β-2 Microglobulin in Renal Disease

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In 1968, Berggard and Bearn (1) isolated β2-microglobulin (β2m). It is a 100-amino acid single polypeptide chain, with a globular structure maintained by a disulfide bond linking two cysteins in positions 25 and 80, respectively. It has a molecular weight of 11,815 daltons and a stokes radius of 16 Å. Its tridimensional structure, as well as its amino acid sequence, is homologous to immunoglobulin constant domains (2). β2m is found on the surface of human cells expressing MHC class I (3). The MHC class I molecule is made up of a heavy chain carrying the allotypic determinants of its specificity and a noncovalent bound light chain, β2m. Like immunoglobulin light chains, β2m stabilizes the structure of the heavy chain and allows surface MHC class I antigen expression (3).

β2m is produced by all cells expressing MHC class I antigen. Lymphocytes and tumor cells synthesize large amounts of β2m in vitro and are thus presumably major biosynthetic sites. In vitro stimulation of lymphocytes by mitogens or specific antigens increases β2m production (4,5). Shedding from cell surface of the MHC occurs through proteolysis, requires metabolic energy, and is inhibited by colchicine (3). β2m instantly dissociates from the heavy chain and enters the circulation as a monomer. β2m is released not only in blood but also in synovial, cerebrospinal, amniotic, and seminal fluids, and in the aqueous humor, the colostrum, and the saliva.

Endogenous production depends partly on the activity of β2m synthesizing cells. In the healthy subject, 125I-labeled β2m turnover studies suggest a daily synthesis of 150 to 200 mg (6). β2m synthesis is stimulated in various conditions and is characterized by monoclonal or polyclonal activation and proliferation of lymphoid cells such as malignant tumors, lymphoproliferative B cell disorders and various chronic inflammatory diseases, e.g., rheumatoid arthritis, systemic lupus erythematosus, Crohn’s disease, viral hepatitis, and other viral infections (7-9).

β2m is readily filtered through the glomerulus and almost completely reabsorbed and destroyed by proximal tubular cells, with less than 400 ng of intact β2m appearing daily in the urine (10).

Decreased renal function results in a proportional rise in serum β2m levels; hence, serum β2m concentration was advocated 15 yr ago as an index of glomerular filtration (11). It has now been abandoned because β2m production and therefore the serum level is influenced by many additional factors. Serum β2m may increase up to 60-fold in dialysis patients, with lower values being observed in subjects with residual renal function (12).

Impairment of β2m tubular uptake results in a raised intact β2m urinary excretion (1). Urinary β2m levels have thus been taken as a marker of proximal tubular dysfunction. Recent interpretations of an augmented urinary β2m excretion revealed that β2m shares with other proteins a common reabsorption pathway and that its tubular transport is rate-limited. Increased β2m urinary levels are thus found as a result of nephrotic-range proteinuria or as a consequence of an elevated filtered load as observed in renal failure. It is only when β2m production and glomerular filtration are normal and proteinuria is minimal that an elevated β2m excretion in the urine proves an adequate marker of proximal tubular function (13-16).

Dialysis-Related Amyloidosis

Dialysis-related amyloidosis (DRA) first appeared on the medical scene in 1980 in a letter to the editor of La Presse Médicale in which Assenat et al. (17) mentioned the presence of amyloid deposits in the tissue removed during carpal tunnel syndrome surgery in patients on long-term dialysis. The subsequent description of similar amyloid deposits in joints and bones and eventually in various organs established that DRA was a systemic complication of long-term dialysis. The discovery that the amyloid fibrils were composed of β2m molecules eventually identified a new entity, among the various types of amyloidosis: β2m amyloidosis (Aβ2m) (18,19). This type of deposit is characterized by bundles of tightly packed, curvilinear fibrils approximately 10 nm in diameter (20).

Clinical Characteristics

Aβ2m has a marked affinity for joint tissues (cartilage, capsule, synovium). Hence, its clinical picture usually includes the carpal tunnel syndrome (CTS), chronic invalidating arthropathies associated with amyloid bone cysts, leading occasionally to bone fractures. Amyloid deposits present, mostly in minute amounts, in various organs are usually asymptomatic.

