MINIREVIEW

Bone and the Immune System (43249)

HELEN E. GRUBER[1]

Skeletal Dysplasia Morphology Laboratory, Medical Genetics-Birth Defects Center, Cedars-Sinai Medical Center, Los Angeles, California 90048, and Department of Pediatrics, University of California at Los Angeles, Los Angeles, California 90024

Several lines of evidence provide insight into the relationship of bone and the immune system: (i) the effects of immune cell products on bone cells; (ii) the bone histologic features that characterize the nude mouse and rat animal models; (iii) the chondro-osseous changes present in immunodeficiency syndromes; and (iv) the immune system alterations which have been reported to be present in osteoporosis.

The present article will review these current perspectives on the interactions of bone and the immune system and show that this is an extremely important interaction that influences both osteoblast and osteoclast function.

Effects of Immune Cell Products on Bone Cells

Studies over the last 20 years have developed this area of research and opened the field to direct observations of the influence of products of the monocyte/macrophage cell line (termed "monokines") or the lymphocyte (termed "lymphokines") upon bone formation by osteoblasts and bone resorption by osteoclasts (1). For the monokines, interleukin 1, tumor necrosis factor-α, macrophage-derived growth factors, and platelet-derived growth factor (PDGF), and for the lymphokines, lymphotoxin and γ-interferon have been studied in greatest detail, with an influence shown on bone remodeling (2, 3).

Some of these peptide products are frequently discussed under the heading "growth regulatory factors," even though this term is misleading since it is now recognized that such substances can act as growth stimulators, inhibitors, or regulators of cellular functions other than actual growth (4). It is beyond the scope of this Minireview to cover all recent reports of the interactions of immune system cells with bone cells. Rather, the intent here is to provide a perspective of how these cells are believed to interact with bone that will be useful to the reader as new reports are published.

The reader is referred to several recent, detailed reviews devoted to the response of bone cells to specific immune cell substances (5–7). Basic to all such studies is the physiologic process of bone turnover, reviewed in special detail by Baron *et al.* (5). In brief, a resorptive stimulus first triggers recruitment of osteoclasts to a site on the bone surface. This is followed by active resorption by osteoclasts, after which cells withdraw from the bone surface and mononuclear phagocytic cells (MNP) appear on the newly resorbed surface. These cells are then followed by young osteoblasts which begin the bone formation phase. After the resorbed Howship's lacunar pit is filled with new osteoid, osteoblasts become flatter and less active, with the final newly remodeled bone surface lined by flat lining cells.

It is this process of bone remodeling that makes bone unique among organ tissues and that also adds so many levels of complexity, with respect to interactions along the remodeling sequence by systemic influences (hormones), stress action on trabecular and cortical systems (physical activity/weight bearing), growth factors produced by the bone cells themselves (which act locally on their own cell types and on the other bone cell types, or which are bound into the newly formed or resorbed bone matrix itself), or factors which come from nearby cells present in the marrow tissue itself.

Mohan and Baylink (8) have recently reviewed the autocrine and paracrine aspects of bone metabolism. As shown in Figure 1, the authors propose that bone acts as a major storage site for growth factors. Their model hypothesizes that growth factors produced by

[1] To whom correspondence and requests for reprints should be addressed at Skeletal Dysplasia Morphology Laboratory, Medical Genetics-Birth Defects Center, Spielberg Building, Third Floor, Cedars-Sinai Medical Center, 8700 Beverly Boulevard, Los Angeles, CA 90048.

0037-9727/91/1973-0219/$3.00/0

osteoblasts diffuse into newly deposited osteoid. Human bone cells are also now known to produce a number of growth factors which are stored in the bone matrix, including insulin-like growth factors I and II, transforming growth factor-β and PDGF. It is also possible that factors originating from nearby cells in the marrow may also be deposited in the new bone matrix at the time of bone formation. Mohan and Baylink (8) hypothesize that these bone-derived factors, which can be liberated during subsequent periods of bone resorption, act in an autocrine, paracrine, or delayed paracrine fashion in the local microenvironment of the bone surface.

