# Cytokines and Osteoporosis

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## INTRODUCTION

Osteoporosis, defined as a decrease of bone mass with preservation of normal bone composition, is thought to be the consequence of an imbalance between bone resorption and formation, in favor of the former. In addition to systemic factors such as estrogen lack, calcitonin deficiency, and decrease of $1,25(OH)_2$vitamin D synthesis leading to a fall of intestinal calcium absorption and secondary hyperparathyroidism, local factors have been suspected to play a role in the development of osteoporosis. Osteoclast-activating factor (OAF) secreted from mononuclear leukocytes augments bone resorption[1,2] and is thought to be a heterogenous mixture consisting of IL-1, TNF$\alpha$, and possibly other local factors.[3,4] Since osteoclasts and macrophages apparently originate from a common source of hematopoietic-immune stem cells, the possibility that these two cell types share the same control system is not only conceivable, but also likely. Therefore, it appeared worthwhile to examine the potential role of cytokines and other local factors in the development of osteoporosis.

### *Background for Osteoporosis*

Age-dependent bone loss is a universal phenomenon in humans.[5] Unlike fish, which constantly take up calcium from seawater through their gills, humans like other creatures on land depend on food as the only source of calcium supply, and thus are always in danger of becoming deficient in calcium. Since 99% of calcium in the body is located in the bone, any acquired calcium deficiency would affect bone calcium content and could explain the universal bone loss in aging.[6] Intestinal calcium absorption falls with advance in age, especially in osteoporotics, due to the aging of both the intestinal tract itself and the kidney, with decreasing renal $1,25(OH)_2$vitamin D synthesis. Estrogen lack, emphasized as the cause of postmenopausal accelerated bone loss in females, may also act through interference with calcium availability in view of the negative calcium balance after menopause. Calcitonin deficiency and secondary hyperparathyroidism may also contribute to the bone loss in aging. These systemic factors exert their actions through their influence on the activity, proliferation, and differentiation of bone cells.

In addition to the classical action of stimulating intestinal calcium absorption, $1,25(OH)_2$vitamin D apparently controls bone remodeling through its action on bone cell differentiation. Decreased activity of $1,25(OH)_2$vitamin D may therefore lead to derangement of bone remodeling and osteoporosis. The interaction of $1,25(OH)_2$vitamin D with the immune system has been a subject of considerable interest in recent years. Following the first report on the action of $1,25(OH)_2$vitamin D on the differentiation of mouse myelogenous leukemia cell line M1 and human promyelocytic cell line HL-60,[7,8] data have accumulated to demonstrate the immunoregulatory action of $1,25(OH)_2$vitamin D. A

change in lymphocyte subsets has been noted in osteoporosis, in particular an increase in the CD4/CD8 lymphocyte ratio in peripheral blood. As the forearm bone mineral content decreases and spinal compression fractures increase, the CD4/CD8 ratio rises. Administration of 1 μg/day 1α(OH)vitamin $D_3$ for 1 month, however, reversed the change[9] (TABLE 1). In order to define the role of lymphocyte subsets on each aspect of bone remodeling more precisely, we carried out multiple regression analysis on age, histomorphometric parameters, and lymphocyte subsets.[10] As shown in TABLE 2, parameters indicating bone formation, such as osteoid volume and osteoblastic surface, tended to decline with age without apparent correlation with lymphocyte subsets. Parameters indicating bone resorption such as osteoclastic surface and osteoclast number, on the other hand, were inversely correlated with the proportions of CD4 and CD8 subsets in lymphocytes, especially the former. Similar effect of $1,25(OH)_2$vitamin D in peripheral lymphocytes was also shown *in vitro*. When human peripheral blood mononuclear cells were incubated with $1,25(OH)_2$vitamin $D_3$, a significant decrease of $OKT_4$ and $OKT_9$ positive lymphocyte subsets was noted, with a consequent decrease of the $OKT_4/OKT_8$ ratio.[11] In the complex interaction between macrophages and lymphocytes for the control of immune function, $1,25(OH)_2$vitamin D tends to stimulate the former and suppress the latter, stimulating IL-1 production and inhibiting IL-2 production. Macrophages possess $1,25(OH)_2$vitamin D receptors and even 25(OH)vitamin D 1-hydroxylase, being not only

