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PERSPECTIVES IN CLINICAL NEPHROLOGY

Is β_2-microglobulin a mediator of bone disease?

Elevated serum and tissue concentrations of β_2-microglobulin are universally found among patients with chronic renal insufficiency. A new type of amyloidosis affecting patients undergoing long-term dialytic therapy is related to the accumulation of β_2-microglobulin [1–19]. This amyloidosis, also termed AB-amyloidosis, β_2-microglobulin amyloidosis, or dialysis related amyloidosis, is manifested clinically by the carpal tunnel syndrome, erosive and destructive osteoarthropathies, and cystic bone lesions [6, 10, 14, 19, 20]. Although most commonly seen in patients following long-term hemodialysis therapy, β_2-microglobulin amyloidosis also occurs in patients treated exclusively by continuous ambulatory peritoneal dialysis [1, 2] or prior to the initiation of dialytic therapy [19]. The incidence of these complications increases with the age of the patient and the duration of renal failure and/or replacement therapy [21–23]. Biochemical and immunohistologic studies have demonstrated that β_2-microglobulin is the major protein constituent of the amyloid fibrils in these patients [3, 8, 17]. However, the pathologic role of β_2-microglobulin in amyloid formation and development of osseous lesions is poorly understood. Many issues concerning β_2-microglobulin amyloidosis remain unresolved. The following discussion will focus on two fundamental questions regarding the etiology of the β_2-microglobulin associated lesion: (1) is the formation of amyloid fibrils a primary event resulting from increased circulating concentrations of β_2-microglobulin; and (2) does β_2-microglobulin or the β_2-microglobulin-amyloid fibril play an active role in inducing bone destruction or is it an innocent bystander?

Metabolism

β_2-microglobulin is a nonglycosylated polypeptide composed of 100 amino acids arranged in a single polypeptide chain with a molecular weight of 11,800 Daltons. The synthesis of β_2-microglobulin has been estimated to range between 50 and 200 mg/day [24]. It is present on the surface of all nucleated cells as an integral part of the HLA class I antigen complex but may also be found in non-HLA-associated forms [25–27] with lymphoid cells, predominantly T cells, having the greatest surface density [28]. Hepatocytes appear to possess the greatest synthetic capacity, thus the liver is most probably the main organ responsible for circulating β_2-microglobulin concentrations under both normal and pathologic conditions [29]. Intracellular levels of β_2-microglobulin are regulated by endogenous mediators, predominantly α- and γ-interferon [29–31]. After shedding from cell surfaces or intracellular release, more than 90% of the β_2-microglobulin circulates in the monomeric form that is not protein bound [18, 32].

β_2-microglobulin is almost exclusively catabolized within the kidney. At least 95% and possibly 100% of the circulating β_2-microglobulin is eliminated via glomerular filtration [24], reabsorbed in the proximal tubule and subsequently degradated by proteolytic enzymes [33–35]. Thus, approximately 150 to 200 mg of β_2-microglobulin are metabolized daily by normal kidneys. Although extrarenal sites of β_2-microglobulin catabolism may exist, none have been clearly demonstrated. In normal individuals the serum concentration of β_2-microglobulin is usually less than 2 mg/liter and the urinary excretion less than 400 μg/24 hr [34, 36, 37].

Increased synthesis and release of β_2-microglobulin, as indicated by an elevation of serum β_2-microglobulin concentrations, occurs in inflammatory and malignant diseases including rheumatic disorders, infectious diseases, acquired immunodeficiency and lymphoproliferative disorders [38–41]. Both T and B cell antigens increase cellular β_2-microglobulin release [42, 43]. *In vitro* and *in vivo* studies demonstrated that the synthesis and release of β_2-microglobulin are mediated by various cytokines. Tumor necrosis factor (TNF), interleukin-2 (IL-2), and both α- and γ-interferon (INF) stimulate the synthesis and release of β_2-microglobulin from various cell types in culture [11, 29, 44, 45]. *In vivo* application of TNF or α- and γ-INF increases serum β_2-microglobulin concentrations [29, 45]. Whereas IL-1 has not been shown to have an stimulatory effect on β_2-microglobulin synthesis or release *in vitro* [11, 29, 44, 45], a stimulatory effect of IL-1 via the *in vivo* induction of other cytokines is probable [46]. The most important cause of increased β_2-microglobulin concentrations is the reduced clearance associated with renal failure, where serum concentrations are increased to more than 50-fold the normal range [4, 21].