Rifas *et al.* (9) have recently focused upon how monokines produced by macrophages stimulate the growth of osteoblasts. They hypothesize that macrophages may act not only during bone repair, but also to ensure the coupling of bone formation to bone resorption during bone remodeling. As shown in Figure 2, there are a number of ways by which monocyte/macrophage cell products can influence bone cells.

Although the macrophage line is usually discussed with respect to its well-documented impact on bone resorption (5), Rifas *et al.* (9) have determined that two factors, which were recognized based on their heparin-agarose column elution pattern, secreted by macrophages stimulate osteoblast growth: HEP I (identified as PDGF) and HEP II, an apparently unique mitogen secreted by macrophages. The authors hypothesize that HEP I may act to recruit osteoblast progenitors by causing a proliferation of "stromal fibroblastic osteoprogenitor cells," and that HEP II may act by causing migration and proliferation of more differentiated osteoblasts. Thus, the macrophage-like cells, which inhabit the empty Howship's lacunae after the departure of the osteoclast, may have a very strategic and influential cellular location and function.

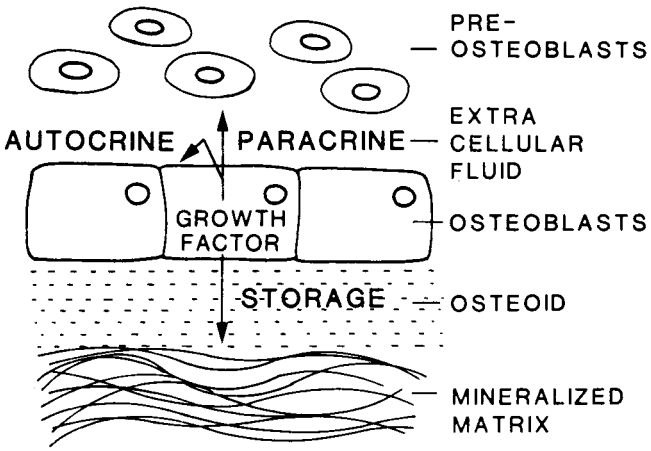

Figure 1. Diagram outlining potential storage of growth factors in bone and their action on osteoblasts. In a like manner, growth factors from other cells could also potentially diffuse toward sites of new bone formation and be deposited in new bone matrix or act on bone cells. (Reprinted from Growth—Genetics and Hormones (Ref. 8), with permission.)

There are relatively few studies which look at the relationship of the immune system and bone using *in vivo* animal models. One recent report has employed subcutaneous injection of nonspecific irritants (talcum or cotton wool) (10). Bone loss associated with the inflammation was reported to occur independently of parathyroid hormone secretion and vitamin D metabolism.

It is more common for studies on this topic to be carried out *in vitro* using bone cell populations isolated from rat bones, an intact calvarial model in which to assay results, or tumor-derived bone cells. It is important for the reader to carefully note the models and protocols used in newly published reports, since findings are often very specific for cell type, culture conditions, and specific models employed.

Bone Changes in the Nude Mouse and Rat Models

Athymic animals provide interesting models in which to evaluate the effect of a defective immune system. The best understood model is that of the athymic mouse (*nu/nu*), a spontaneous mutant which shows a T lymphocyte immune deficiency. Previous work with this model has shown that about 2% of the athymic mouse spleen cells are phenotypically mature T cells, and that there is present a group of cells with "incomplete" T cell features (11, 12). Another animal model is that of the motheaten (*me/me*) mouse, a mutant in which multiple defects are present, especially an abnormality in B lymphocyte differentiation.

Unfortunately, there are very few studies which have evaluated bone turnover in the nude mouse. Vignery *et al.* (13) have studied bone from the tail vertebrae from both of these mouse mutants and found distinctive bone changes in each mutant. Bone turnover was markedly reduced in the nude mouse: Bone formation was 10 times lower than that seen in normal littermates, and osteoclasts were less numerous and less active. The motheaten mutant, however, showed markedly increased bone turnover. These findings suggest potentially different roles for T and B lymphocytes with respect to their relationship with bone cells and bone turnover.

McCauley *et al.* (14) have recently examined bone turnover *in vivo* in the athymic mouse and also tested *in vitro* the bone resorbing activity of conditioned medium from splenic lymphocytes from these animals (15).