**TABLE 1.** Peripheral Lymphocyte Subset CD4/CD8 Ratio and Osteopolosis

| CD4/CD8 | −0.9 | 1.0–1.4 | 1.5–1.9 | 2.0– |
|---|---|---|---|---|
| % compression fracture | 53 | 69 | 88 | 80 |
| Radial BMD g/cm2 | 0.46 ± 0.04 | 0.44 ± 0.02 | 0.47* ± 0.03 | 0.40 ± 0.01 |

*$p < 0.05$.

effectively stimulated by $1,25(OH)_2$vitamin $D_3$, but also capable of synthesing $1,25(OH)_2$vitamin D by themselves to augment its effect in an autocrine fashion. Lymphocytes, on the other hand, acquire $1,25(OH)_2$vitamin D only after activation, to be inhibited by $1,25(OH)_2$vitamin $D_3$with a decrease in the release of IL-2. In vitamin D-resistant osteomalacia with limited $1,25(OH)_2$vitamin D synthesis, $OKM_1$, Leu7, and Leu11 positive cells and NK cell activity and adenine deaminase activity significantly increased, and all these changes were reversed on administration of large doses of 1α(OH)vitamin $D_3$.[12]

### *Plasma Interferon α*

A sensitive and specific radioimmunoassay for the measurement of plasma interferon α was developed in our laboratory utilizing α-interferon produced by human leukemic BALL-1 cells infected by Sendai virus. Antibody to α-interferon was raised by immunizing rabbits with this antigen. Using α-interferon radioiodinated by the lactoperoxidase technique, α-interferon at a minimum concentration was detected, without cross reactivity against β-interferon, γ-interferon, and ACTH. Circulating α-interferon was extracted from plasma and concentrated by silisic acid. Intra- and inter-assay coefficient of variation

TABLE 2. Multiple Regression Analysis between Lymphocyte Subsets

| Purpose Variables | Age | Number of Lymphocytes | OKT3+ | OKT4+ | OKT8+ | R |
|---|---|---|---|---|---|---|
| BMC(g/cm2) | −5.907** | −1.456 | −2.853 | 3.989** | 4.198** | 0.882** |
| Bone volume/ total Volume (%) | 0.564 | −0.353 | −0.035 | −0.598 | −0.552 | 0.322 |
| Osteoid volume /bone volume (%) | −2.231* | −0.359 | −0.208 | −1.244 | −0.382 | 0.649* |
| Osteoblastic surface/bone surface (%) | −3.053** | 0.548 | −0.310 | −1.910 | 0.796 | 0.805** |
| Osteoclastic surface/bone surface (%) | −1.026 | −0.382 | 1.549 | −4.331** | −2.552* | 0.803** |
| Number of osteoclasts/ total area | −0.613 | 0.014 | 1.855 | −5.558** | −3.842* | 0.859** |

was 0.21 and 4.12%, respectively. An age-bound decline of plasma α-interferon was found in normal male and female subjects from 230 ± 10 to 150 ± 15 pg/ml. Although no significant correlation was demonstrated between plasma IFNα and radial bone mineral density, a significant rise from 121 ± 15 to 178 ± 20 pg/ml was noted in osteoporotics after 1 month treatment with 1 μg 1α(OH)vitamin $D_3$/day. Although a direct production of IFNα by bone cells does not appear likely, the age-related fall of plasma IFNα suggests a decrease of macrophage function associated with vitamin D deficiency in aging. This is confirmed by the effect of 1α(OH)vitamin $D_3$ increasing plasma IFNα.[13] In view of the therapeutic effect of 1α(OH)vitamin $D_3$ on osteoporosis, such macrophage activation may well be associated with bone cell activation.

### *Vitamin D, Cytokines, and Osteoporosis*

In conclusion, deficiency and decreased biosynthesis of vitamin D or its active metabolites progressing with advancing age appears to cause an imbalance between macrophage and lymphocyte functions as shown in TABLE 3. Macrophage hypoactivity in vitamin D deficiency may be associated with decreased activity of osteoclasts sharing a common origin, through the lack of vitamin D action on differentiation and activation of this line of cells. The action of $1,25(OH)_2$vitamin D on osteoclasts is mediated by osteoblasts. Such osteoblast-osteoclast interaction would cause a parallel response of osteoblasts and osteoclasts to $1,25(OH)_2$vitamin D. Cytokines which stimulate bone resorption such as IL-1 and TNFα mainly released from monocytes are likely to decrease in vitamin D deficiency causing low turnover osteoporosis, whereas IL-2 increases in a compensatory fashion, as seen in patients with vitamin D-resistant osteomalacia. Interferon α released from macrophages would decrease in vitamin D deficiency and interferon γ inhibited by vitamin D probably increases in vitamin D deficiency.[14] Since interferon γ inhibits bone resorption, its increase may contribute to the development of low turnover osteoporosis. Since TGFβ is secreted by osteoblasts and is also released on matrix degradation by bone resorption, decreased osteoclast and osteoblast activities would be associated with decreased TGFβ activity. Although the interactions among various cyto-