Several years ago it was observed that the type of hemodialysis therapy may affect serum β_2-microglobulin concentrations. An increase in β_2-microglobulin concentration during dialysis with cuprophane membranes and a decrease during dialysis with synthetic membranes were observed [9, 47]. The increased levels may result from dialysis membrane bioincompatibility, triggering complement activation and C5a and C3a formation, leading to sequestration and activation of leukocytes, which then promote β_2-microglobulin synthesis and release [32, 48]. Alternatively it has been suggested that increased β_2-microglobulin synthesis occurs as a result of a marked and rapid lowering of plasma osmolality or oncotic pressure during hemodialysis [11, 49]. The role of the hemodialysis treatment and type of membrane remain controversial. Campistol et al evaluated *in vitro* β_2-microglobulin synthesis in lymphocyte cultures and were unable to demonstrate an effect of hemodialysis modality or membrane type on β_2-microglobulin synthesis [50], whereas Zaoui, Stone and Hakim clearly demonstrated that lymphocytes cultured from patients dialyzed with cuprophane membranes produced significantly more β_2-microglobulin than patients dialyzed with synthetic membranes [51]. Similar to the findings of Campistol and colleagues [50], Zingraff et al also demonstrated no effect of the cuprophane

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membrane to stimulate *in vitro* β_2-microglobulin synthesis in whole blood from both normal individuals and hemodialysis patients [47]. Furthermore, the addition of IL-1 and IL-6 to lymphocyte cultures did not increase β_2-microglobulin synthesis, whereas the addition of normal rabbit serum did [50]. More work is required to elucidate which factors are important in inducing β_2-microglobulin synthesis during dialysis.

β_2-Microglobulin as an amyloidogenic protein

Serum β_2-microglobulin levels in patients with chronic renal failure with and without clinical or histological amyloidosis are similar. Thus, additional factors other than the increased circulating concentrations of β_2-microglobulin must be involved in the development of amyloidosis [4, 5, 12, 13, 16, 21, 52, 53]. The β_2-microglobulin molecule contains two anti-parallel β-pleated sheets, and its three-dimensional structure resembles the domains of IgG [25]. The β-structure of the molecule suggests an intrinsic capacity for the formation of amyloid fibrils. In fact, the spontaneous generation of β_2-microglobulin amyloid fibrils has been achieved *in vitro* with high concentrations of β_2-microglobulin in salt-free solutions [54]. Spontaneous *in vitro* aggregation of β_2-microglobulin into amyloid fibrils has also been demonstrated with bovine β_2-microglobulin [55] and from protein originating from mononuclear cells of dialysis patients [56]. These findings suggest that, unlike other types of amyloidosis where a proteolytic process is required, β_2-microglobulin may not require a proteolytic phase or cell processing.