The *in vivo* studies of the nude and euthymic mice identified decreased vertebral tissue area and shorter tibia in the nude animals (14). Vertebral endosteal bone surface showed reduced tetracycline labeling and reduced osteoclast index at 6 weeks of age. In terms of

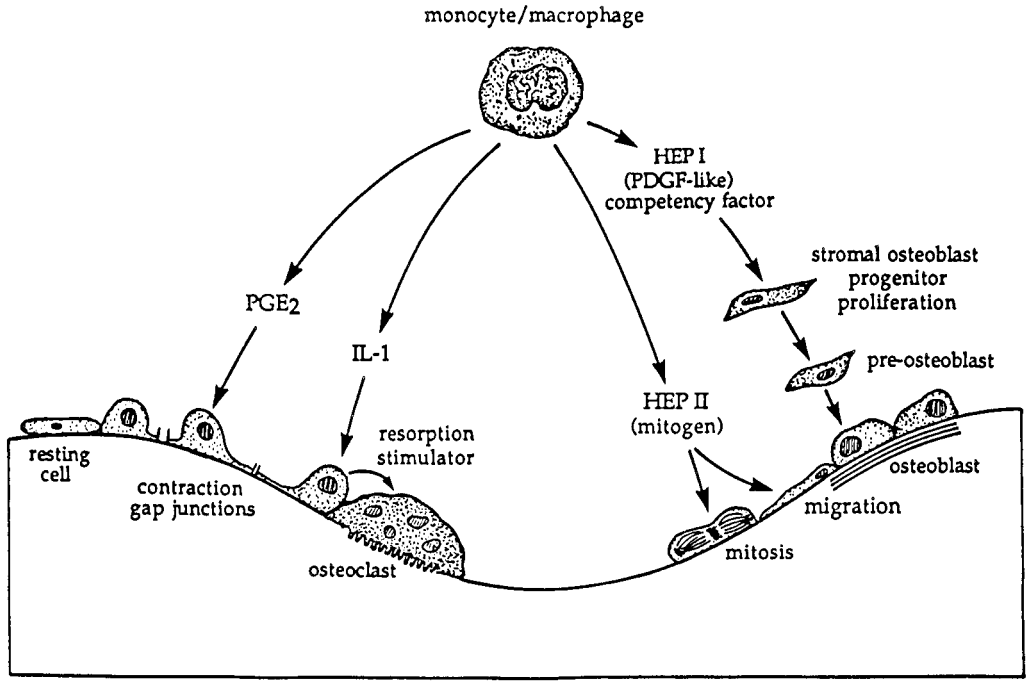

Figure 2. Model describing the interaction of monocyte/macrophage products on the coupling of bone resorption (depicted on the left of the diagram) and bone formation (depicted on the right). Prostaglandin E_2 (PGE_2) from mononuclear phagocytes acts first to cause contraction of osteoblasts. This allows bone surface to be "exposed," followed by attachment of osteoclasts at this site. IL-1 may cause "activated" osteoblasts to secrete factors which stimulate osteoclastic bone resorption. The PDGF-like HEP I may act to stimulate osteoblast progenitor cell division; these cells then differentiate into osteoblasts. HEP II may help signal early osteoblasts to come to Howship's lacunae, to proliferate there, and to produce new bone matrix. (Reprinted from Connective Tissue Research (Ref. 9), with permission.)

bone turnover, the authors concluded that the major differences they found related to decreases in features of bone formation quantified at the organ level.

In a series of *in vitro* studies, these same authors investigated the bone resorbing activity of nude and euthymic mouse splenic leukocytes (15). Techniques used in this study tested the ability of conditioned media from cultured splenic leukocytes from nude and euthymic mice to induce bone resorption in calvaria of normal fetal mice. Conditioned media from the nude mouse had greater bone resorbing activity than did that from euthymic mouse leukocytes. This bone resorbing activity was inhibited by indomethacin and interferon-γ. Conditioned media from nude mouse leukocytes contained higher levels of prostaglandin E than did that from euthymic mouse leukocytes.