kines appear quite complex, vitamin D-deficient and -sufficient states thus appear to cause a contrasting behavior among many cytokines, and this may help to explain the cause of low turnover osteoporosis apparently associated with the state of vitamin D deficiency.

## SUMMARY

Bone remodeling is controlled by systemic factors such as parathyroid hormone (PTH), calcitonin (CT), and $1,25(OH)_2$vitamin D and by local factors including cytokines and growth factors such as IL-1, IL-2, TNFα, TGFβ, IFNα, and IFNγ. Derangement of such control mechanisms leading to an imbalance between osteoclastic bone resorption and osteoblastic bone formation could cause osteoporosis. Conditions associated with immune dysfunction such as aging, corticosteroid therapy, and rheumatoid arthritis are associated with osteoporosis, which is also more common in females than in males, like most of the autoimmune-collagen diseases. Peripheral lymphocyte subsets CD4/CD8 were higher in patients with senile osteoporosis than in the age-matched controls, and returned to normal after 1 month of $1\alpha(OH)$vitamin $D_3$ treatment. On multiple regression analysis of histomorphometric data and lymphocyte subsets, a negative correlation was found between CD4 lymphocytes and bone resorption. High CD4 is thus associated with a low level of osteoclastic bone resorption or low turnover osteoporosis. Plasma interferon reflecting macrophage function decreased with advance in age and increased in response to $1\alpha(OH)D_3$ treatment. As one of the immunoregulators, vitamin D tends to stimulate the

**TABLE 3.** Osteoporosis, Cytokines, and Vitamin D[a]

| | MØ OC OB | Lymphocyte CD4 (CD4) CD8 | IL-1 IL-2 | IFNα IFNγ | TGFβ TNFα |
|---|---|---|---|---|---|
| Vitamin D deficiency [VD(−)] | | | | | |
| Low turnover osteoporosis | ↓ ↓ | ↑ ↑ | ↓ ↑ | ↓ ↑ | ↓ ↓ |
| Old age | ↓ | | | | |
| Vitamin D sufficiency [VD(+)] | | | | | |
| Normal | ↑ ↑ | ↓ ↓ | ↑ ↓ | ↑ ↓ | ↑ ↑ |
| Young age | ↑ | | | | |

[a]The proposed relationship between osteoporosis, cytokines, and vitamin D is summarized. VD(−) stands for vitamin D deficiency and VD(+) for vitamin D sufficiency. In the upper part of the table, association of vitamin D deficiency with low turnover osteoporosis and old age is indicated, whereas the normal state, young age with sufficient vitamin D is indicated in the lower part. Since vitamin D stimulates macrophage activity, vitamin D deficiency is shown by a *downward arrow* for MØ and vitamin D sufficiency by an *upward arrow*. OC and OB, standing for osteoclasts and osteoblasts, respectively, parallel the macrophage activity. Decrease of both OC and OB activity causes low turnover osteoporosis. CD4 helper lymphocyte and CD4 helper/CD8 suppression ratio increases in the vitamin D deficient state and is restored on sufficient vitamin D supply. As to the cytokine activity, that of IL-1 and IFNα released from macrophage falls, and IL-2 (and IFNγ) released from lymphocytes rises in vitamin D deficiency, TGFβ and TNFα being parallel with macrophage activity.

macrophage-natural killer system and suppress the lymphocyte system, stimulating TGFβ and TNFα activity. Senile osteoporosis of low turnover thus appears to be associated with vitamin D deficiency, low macrophage function, high CD4 lymphocyte proportion, low IL-1 and high IL-2 activity, low IFNα and high IFNγ activity, and low TGFβ and TNFα activity. Treatment with vitamin D derivatives tends to reverse these changes.

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