Several observations, however, strongly argue against simple precipitation of β_2-microglobulin as the mechanism of amyloidogenesis. It has been observed that not only intact β_2-microglobulin, but also a 24 kDa dimer and various fragments of β_2-microglobulin are involved in amyloid formation [52]. Linke and colleagues [52] demonstrated that proteases can cleave the lysine residue located at the amino-terminal of β_2-microglobulin, thus increasing its hydrophobic tendency and inducing amyloid formation. They have subsequently demonstrated the presence of protease modified β_2-microglobulin fragments *in situ* in amyloid deposits isolated from chronic dialysis patients [53]. Gejyo, Homma and Arakawa have identified a 7.4 kDa fragment consisting of β_2-microglobulin residues from amyloid renal calculi in chronic dialysis patients, which was not due to a lysine-specific cleavage [6]. In addition to intact β_2-microglobulin, a "novel β_2-microglobulin" with a reduced molecular weight has been identified from solubilized amyloid fibrils as well as in the plasma of hemodialysis patients with clinical β_2-microglobulin amyloidosis [16]. This protein exhibited a more acidic isoelectric point than normal β_2-microglobulin. Subsequently, other investigators have identified several acidic isoforms of β_2-microglobulin in serum and urine from both uremic and nonuremic individuals [57, 58]. Other than a circulating dimeric form of β_2-microglobulin isolated only from uremic serum [57], it appears that these circulating acidic isoforms are not necessarily specific for amyloidogenisis [57, 58]. Miyata et al [12] have also isolated an acidic β_2-microglobulin from amyloid fibrils, serum and urine of affected hemodialysis patients which was not present in normal individuals. This acidic isoform comprised the majority of β_2-microglobulin found in the amyloid fibrils. The electrogenic heterogeneity of β_2-microglobulin was not due to deamination as previously proposed [8, 16], but a result of Maillard reaction causing advanced glycation end product modification of circulating β_2-microglobulin [12].

In addition to the post translational modification of β_2-microglobulin, substances other than β_2-microglobulin may facilitate the amyloidogenesis of β_2-microglobulin. We have observed increased incidence of β_2-microglobulin amyloidosis with decreased circulating concentrations of amyloid P component, suggesting increased tissue uptake of amyloid P component in the pathogenesis of the amyloidosis [21]. The glycosaminoglycans (GAG) composition of the interstitial matrix of the tissue may also be a local factor favoring amyloid formation, as suggested by ultrastructural and immunohistochemical studies of β_2-microglobulin amyloid deposits in hemodialysis patients [15]. Electron probe analysis has demonstrated the presence of calcium within the amyloid deposition, suggesting the importance of calcium in the amyloidogenic process [7]. Other proteins, in addition to β_2-microglobulin and its fragments, have also been identified from amyloid deposits. Argiles and colleagues identified α_2-macroglobulin in amyloid deposits and suggested that the progression of the amyloid was due to suppressed decomposition of amyloid fibrils by α_2-macroglobulin [59]. Brancaccio and colleagues have identified a new 85 kDa protein contained in synovial amyloid deposits of hemodialysis patients at a molar ratio equivalent to β_2-microglobulin, however whether this protein is involved with amyloidogenesis has yet to be determined [60].

The predilection for β_2-microglobulin amyloid deposits to osteo-articular tissues, which are rich in collagen, suggests an affinity of β_2-microglobulin for collagen. In fact it has been demonstrated that β_2-microglobulin preferentially binds to various types of collagen in a dose dependent manner [61]. The affinity of β_2-microglobulin for collagen is greater than for proteoglycan or heparan sulfate. Spiegel and co-workers have also observed that both amyloid P component and β_2-microglobulin are found associated with collagen in the dermis of dialysis patients, and their relative concentrations are related to the duration of dialysis [62].

β_2-Microglobulin as a bone growth factor

Bone cells produce β_2-microglobulin and the so-called bone-derived growth factor (BDGF). BDGF, a bone growth factor originally isolated from rat calvariae and subsequently isolated from bovine bone matrix, is now known to be β_2-microglobulin [63, 64]. The amino acid composition of BDGF is compatible with murine β_2-microglobulin and the amino-terminal sequence is identical with murine β_2-microglobulin. β_2-microglobulin appears to have a mitogenic effect on murine bone cells as human β_2-microglobulin and isolated BDGF similarly stimulate DNA production, collagen and non-collagen protein synthesis in isolated osteoblast cultures [63, 65, 66]. However, utilizing isolated chicken osteoblasts, Jennings, Mohan and Baylink were unable to demonstrate a mitogenic effect of human β_2-microglobulin, and the mitogenic effect of bovine bone matrix-derived β_2-microglobulin was attributable to contamination with transforming growth factor-β [64]. In murine calvarial cultures, neither we [67] nor others [63] were able to demonstrate a mitogenic effect of human β_2-microglobulin. The incongruity observed in [^{3}H]-thymidine incorporation between isolated osteoblast and calvarial cultures suggests that additional components of bone may also be affected by β_2-microglobulin, complicating the interpretation of