An athymic nude mutation is also available for the rat (16). To date, this model has been used mainly to evaluate metastasis of tumor cells to bone and bone marrow (17, 18); much remains to be learned about the features of bone turnover and the bone resorbing activity of cultured splenic leukocytes from the nude rat.

Chondro-Osseous Changes in Immunodeficiency Syndromes

There are several major primary immune deficiency disorders. These are usually classified according to cause (B cell deficiency, T cell or cell mediated deficiency, neutrophil disorders, combined T and B cell disorders, and disorders of the complement system). Herman and Kirkpatrick (19) have reviewed the major skeletal findings in terms of radiologic features, and Hong (20) has recently published a brief review article on the general clinical bone features of selected conditions. It is useful to consider these syndromes in the present review because they provide examples in clinically delineated conditions of interactions between bone and the immune system.

Several of the immunodeficiency syndromes (adenosine deaminase deficiency, cartilage hair hypoplasia, and the DiGeorge syndrome) have clinical features especially pertinent to the present discussion.

Adenosine Deaminase (ADA) Deficiency. ADA deficiency is inherited as an autosomal recessive and X-linked condition (21). Thirty-five families have been studied with this condition; about 50% of cases show non-X-linked severe combined immunodeficiency. ADA deficiency can now be identified by prenatal chorionic villus biopsy analysis (22) and by staining fetal blood obtained at fetoscopy with monoclonal antibodies (23). This condition is usually fatal. Since bone marrow transplantation is not an available treatment for many patients, other therapies have been developed, including injection of bovine adenosine deaminase

modified enzyme (24) and, most recently, somatic cell gene replacement (25). With the heightened interest in this condition, it is timely to review the chondro-osseous changes in ADA deficiency.

Radiologic features of ADA deficiency include irregularities of the metaphyseal ends of long bones, splayed metaphyses, and metaphyseal spurs. Ribs are short, wide, and cup-shaped at the costochondral junctions. Vertebrae show a "bone within a bone" appearance and mild platyspondylia. Iliac wings are square-shaped and the acetabular roofs flat (21). Some studies have reported a partial resolution of these abnormalities following enzyme replacement therapy (26).

There are only two reports of the chondro-osseous histopathologic changes present in ADA deficiency (27, 28). This group documented autopsy findings in three children with bone and cartilage changes distinctive from findings in previously studied metaphyseal dysplasias. Normal chondrocyte column formation in the growth plate was almost completely absent. No zone of proliferative chondrocytes was present. Primary spongiosa were almost entirely absent; when present there appeared to be an unusual transition of cartilage matrix to osteoid on the trabecular surface. Few osteoblasts or osteoclasts could be found in the metaphyseal area. Periosteal bone formation appeared to be normal, but there was a bony projection over the epiphyseal ridges (which resulted in the metaphyseal cupping evident radiographically). There appear to be at least three abnormalities which contribute to growth failure and metaphyseal alterations in ADA deficiency: a major block in chondrocyte proliferation, an abnormal endochondral ossification, and a decreased number of bone cells. The cause(s) of these skeletal abnormalities, and how they relate to the immunologic impairment present in this condition, remains unknown.

DiGeorge Syndrome. The DiGeorge syndrome is an uncommon syndrome of congenital absence of the thymus and parathyroid glands; several forms of the syndrome are recognized (21). In the complete DiGeorge syndrome, parathyroid glands are absent and there is no T cell function. In the partial DiGeorge syndrome, some T cell function is present. Skeletal radiologic abnormalities include micrognathia and aplasia of the mandible (19). There appear to be no quantitative histopathologic data available to determine if there are changes in osteoclast or osteoblast number or size, or alterations in bone turnover in this syndrome.

