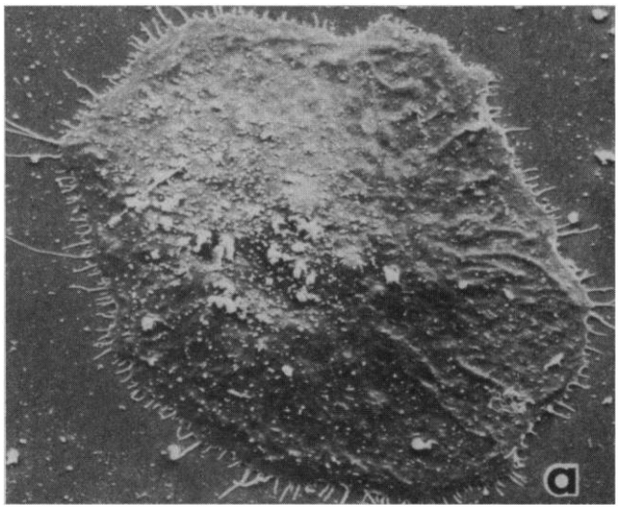
"We have demonstrated evidence of estrogen receptors in osteoblasts," says Riggs. "Other groups were not able to find these receptors and it was therefore assumed that the effects of estrogen on bone were mediated indirectly." The new data suggest that, because estrogen may bind to osteoblasts, the hormone may affect the bone-forming cells directly.

Two techniques used by Riggs, Thomas Spelsberg, and Eric Erickson, also of the Mayo Clinic, allowed them to detect evidence of specific estrogen binding in osteoblast-like cells. First, the researchers modified a culture system for growing human bone cells, about 90% of which are osteoblast-like. "These are not mature osteoblasts, because, by definition, the mature cells are terminally differentiated and will not grow in culture," says Riggs. "But the cells we grow have all the phenotypic characteristics of mature osteoblasts." These include the marker proteins osteocalcin, osteonectin, and type I collagen.

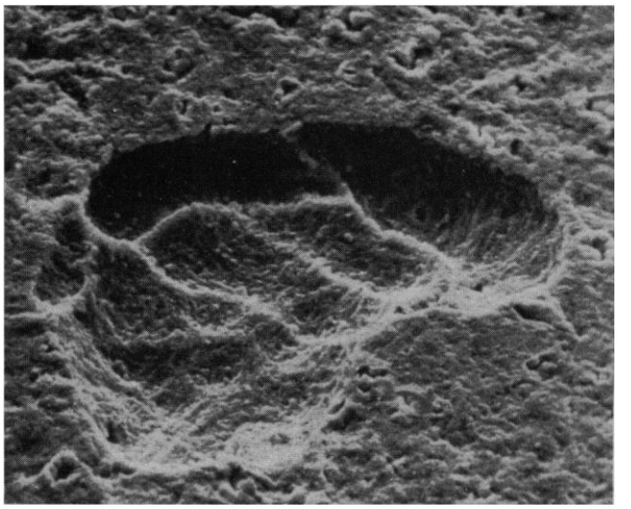
The second technique, devised by Spelsberg, increases the sensitivity of the estrogen receptor assay. "We do not just measure binding," says Riggs. "To be positive in our assay the radioactively labeled estrogen must bind and also be transported to a nuclear receptor site." Riggs and his co-workers do not yet know how estrogen mediates its effects at the cellular level, but the well-documented effects of the hormone at the tissue level are to decrease bone loss.

Currently, some women with osteoporosis receive estrogen as a treatment, but the therapy is limited by its lack of effectiveness in older women and its side effects. "If estrogen is given after the 8- to 10-year period following menopause it is not effective as a treatment for osteoporosis," says Riggs. Two complications of long-term estrogen therapy are cancer of the endometrium that lines the uterus and blood clots. But researchers are designing ways to counterbalance these effects, and Riggs indicates that the trend is to use estrogen more often as a treatment for osteoporosis. ■ **D.M.B.**

The new results will be presented at the Annual Meeting of the American Society for Bone and Mineral Research, to be held 7–9 June in Indianapolis, IN.



A giant multinucleated osteoclast *growing in tissue culture sends out processes as it would in vivo during bone resorption. (×1000)* [*Courtesy of M. J. Oursler, L. V. Bell, B. Clevinger, and P. Osdoby,* J. Cell Biol. **100**, *1592 (1985).*]



A moonlike crater in bone, *called a resorption lacuna, is created by an osteoclast and would be filled as osteoblasts make new bone.* [*Courtesy of A. Boyde, N. Ali, and S. Jones,* Scanning Electron Microsc. **3**, *1259 (1985).*]

be a major product of bone and stimulates both bone resorption and formation under the right conditions," says Raisz. "Prostaglandins are very complex. They clearly stimulate bone resorption after chronic exposure, stimulate bone formation at low concentrations, and inhibit it at high concentrations."