this experimental measurement. Further supporting such complexity, Evans, Thavarajan and Kanis demonstrated that, although osteoblasts respond to and produce β_2-microglobulin, β_2-microglobulin does not affect the production of alkaline phosphatase or osteocalcin by isolated osteoblasts [66]. Similarly, we were unable to demonstrate an effect of β_2-microglobulin on alkaline phosphatase release [67]. Whether the mitogenic effect of β_2-microglobulin on osteoblasts is a direct effect or a result of contamination or augmentation of other growth factors requires further evaluation.

Molecules of the major histocompatibility complex interact with hormonal receptors, and some investigators believe that β_2-microglobulin might not be a typical growth factor but a regulator of the growth-promoting effects of other growth factors. Centrella, McCarthy and Canalis have demonstrated that the mitogenic effect of insulin-like growth factor I (IGF-I) on bone cells is augmented by β_2-microglobulin. In osteoblast enriched cell cultures, β_2-microglobulin enhances IGF-I receptor number, increases IGF-I transcripts and polypeptide levels [65]. The stimulatory effects of β_2-microglobulin appears to be selective since it does not enhance transforming growth factor β (TGF-β) receptors or transcripts [65]. Although still controversial, the evidence accumulated thus far suggests that β_2-microglobulin has a physiologic function in bone remodeling.

β_2-Microglobulin as a bone resorbing agent

Experimental data demonstrating that, in addition to the deposition of β_2-microglobulin-amyloid at sites of osteo-articular damage, β_2-microglobulin is directly involved in causing osteoarticular disease has been provided by several investigators. Subcutaneous injections of β_2-microglobulin induces histologic evidence of bone resorption in neonatal mice [68]. It is unclear if this observation following local injection is analogous to the effects of circulating β_2-microglobulin. However, we have demonstrated that purified human β_2-microglobulin induces a dose- and time-dependent net calcium efflux from cultured neonatal mouse calvariae [67, 69]. The observation that the β_2-microglobulin-induced calcium efflux could be inhibited by calcitonin, required an incubation period of at least 48 hours and is associated with the release of the osteoclast enzymes, β-glucuronidase and acid phosphatase, is consistent with a cell-mediated phenomenon and suggests stimulated osteoclastic bone resorption. The significant time delay supports the hypothesis that β_2-microglobulin acts via the activation of other factors in the complex regulation of bone remodeling [65, 70].

Bone remodeling is controlled by hormonal and local factors in a complex regulatory system and β_2-microglobulin probably interacts via several of these pathways. Using both submaximal and pharmacologic concentrations of parathyroid hormone, we were unable to demonstrate augmentation of calcium efflux from calvariae incubated with parathyroid hormone and β_2-microglobulin [67, 69]. Moreover, other studies were unable to demonstrate an effect of parathyroid hormone on β_2-microglobulin transcription [71] or an effect of $1,25(OH)_2D_3$ on β_2-microglobulin immunoreactivity [66] in osteoblast cultures. These preliminary data suggest that calciotropic hormones may not influence β_2-microglobulin synthesis or action in a direct manner.