Cartilage Hair Hypoplasia. Cartilage hair hypoplasia (metaphyseal chondrodysplasia, McKusick type) is an autosomal recessive condition which has a common occurrence, especially among the Amish (1.5 per 1000) and in Finland (21). Radiologic features include flaring, cupping, marginal serration, and scalloping of the metaphyses of the tubular bones, especially at the knees. Irregular, cyst-like radiolucencies may occur in the metaphyses with extension into the diaphyses of long bones. Epiphyseal shape is abnormal. The carpal and tarsal bones are small and have an irregular contour. Metacarpals, metatarsals, and phalanges are markedly short. Cupping and cyst-like regions may be present at the costochondral junctions. Vertebrae, pelvis, and the femoral heads are small. Chondro-osseous histopathologic findings include clusters or nests of chondrocytes at the growth plate (replacing orderly columns), and nests of hypertrophic cartilage which extend in a tongue-like fashion into the metaphysis (29).

The combination of metaphyseal abnormalities and immune deficiency is present in at least two additional syndromes: the Schwachman syndrome (skeletal lesions are associated with pancreatic exocrine insufficiency and neutropenia), and the metaphyseal chondrodysplasia-thymolymphopenia syndrome.

The association of immune deficiency syndromes and the chondro-osseous abnormalities described above raises many interesting questions which as yet remain unanswered.

Immune Alterations and Osteoporosis

There is an interesting body of literature which suggests a relationship between some forms of osteoporosis/osteopenia and abnormalities in the immune system.

Rubin *et al.* (30) recently presented the clinical course of a 29-month-old girl with recurrent infections and multiple fractures who died at the age of 40 months. The authors suggested that this case might represent a new congenital disorder with severe osteopenia (with increased osteoclast activity) in association with a defect in T cell immunoregulation. Clinical studies showed normal serum immunoglobulins; *in vitro* polyclonal-induced immunoglobulin production was decreased and there was a markedly increased suppressor T cell activity. Analysis of T cell subsets showed 90% $T3^+$ cells, 55% $T4^+$ cells, and 36% $T8^+$ cells. Studies showed that the increased suppressor activity resided in the T cell population. Iliac crest bone biopsy showed marked osteopenia and the presence of small, atypical osteoclasts. The authors hypothesize that the increased bone resorption, decreased bone formation, and suppressor activity could be linked in a pathway involving the abnormal function of immune cells.

Several recent clinical reports have evaluated lymphocytes in populations of osteoporotic patients. Rosen *et al.* (31) studied surface markers on T lymphocytes obtained from groups of 16 postmenopausal osteoporotic women and 12 symptomatic osteoporotic men. The ratio of CD4-bearing (T helper) cells to CD8-bearing (T cytotoxic suppressor) cells was increased in the group of osteoporotic women. When data from normal and osteoporotic women were analyzed, a significant negative correlation was found between the

CD4/CD8 ratio and spinal bone mineral density (derived from dual beam photon densitometric analysis). These findings did not hold for the male osteoporotic patients included in the study. The authors suggest that their data support a potential link between immune activation and bone alterations in postmenopausal osteoporosis.

In another study, Imai *et al.* (32) and Fujita *et al.* (33) found that osteoporotic women showed lowered $OKT3^+$ and $OKT8^+$ counts and a higher $OK4^+$ to $OK8^+$ ratio than controls. Based on the bone data from their patients, the authors concluded that abnormalities in peripheral T lymphocyte subsets, especially $OK4^+$ and $OK8^+$, were closely associated with the decreased bone mass. They concluded that their study suggested a causal relationship between T lymphocyte functions and the development of postmenopausal osteoporosis.

Duke-Cohan *et al.* (34) have reported a defect in the ability of osteoporotic patients to respond to foreign histocompatibility antigens in the mixed leukocyte reaction (34). This was due both to a poorly responding lymphocyte population and to an as yet unidentified suppressor factor in osteoporotic sera. The relative and absolute numbers of T cells were found to be increased.

The final topic meriting discussion in this section focuses upon bone morphogenetic protein (BMP), a bioactive factor in bone which has been isolated and studied extensively by Dr. Marshall Urist and his collaborators. BMP is a noncollagenous protein which is present in the bone matrix and is released during bone resorption. Relevant to this present discussion, BMP has now been identified by radioimmunoassay in circulating blood (35). Urist *et al.* (36, 37) have formulated the hypothesis that autoimmune responses against BMP might be formed by autostimulation of B cells, or by regulatory T cells and/or of effector T cells. Since BMP can elicit new mesenchymal cells to participate in bone formation, this hypothesis has implications for osteoporosis. The proposed autoimmune hypothesis states that patients with osteoporosis develop a specific humoral immune response to endogenous BMP or to an immunoreactive portion of BMP. This results in autoimmunization, which, in turn, has the consequence that BMP cannot recruit new mesenchymal cells to develop into osteoblasts and function during bone turnover.

