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β_2-Microglobulin and bone cell metabolism

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Introduction

Dialysis-related amyloidosis (DRA), also referred to as β_2-microglobulin (β_2M) amyloidosis, is a major cause of skeletal morbidity in patients with end-stage renal disease. The DRA syndrome results in a progressive destructive periarticular osteoarthropathy. The pathological lesions of DRA consist of cystic lesions and localized areas of β_2M-amyloid deposition. Approximately 70% of adult patients who undergo dialysis for more than 10 years develop radiographic evidence and/or symptomatic pathology associated with β_2M-amyloid deposition [1]. With advances in the treatment of the cardiac and cerebrovascular complications associated with end-stage renal disease, it is anticipated that the life expectancy of dialysis patients will continue to increase. Thus, morbidity from bone disease in general and DRA in particular will become more prevalent. Over the past decade numerous hypotheses have been put forward in an attempt to explain how β_2M might affect bone cell metabolism and play a role in the development of DRA. However, much of the reported experimental data has been difficult to interpret, resulting in much controversy regarding the role of β_2M in normal and abnormal bone physiology. Although there is substantial evidence suggesting that there is a specific effect of β_2M on bone-cell metabolism, a recurrent argument has been that the effect was the result of other growth factors or undefined contamination of the β_2M preparations tested. There are two major facts forming the basis for this argument: a lack of an identified receptor for β_2M, and continued disagreement among investigators concerning the mitogenic effect of β_2M on bone cells (Table 1). Thus, the question that needs to be addressed is whether β_2M plays an active role in bone metabolism or whether β_2M is a passive participant, being incidentally deposited in the form of amyloid at sites of bony destruction.

Effect of β_2M on bone

Although the effect of β_2M on osteoblast proliferation is controversial, multiple other effects of β_2M on bone-cell metabolism have been observed. Osteoblasts produce β_2M [2]. Subcutaneous injection of β_2M induces histological evidence of bone resorption in neonatal mice [3], and purified human β_2M induces a dose- and time-dependent net calcium efflux in cultured murine calvariae [4,5]. This calcium efflux is

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Table 1. Selected effects of β_2-microglobulin on bone and bone-cell metabolism

Reference	Species	Target	Developmental stage	β_2M Dose	β_2M Source species	Results
Kataoka 1986[23]	Mouse	MC 3T3-E1	Osteoblast Phenotype	10^{-6}–10^{-5} M	Human: GF, IEC	Calcification↓, cell proliferation→, collagen synthesis→, ALP→
Canalis 1987[22]	Rat	Calvariae, Osteoblasts	?	10^{-6}–10^{-4} M	Human: Sigma Rat: GP, HPLC	Cell proliferation↑
Jennings 1989[20]	Chicken; Mink	Calvarial cells; Lung epithelial cells	?	10^{-8}–10^{-5} M	Human, Calbiochem Bovine: GF, IEC, HPLC	Purification diminishes mitogenic activity
Centrella 1989[19]	Rat	Calvarial Osteoblasts	?	10^{-7}–10^{-6} M	Human: Sigma	DNA synthesis↑, synergy with IGF-I
Evans 1991[2]	Human	Bone-derived cells	Osteoblast Phenotype	10^{-6}–10^{-5} M	Human: Sigma	Cell proliferation↑, osteocalcin→, ALP→, PGE_2→
Moe 1992[4]	Mouse	Calvariae	?	10^{-9}–10^{-7} M 10^{-8}–10^{-6} M	Human: Sigma	DNA synthesis→ Bone resorption↑
Moe 1995[6]	Mouse	Calvariae	?	10^{-7} M	Human: Sigma Human: IEC, GF	PGE_2→ Bone resorption↑

GF, gel filtration; IEC, ion exchange chromatography; HPLC, reversed phase HPLC, Sigma purified by GF and IEC; ALP, alkaline phosphatase; ↑ increase; ↓ decrease; → no effect; ? unknown.