The factors that promote bone mineralization are not known precisely, but may include osteocalcin and osteonectin. "There is no question that both osteonectin and osteocalcin are made by osteoblasts," says Riggs. "These substances may soon be used as diagnostic markers to identify active bone reformation." For example, by determining whether an osteoporosis patient has a high rate of bone turnover or a low rate, doctors could either give drugs that inhibit or stimulate bone formation. In addition to these locally acting factors are circulating compounds, vitamin D and parathyroid hormone, which have more general effects on bone production.

More unanswered questions concern what cell types and mechanisms couple the osteoclast-mediated process of bone resorption with the osteoblast-mediated processes of bone formation. Osteoblasts themselves may link the processes. For example, they make collagenase, the enzyme that degrades the organic matrix of bone after the calcium has been leached out. Osteoblasts may also be the mysterious intermediate cell type stimulated directly by osteoclast-activating factors. They may then secrete a separate factor which activates the osteoclast to dissolve bone, according to the model proposed by Mundy and Roodman.

Preserving the balance between the processes of bone resorption and formation "is the key to maintaining bone mass," says Mundy. "Osteoporosis is the consequence of an imbalance between the two. The main problem in the form of osteoporosis that affects postmenopausal women between 50 and 60 years of age is that, after a decrease in estrogen levels, bone resorption increases and the formation process cannot keep up. But after the age of 60 or so the main problem is a decrease in osteoblast activity. Whether the osteoblasts are not responding to normal signals adequately or the signals aren't there is still a question. But the osteoblasts simply are not making enough new bone" (see box).

Other examples of an imbalance between bone resorption and formation occur with persistent infections. Three of the factors that mediate bone resorption, lymphotoxin, interleukin-1, and tumor necrosis factor α, are probably responsible for bone destruction wherever chronic infection occurs, says Mundy. For example, they may mediate abnormal resorptive activity in rheumatoid arthritis and periodontal gum disease, a major cause of tooth loss. And in patients with myeloma, a B cell tumor that makes lymphotoxin, bone is also destroyed.

Although the origin of such bone diseases is not well understood now, a combination of factors—age, level of physical activity, family history, use of alcohol and cigarettes, and nutrition—contribute to at least the development of osteoporosis. That is why singling out one factor, such as nutrition, as a method of prevention or treatment is probably not the answer. "It is fairly clear that you cannot prevent lumbar vertebral bone loss in postmenopausal women [the group most likely to get this form of osteoporosis] with increased dietary calcium," says Raisz. He indicates that "adequate dietary calcium is important in achieving peak bone mass," which occurs before the age of 25. Calcium supplements given alone later in life may slow the rate of age-related bone loss somewhat. But according to a recent study by Claus Christiansen and his colleagues of the University of Copenhagen in Denmark, estrogen therapy is probably more effective than calcium in early postmenopausal women.

In the mature adult, local factors produced by lymphocytes, monocytes, and macrophages that reside temporarily in the bone marrow, as well as circulating hormones, regulate bone turnover. "My hunch is that the major job of the immune system factors is to regulate bone mass, whereas the systemic factors control calcium homeostasis," says Mundy. Understanding how these substances and their target cells interact during bone remodeling may lead researchers to devise preventions or treatments for bone diseases. ■ **DEBORAH M. BARNES**

ADDITIONAL READING

G. R. Mundy and G. D. Roodman, "Osteoclast ontogeny and function," in *Bone and Mineral Research*, W. Peck, Ed. (Elsevier, New York, 1987), vol. 5, p. 209.

B. L. Riggs and L. J. Melton, "Involutional osteoporosis," *N. Engl. J. Med.* **314**, 1676 (1986).

L. G. Raisz and T. J. Martin, "Prostaglandins in bone and mineral metabolism," in *Bone and Mineral Research*, W. Peck, Ed. (Elsevier, New York, 1984), vol. 2, p. 286.

R. Baron, L. Neff, C. Roy, A. Boisvert, M. Caplan, "Evidence for a high and specific concentration of (Na^+, K^+)ATPase in the plasma membrane of the osteoclast," *Cell* **46**, 311 (1986).

M. Centrella and E. Canalis, "Transforming and nontransforming growth factors are present in medium conditioned by fetal rat calvariae," *Proc. Natl. Acad. Sci. U.S.A.* **82**, 7335 (1985).

B. Riis, K. Thomsen, C. Christiansen, "Does calcium supplementation prevent postmenopausal bone loss?" *N. Engl. J. Med.* **316**, 173 (1987).