During bone resorption, Urist *et al.* (36) hypothesize, the released BMP may be degraded, and may then combine with specific receptors on B lymphocytes in the marrow. The degraded BMP may stimulate production of elevated anti-BMP in localized regions adjacent to bone resorption sites. The anti-BMP might then act as an immunoglobulin secreted by sensitized B lymphocytes into body fluids (36). Data from Urist *et al.* (36, 37) show that women with severe osteoporosis exhibited anti-BMP levels approximately two times

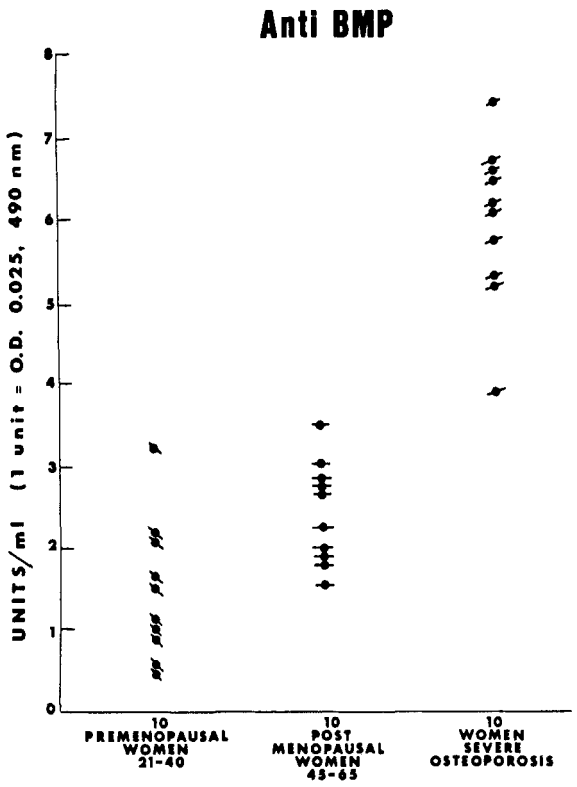

Figure 3. Elevated levels of anti-BMP have been identified in patients sera in a group of severely osteoporotic women. (Reproduced from Normal and Abnormal Bone Growth: Basic and Clinical Research (Ref. 36), with permission.)

greater than those seen in postmenopausal women, and two to eight times greater than those of premenopausal women (Fig. 3) (36, 37).

Summary

There are several lines of evidence which provide support for an important relationship between immune cells and bone. Clinical studies of immunodeficiency syndromes have shown that abnormalities in bone shape are evident on x-rays, and peculiarities in the structure of the growth plate have been identified by histopathology. Studies of bone histology, and quantitation of cellular abnormalities, are scarce. Abnormalities in bone turnover, have, however, been identified in the nude mouse model. Many lines of evidence derived from *in vitro* bone studies have shown that lymphokines and monokines can influence bone formation and bone resorption. Some clinical studies of postmenopausal osteoporosis have indicated the possible presence of immune cell changes in this condition.

Although several hypotheses have been formed regarding the exact mechanisms of the effect of immune cytokine on bone, this is clearly a very large area of study and there is a need for additional carefully controlled experiments with special emphasis on bone cells and bone matrix, especially in the human. As knowl-

edge progresses regarding immunology and hematology, a clearer understanding of the lineages of the osteoblast and osteoclast will emerge and we will better understand how specialized bone cells interact with and react to their immune cell neighbors in the bone marrow and to immune system signals. These findings will have especially important implications for the local bone loss seen in rheumatoid arthritis, periodontal disease, and chronic osteomyelitis (38).

This work was supported in part by NIH Grant 1 PO1 HO 22657-05. The author gratefully acknowledges Drs. David Baylink, Marshall Urist, and Leonard Rifas for their help and kind permission to reproduce illustrations. The author thanks Gail Reyburn for her assistance with preparation of the manuscript, Dolly Tyan for her helpful discussions about the immune system, and David Rimoin, M.D., Ph.D., for his support and encouragement.