mediated in part by interleukin-1β (IL-1β) [6]. β_2M also stimulates the synthesis of IL-6, a potent bone-resorbing cytokine, leading to an increase in mRNA and protein levels in osteoblasts [7]. β_2M has also been shown to stimulate synovial fibroblasts to produce stromelysin, a neutral matrix metalloproteinase (MMP), which is believed to be a key enzyme causing articular destruction in inflammatory joint diseases [8]. The findings that β_2M induces the synthesis of collagenase-1 from rabbit synovial fibroblasts and the preferential collagen binding capacity of β_2M also supports the hypothesis that β_2M has a principal role in modulating connective-tissue breakdown [9,10]. Migita and colleagues [11] demonstrated that β_2M increases cyclo-oxygenase-2 (COX-2) protein and mRNA expression in a dose-dependent manner from human synovial cells; however, utilizing the mouse calvarial resorption model, we were unable to demonstrate an effect of β_2M on prostaglandin E_2 production [6].

Although β_2M has a significant bone-resorbing effect, advanced glycation end-product (AGE) modification of β_2M appears to further increase bone resorption and cytokine production [12,13]. Compared to unmodified β_2M, the number of resorption pits formed by isolated osteoclasts are significantly increased by AGE-modified β_2M [13]. AGE modification of β_2M seems to alter bone metabolism in a number of ways, not only increasing bone resorption, but also decreasing fibroblastic collagen deposition. AGE modification of β_2M compared with unmodified β_2M decreases fibroblastic synthesis of type I collagen [14]. Interestingly, the amyloid deposits and surrounding macrophages from patients with DRA react with a monoclonal anti-AGE antibody [15]. AGE-modified β_2M stimulates chemotaxis of monocytes and macrophages and enhances the secretion of cytokines [16]. The biological effect of AGE-modified β_2M on monocytes and macrophages is thought to be mediated by the receptor for AGE (RAGE). The finding that AGE-modified β_2M further induces bone resorption and osteoblastic cytokine release as well as reducing type I collagen synthesis by fibroblasts compared to unmodified β_2M could be the result of cellular RAGE recognition, as RAGE has been described on osteoblasts [17].

Is β_2M a growth factor?

It was in the late 1980s when β_2M was proposed as a potential bone growth factor. However, the mitogenic effect of β_2M continues to be one of the most controversial and hotly debated issues concerning the effect of β_2M on bone. As shown in Table 1, studies evaluating the effect of β_2M have been performed utilizing different experimental models with cells and tissues obtained from different animal species, and using varying doses of β_2M. In most of the studies utilizing bone cells, the developmental stage of the bone cells was not defined. Bone cells undergo a series

of developmental stages, such as proliferation, differentiation, and apoptosis, and each of them involves induction and suppression of various genes. The response of osteoblasts to various factors has been shown to be dependent on their developmental stage [18]. This may shed some insight as to why different experimental systems yield various results depending on the maturational stage of the osteoblasts. The variability in bone-cell response has led some investigators to suggest that β_2M might not be a typical growth factor, but a regulator of the growth-promoting effects of other growth factors [19].