Several cytokines are known to induce bone resorption [70, 72]. Both IL-1 and TNF-β have multiple effects on bone including stimulation of cell mediated resorption [73–75]. Enhanced production with elevated basal concentrations of these cytokines has been demonstrated in patients undergoing chronic hemodialysis [76, 77]. It is possible that these cytokines could initiate bone resorption, thus predisposing osteo-articular structures to the deposition of β_2-microglobulin. Contrarily, the bone resorbing effect of β_2-microglobulin may be mediated by these cytokines. It has recently been demonstrated that advanced glycation end product-modified β_2-microglobulin purified from chronic dialysis patients stimulates production of TNF-α and IL-1β by macrophages [13]. This is consistent with our recent observation that β_2-microglobulin induced calcium release from neonatal calvariae can be completely prevented with IL-1β antibody, whereas prostaglandin inhibition with indomethacin has no effect on the β_2-microglobulin-induced calcium release from cultured bone [78].

β_2-microglobulin has also been demonstrated to induce the synthesis of fibroblast collagenase, suggesting its role in modulating connective tissue breakdown in both normal and disease states [79]. Miyata et al demonstrated that advanced glycation end product-modified β_2-microglobulin stimulates a sufficient amount of TNF-α and IL-1β to cause production of collagenase in cultured human synovial cells [13]. Clearly more work is required to further elucidate the relationship between the accumulation of β_2-microglobulin and the evolution of progressive tissue destruction.

Conclusion

In summary, osteo-articular tissue, which is rich in collagen, most likely has a high affinity for β_2-microglobulin accumulation. The deposition of β_2-microglobulin as amyloid fibrils is not only the result of elevated tissue concentrations and interaction with other compounds such as P component, collagen and glycosaminoglycans, but most likely is the result of metabolic processing including proteolysis and/or advanced glycation end-product modification. Within osteo-articular tissues which have high endogenous enzymatic activity and metabolically-altered bone remodeling from underlying renal disease, β_2-microglobulin most likely further interferes with the remodeling process. Based upon the available data we propose the following model of β_2-microglobulin osteo-articular destruction (Fig. 1). The increased circulating serum concentrations of β_2-microglobulin become modified into advanced glycation end products with time. These proteins then induce the production of IL-1 and TNF-α which stimulate bone resorption and inhibit bone formation. The TNF-α, and possibly IL-1, also increase the local production of β_2-microglobulin. In addition, synovial cells are stimulated to produce collagenase which further facilitates bone resorption and soft tissue destruction. The local tissue destruction induces the migration of inflammatory cells and the subsequent production of cytokines leading to increased tissue destruction. The resorbed bone and surrounding tissues, which are rich in collagen, becomes the site of deposition and formation of β_2-microglobulin amyloid. Further studies are required to substantiate this hypotheses and answer the question as to why this form of amyloidosis has such a predisposition to osteo-articular structures.

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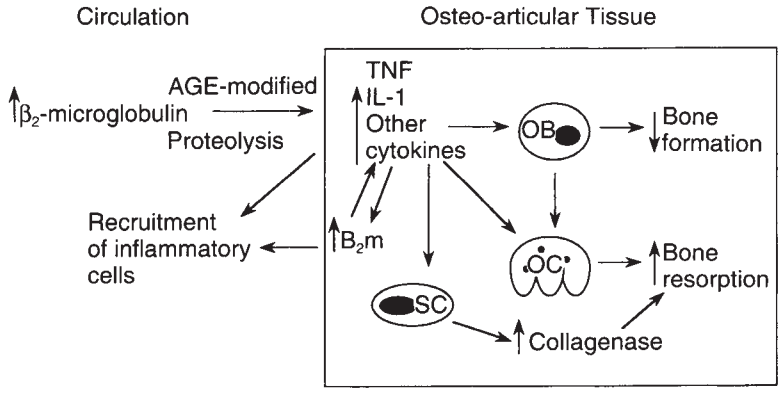

Fig. 1. *Proposed mechanism of β_2-microglobulin tissue destruction in the pathogenesis of β_2-microglobulin amyloidosis.* Abbreviations are: AGE, advanced glycation end product; OB, osteoblast; OC, osteoclast; SC, synovial cell.

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