1. Gowen M. The relationship between the immune system and bone formation and destruction. Bone Clin Biochem News Rev **5**:34–36, 1988.
2. Canalis E, McCarthy T, Centrella M. Growth factors and the regulation of bone remodeling. J Clin Invest **81**:277–281, 1988.
3. Krane SM, Amento EP, Goldring SR. Lymphocytes, monocytes and metabolic bone disease. In: Cohn DV, Fujita T, Potts JT Jr., Talmage RV, Eds. Endocrine Control of Bone and Calcium Metabolism. Amsterdam: Elsevier Science Publishers BV, pp3–14, 1984.
4. Cross SL. Busy signals. The New Biologist **1**:32–34, 1989.
5. Baron R, Vignery A, Horowitz M. Lymphocytes, macrophages and the regulation of bone remodeling. Bone Miner Res **2**:175–243, 1984.
6. Canalis E, McCarthy T, Centrella M. The regulation of bone formation by local growth factors. Bone Miner Res **6**:27–56, 1989.
7. Mundy GR, Roodman GD, Yoneda T, Bonewald L, Oreffo R. Growth regulatory factors and bone cell function. In: Cohn DV, Glorieux FS, Martin TJ, Eds. Calcium Regulation and Bone Metabolism: Basic and Clinical Aspects. Amsterdam: Elsevier Science Publishers, Vol **10**: pp257–269, 1990.
8. Mohan S, Baylink DJ. Autocrine and paracrine aspects of bone metabolism. Growth—Genetics and Hormones **6**:1–9, 1990.
9. Rifas L, Cheng S-L, Shen V, Peck WA. Monokines produced by macrophages stimulate the growth of osteoblasts. Connect Tissue Res **23**:163–178, 1989.
10. Minne HW, Pfeilschrifter J, Scharla S, Mutschelknauss S, Schwarz A, Krempien B, Ziegler R. Inflammation-mediated osteopenia in the rat: A new animal model for pathological loss of bone mass. Endocrinology **115**:50–54, 1984.
11. Chen W, Scollay R, Shortman K, Skinner M, Marbrook J. T-cell development in the absence of thymus: The number, the phenotype, and the functional capacity of T lymphocytes in nude mice. Am J Anat **170**:339–347, 1984.
12. Rangers GE, Palladino MA, Scheid MD. Development of T-cell function in relation to T-cell set diversification in nu/nu mice. Cell Immunol **98**:496–505, 1986.
13. Vignery A, Silverglate A, Horowitz M, Shultz L, Baron R. Abnormal bone remodeling activity in the immunodeficient nude (Nu/Nu) and motheaten (me/me) mice [Abstract]. Calcif Tissue Int **33(3)**:301, 1981.
14. McCauley LK, Rosol TJ, Capen CC, Horton JE. A comparison of bone turnover in athymic (nude) and euthymic mice: Biochemical, histomorphometric, bone ash and *in vitro* studies. Bone **10**:29–34, 1989.
15. McCauley LK, Rosol TJ, Capen CC, Horton JE, Shanfeld J. Investigations on *in vitro* bone resorbing activity from athymic (nude) and euthymic mouse splenic leukocytes. Bone **10**:389–393, 1989.
16. Festing MRS, May D, Connors TA, Lovell D, Sparrow S. An athymic nude mutation in the rat. Nature **274**:365–366, 1978.
17. Kjonniksen I, Nesland JM, Pihl A, Fodstad O. Nude rat model for studying metastasis of human tumor cells to bone and bone marrow. JNCI **82**:408–412, 1990.
18. Olden K. Human tumor bone metastasis model in athymic nude rats. JNCI **82**:340–341, 1990.
19. Herman TE, Kirkpatrick JA. Radiology of immunodeficiency syndromes. Postgrad Radiol **1**:99–121, 1981.
20. Hong R. Associations of the skeletal and immune systems. Am J Med Genet **34**:55–59, 1989.