To further complicate the interpretation of the various results, the β_2M utilized in these studies was isolated by different techniques and from different sources. The possible impurity of some preparations of β_2M has resulted in at least one group proposing that the mitogenic effect of β_2M is the result of growth factor contamination. Jennings *et al.* [20] further purified β_2M and observed that the mitogenic activity of the original protein was diminished after purification by reverse-phase high-performance liquid chromatography (RP-HPLC). Maximal mitogenic activity was detected in fractions different from their ultra-purified β_2M; thus they concluded that the original mitogenic activity was the result of growth factor contamination [20]. Although RP-HPLC ensures analytical purity, it may cause denaturation and loss of protein function. This denaturation might explain loss of mitogenic activity in the β_2M fraction. Furthermore, the β-sheet structure of β_2M favours amyloid fibril formation and spontaneous precipitation [21], thus high concentrations of β_2M in a salty environment, as occurs during RP-HPLC, may result in precipitation and dimer formation, with a consequent decrease in the amount of bioavailable β_2M. This could serve as an alternative interpretation as to why RP-HPLC leads to a loss of mitogenic activity of β_2M. Since growth-factor contamination has been suggested as being responsible for some of the mitogenic effects of β_2M, the purification method has become a crucial issue. The majority of studies, as listed in Table 1, have been performed using β_2M purified by gel filtration and ion exchange chromatography. Thus the possibility of growth factor contamination raised serious concerns as to whether the accumulated data concerning β_2M is reliable. Although the majority of the experimental data supports a role of β_2M in both normal and abnormal bone metabolism, it is not surprising that defining this role has been elusive.

Summary

β_2M is the small extracellular subunit of the MHC Class I molecule, and is present on the surface of all nucleated cells. As molecules of the MHC complex commonly do, β_2M has been suggested to possibly interact with hormonal and/or growth factor receptors [22]. This theory is further supported by the fact that despite the intensive research and focus on β_2M, a receptor for it has not been identified. Interaction of β_2M with various receptors may potentially induce various signal transduction pathways and genes depending on the experimental system utilized and the differentiation stage of osteoblasts and could explain some of the controversial findings (Table 1).

Initial work on β_2M offered a promising role in cell regulation, until questions were raised about possible contamination of β_2M preparations. This cloud of suspicion shed significant doubt primarily on the mitogenic effect of β_2M. Since the amyloid deposits contain up to 95% β_2M, it is tempting to blame β_2M for the altered bone metabolism observed in DRA. The presence of β_2M in the immediate environment throughout the development of the osteoblast might alter maturation and differentiation. The cumulative data demonstrate that β_2M has biological activity far beyond that which could be explained by growth factor contamination. Nonetheless, the proposed roles of β_2M are so numerous that is difficult to imagine one molecule having such a variety of specific effects, unless β_2M acts through a variety of receptors in diverse ways depending on the developmental phase of cells and the availability of receptors. The current theory about the mechanism of β_2M causing DRA invokes the release of β_2M and the activation of monocytes by AGE-modified β_2M. After being shed from cell surfaces, the free AGE–β_2M could trigger cell migration and release of bone-resorbing cytokines at osteoarticular sites, and the free heavy chains remaining at the cell surfaces may also activate monocytes. Clearly, the β_2M-mediated alteration of bone cell metabolism is very complex and could not be explained by a single metabolic pathway. Despite the accumulating knowledge about β_2M toxicity, strategies for prevention and therapy for DRA have been unsuccessful, and many issues concerning β_2M amyloidosis remain unresolved. Notwithstanding the controversies, determination of the true significance and role of β_2M in bone metabolism is imperative and is critical in regard to preventing long-term skeletal morbidity in dialysis patients.

References

1. Sprague SM, Moe SM. Clinical manifestations and pathogenesis of dialysis-related amyloidosis. *Semin Dial* 1996; 9: 360–369
2. Evans DB, Thavarajan M, Kanis JA. Immunoreactivity and proliferative actions of β_2-microglobulin on human bone-derived cells *in vitro*. *Biochem Biophys Res Commun* 1991; 175: 795–803
3. Petersen J, Kang MS. *In vivo* effect of β_2-microglobulin on bone resorption. *Am J Kidney Dis* 1994; 23: 726–730
4. Moe SM, Sprague SM. β_2-microglobulin induces calcium efflux from cultured neonatal mouse calvariae. *Am J Physiol* 1992; 263: F540–545
5. Moe SM, Barrett SA, Sprague SM. β_2-microglobulin stimulates osteoclastic mediated bone mineral dissolution from neonatal mouse calvariae. *Calcium Regul Hormones Bone Metab* 1992; 11: 302–306
6. Moe SM, Cummings SA, Hack BK, Sprague SM. Role of IL-1β and prostaglandins in β_2-microglobulin induced bone mineral dissolution. *Kidney Int* 1995; 47: 587–591