Carpal Tunnel Syndrome (CTS)

CTS is usually the first manifestation of Aβ2m. It may be observed within 3 to 5 yr after the onset of dialysis. Its
prevalence rises thereafter, reaching approximately 100% after >20 yr of hemodialysis (HD) (21–23). The symptoms are similar to those observed in non-HD patients: paresthesias of the palmar surfaces of the first 3 to 4 fingers, with eventual sensory and motor loss (wasting of the thenar muscles in particular). The pain typically is exacerbated at night and during HD sessions, as well as by tapping over the palmar surface of the wrist (Tinel’s sign) or forcing flexion of the wrist (Phalen’s sign) (21,23). Eventually, it involves both hands. The differential diagnosis includes uremic neuropathy and cervical root compression (21). Electromyography will confirm the diagnosis. Occasionally, amyloid deposits are associated with an ulnar nerve compression, manifested by paresthesias of the palmar surface of the fourth or fifth fingers, the so-called Guyon’s syndrome (24).

Histologic examination of the material removed during surgery reveals mainly fibrous tissue, β2m amyloid deposits, identified in approximately 70% of cases (23), are usually small. Their absence in 30% of patients may be ascribed either to sampling problems or to the fact that Aβ2m is not the only etiology of CTS in HD patients (23): The vascular access and microcrystalline wrist arthritis have been incriminated (21), and it should not be forgotten that non-β2m CTS is not uncommon, especially in middle-aged nonuremic women. It is clear from most pathologic studies that the median nerve is compressed mainly by fibrous tissue and only very rarely by the amyloid deposit itself, a situation analogous to that observed in AL (or light chain-related) amyloidosis. Aβ2m-related CTS should be suspected in long-term (>7 yr) HD patients, especially when its symptoms are associated with chronic arthralgias.

**Amyloid Arthropathy: The Peripheral Joints**

The first Aβ2m deposits appear on the surface of the cartilage. They extend subsequently to the synovia, joint capsules, and attached tendons (Figure 1). The deposits, initially paucicellular (20,25), are eventually surrounded by macrophages (26) (Figure 2). The former stage is usually asymptomatic, whereas the latter is characterized by manifestations of articular inflammation.

The prevalence of arthralgias rises with HD duration. In Charra et al.’s series (22), shoulder pain and stiffness were noted as early as 5 yr after HD onset, to affect 50 and 100% of patients after 13 and 19 yr of HD, respectively. Arthralgias are usually insidious at onset and worsen progressively. Joint mobility becomes restricted (21). Articular involvement, usually bilateral (27), starts often in the shoulder and extends later to the hip, knee, wrist, etc. Pain is exacerbated at night or during HD sessions (23). Chronic tenosynovitis of the finger flexors is associated with mobility restriction, pain, and palmar swelling, and, in some patients, trigger fingers (21).

Joint swelling is not a constant finding, but joint effusions may develop and are usually of the low-grade inflammatory type (21), unless hemarthrosis develops, as observed in a minority of patients (28). Synovial fluid aspiration may yield small synovial fragments containing β2m amyloid deposits.

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**Figure 1.** Ultrasonographic (A) and postmortem (B) findings in a patient dialyzed for 17 yr. Femoral neck capsule is markedly thickened (arrows), measuring 13.6 mm both on ultrasonographic and postmortem. Histologic examination of femoral neck capsule disclosed extensive β2 microglobulin (β2m) amyloidosis. Reprinted with permission. From reference 142.
(29). Unusual manifestations of $\beta_2$m include subcutaneous $\beta_2$m amyloid masses with a periarticular location (elbow, knee, hip) (30,31).

**Amyloid Arthropathy: The Spine**

Kuntz et al. first described (32) a destructive spondylarthropathy developing in long-term HD patients. Radiologic signs include severe narrowing of the intervertebral spaces (especially at the cervical level), erosions, and cysts of the vertebral plates without significant osteophytosis. Clinical signs are usually limited to moderate pain relieved by analgesics (21). This syndrome, subsequently recognized as common in long-term HD patients, is not solely due to amyloid deposits because no $\beta_2$m was found at histology in several typical cases (33). It is probably of multifactorial origin: Age, mechanical stress and/or severe hyperparathyroidism are probably involved (33). Amyloid masses may develop in the epidural space and joints of the cervical spine, especially the atlanto-occipital joint of long-term HD patients (34–36). They eventually result in subacute or acute neurologic compression, with quadriaparesis or occipital nerve neuralgia.