21. Taybi H, Lachman RS. Radiology of Syndromes, Metabolic Disorders, and Skeletal Dysplasias, 3rd ed. Chicago: Year Book Medical Publishers, pp684–686, 1990.
22. Dooley T, Fairbanks LD, Simmonds HA, Rodeck CH, Nicolaides KH, Soothill PW, Stewart P, Morgan G, Levinsky RJ. First trimester diagnosis of adenosine deaminase deficiency. Prenat Diagn **7**:561–565, 1987.
23. Linch DC, Levinsky RJ, Rodeck CH, Maclennan KA, Simmonds HA. Prenatal diagnosis of three cases of severe combined immunodeficiency: Severe T cell deficiency during the first half of gestation in fetuses with adenosine deaminase deficiency. Clin Exp Immunol **56**:223–232, 1984.
24. Hershfield MS, Buckley RH, Greenberg ML, Melton AL, Schiff R, Hatem C, Kurtzberg J, Market ML, Kobayashi RH, Kobayashi AL, Abuchowski A. Treatment of adenosine deaminase deficiency with polyethylene glycol-modified adenosine deaminase. N Engl J Med **316**:589–596, 1987.
25. Culliton GJ. Gene therapy begins. Science **249**:1372, 1990.
26. Yulish BS, Stern RC, Polmar SH. Partial resolution of bone lesions: A child with severe combined immunodeficiency disease and adenosine deaminase deficiency after enzyme-replacement therapy. Am J Dis Child **134**:61–63, 1980.
27. Kaitila I, Rimoin DL, Cedarbaum SD, Stiehm ER, Lachman RS. Chondroosseous histopathology in adenosine deaminase deficient combined immunodeficiency disease. Birth Defects Orig Artic Ser **XII**:115–121, 1976.
28. Cedarbaum SD, Kaitila I, Rimoin DL, Stiehm ER. The chondroosseous dysplasia of adenosine deaminase deficiency with severe combined immunodeficiency. J Pediatr **89**:737–742, 1976.
29. Sillence DO, Horton WA, Rimoin DL. Morphologic studies in the skeletal dysplasias. Am J Pathol **96**:812–870, 1979.
30. Rubin KR, Ballow M, Baron R, Greenstein RM, Raisz LG, Rowe DW. Malignant osteoporosis and defective immunoregulation. J Bone Miner Res **3**:509–516, 1988.
31. Rosen CJ, Usiskin K, Owens M, Barlascini CO, Belsky M, Adler RA. T lymphocyte surface antigen markers in osteoporosis. J Bone Min Res **5**:851–855, 1990.
32. Imai Y, Tsunenari T, Fukase M, Fujita T. Quantitative bone histomorphometry and circulating T lymphocyte subsets in postmenopausal osteoporosis. J Bone Miner Res **5**:393–399, 1990.
33. Fujita T, Matsui T, Nakao Y, Shozawa S, Imai Y. Cytokines and osteoporosis. Ann NY Acad Sci **587**:371–375, 1990.
34. Duke-Cohan JS, Weinberg H, Sharon R, Naor D. Immunological function in osteoporosis. Clin Immunol Immunopathol **35**:125–129, 1985.

35. Urist MR, Hudak RT. Radioimmunoassay of bone morphogenetic protein in serum. A tissue specific parameter of bone metabolism. Proc Soc Exp Biol Med **176**:472–475, 1984.
36. Urist MR, Hudak RT, Huo YK, Rasmussen JK. Osteoporosis: A bone morphogenetic protein auto-immune disorder. In: Dixon AD, Sarnat BG, Eds. Normal and Abnormal Bone Growth: Basic and Clinical Research. New York: Alan R. Liss, pp776–96, 1985.
37. Urist MR, Nillsson DS, Hudak R, Huo Y-K, Rasmussen J, Hirota W, Lietze A. Immunologic evidence of a bone morphogenetic protein in the milieu interieur. Ann Biol Clin **43**:755–766, 1985.
38. Mundy GR, Raisz LG. Disorders of bone resorption. In: Bronner F, Coburn JW, Eds. Disorders of Mineral Metabolism. New York: Academic Press, Vol **III**: p1, 1981.