7. Balint E, Marshall C, Sprague SM. The role of IL-6 in β_2-microglobulin induced bone mineral dissolution. *Kidney Int* 2000; 57: 1599–1607
8. Migita K, Eguchi K, Tominaga M, Origuchi T, Kawabe Y, Nagataki S. β_2-microglobulin induces stromelysin production by human synovial fibroblasts. *Biochem Biophys Res Commun* 1997; 239: 621–625
9. Brinckerhoff CE, Mitchell TI, Karmilowicz MJ, Kluve-Beckerman B, Benson MD. Autocrine induction of collagenase by serum amyloid A-like and β_2-microglobulin-like proteins. *Science* 1989; 243: 655–657
10. Hou FF, Chertow GM, Kay J *et al.* Interaction between β_2-microglobulin and advanced glycation end products in the development of dialysis related-amyloidosis. *Kidney Int* 1997; 51: 1514–1519
11. Migita K, Tominaga M, Origuchi T *et al.* Induction of cyclooxygenase-2 in human synovial cells by β_2-microglobulin. *Kidney Int* 1999; 55: 572–578
12. Miyata T, Sprague SM. Advanced glycation of β_2-microglobulin in the pathogenesis of bone lesions in dialysis associated amyloidosis. *Nephrol Dial Transplant* 1996; 11: 86–90
13. Miyata T, Kawai R, Taketomi S, Sprague SM. Possible involvement of advanced glycation end-products in bone resorption. *Nephrol Dial Transplant* 1996; 11 [Suppl 5]: 54–57
14. Owen WF Jr, Hou FF, Stuart RO *et al.* β_2-microglobulin modified with advanced glycation end products modulates collagen synthesis by human fibroblasts. *Kidney Int* 1998; 53: 1365–1373
15. Niwa T, Miyazaki S, Katsuzaki T *et al.* Immunohistochemical detection of advanced glycation end products in dialysis-related amyloidosis. *Kidney Int* 1995; 48: 771–778
16. Miyata T, Iida Y, Ueda Y *et al.* Monocyte/macrophage response to β_2-microglobulin modified with advanced glycation end products. *Kidney Int* 1996; 49: 538–550
17. Takagi M, Kasayama S, Yamamoto T *et al.* Advanced glycation end products stimulate interleukin-6 production from human bone derived cells. *J Bone Miner Res* 1997; 12: 439–446
18. Siddhanti SR, Quarles LD. Molecular to pharmacologic control of osteoblast proliferation and differentiation. *J Cell Biochem* 1994; 55: 310–320
19. Centrella M, McCarthy TL, Canalis E. β_2-Microglobulin enhances insulin-like growth factor I receptor levels and synthesis in bone cell cultures. *J Biol Chem* 1989; 264: 18 268–18 271
20. Jennings JC, Mohan S, Baylink DJ. β_2-Microglobulin is not a bone cell mitogen. *Endocrinology* 1989; 125: 404–409
21. Conners LH, Shirahama T, Skinner M, Fenves, A, Cohen AS. *In vitro* formation of amyloid fibrils from intact β_2-microglobulin. *Biochem Biophy Res Commun* 1985; 131: 1063–1068
22. Canalis E, McCarthy T, Centrella M. A bone-derived growth factor isolated from rat is β_2-microglobulin. *Endocrinology* 1987; 121: 1198–1200
23. Kataoka H, Geyjo F, Yamada S, Kunitomo T, Arakawa M. Inhibitory effect of β_2-microglobulin on *in vitro* calcification of osteoblastic cells. *Biochem Biophys Res Commun* 1986; 141: 360–366