**Bone Fractures**

$\beta_2$m amyloid invades not only cartilage, synovia, and capsules but also the adjacent bone. The development of typical bone cysts may eventually culminate in pathologic fractures, especially of the femoral neck (23,36–38). Other locations include the scaphoid bone and the C1–C2 junction.

**Systemic (Nonosteoarticular) Manifestations**

$\beta_2$m amyloid deposits are also found in various organs, mostly after more than 12 yr of HD, i.e., much later than in the joints. They are usually small, mostly in blood vessel walls (39), and are usually asymptomatic. Rarely, they enlarge within visceral organs and cause heart failure with pulmonary hypertension, gastrointestinal tract bleeding, bowel perforation, infarction or pseudo-obstruction, chronic diarrhea, macroGLOSSIA, or lingual nodules (40–48). A review of all postmortem reports of systemic $\beta_2$m shows an uneven distribution among various organs: heart, 80%; gastrointestinal tract, 78%; lung, 59%; liver, 41%; kidney, 33%; spleen, 5% (49).

It may be worth mentioning that $\beta_2$m amyloid may be observed in the prostate of patients with normal renal function. No extra prostatic sites have ever been identified. This entity is clearly different from $\beta_2$m (50).

**Diagnostic Tools**

**Histology: The Gold Standard**

The gold standard for the diagnosis of all types of amyloidosis remains histologic examination. In patients with joint effusion, synovial fluid aspiration yielded Congo Red-positive synovial fragments in six of seven tested patients in whom $\beta_2$m was almost certainly present (29).

Histology of affected tissues (mainly joints) remains unavailable in most patients suspected of having $\beta_2$m, whereas biopsies of nonosteoarticular structures (including subcutaneous fat and rectum) have proven insensitive (36,51,52).

At autopsy, the prevalence of histologic $\beta_2$m in the joints is much higher than suspected on clinical grounds: up to 20 to 30% of patients are affected within 2 to 3 yr of HD, and more than 90% beyond 7 yr of HD, both in peripheral joints (25) and vertebrae (53). In these series, the sternoclavicular joints and the cervical vertebrae are preferentially involved compared with shoulders or thoracic vertebrae. The prevalence of nonosteoarticular $\beta_2$m remains ill-defined. It develops mostly after more than 12 yr of HD.
Skeletal X-Rays

Bone cysts have been recognized since 1985 as a hallmark of Aβ2m (37). The radiologic pattern of bone lesions is similar in Aβ2m and in AL amyloidosis (54). It includes swelling of soft tissues, with a preserved or widened joint space, and juxtaarticular cystic bone defects located mainly in the wrist (Figure 3), shoulder, and hip (23). Bone cysts increase in size and number over time, together with displacement of fat pads (reflecting the soft tissue swelling). Skeletal x-rays may allow a specific diagnosis of bone Aβ2m provided that strict criteria are applied (54,55). Their size should exceed 10 mm (hip, shoulder) or 5 mm (wrist). Cysts have to be located outside areas prone to synovial inclusions such as the femoral neck and outside the weight-bearing area of the acetabulum. Joint space adjacent to the bone defect should be normal, in order to exclude osteoarthritic bone cysts. When defects of an adequate size affect a weight-bearing area or sites of synovial inclusions, only defects whose diameter increases by >30% per year are considered significant (54,55). For Aβ2m to be diagnosed, at least two joints should be involved (55). When the two affected joints are the wrists, at least two significant bone defects must be detected in one of them. These criteria allow, in our experience, a specific diagnosis of Aβ2m. Unfortunately, their sensitivity is low.

Ultrasonography

Capsulosynovial ("soft tissue") swelling precedes the development of bone cysts in Aβ2m. This is demonstrated by the early displacement of the fat pad, prior to the appearance of typical bone cysts in the shoulder (23).

Technical developments now permit an accurate assessment of capsules and tendons by joint ultrasonography, thus yielding a potentially promising, more sensitive tool for Aβ2m diagnosis. Indeed, thickening of supraspinatus shoulder tendon and of the femoral neck capsule have been documented (56–59) (Figure 1).

SAP and β2m Scintigraphy

SAP Scintigraphy. Serum amyloid P component (SAP), a nonfibrillar plasma glycoprotein synthesized by the liver, undergoes noncovalent calcium-dependent binding to almost all types of amyloid fibrils. This avidity has been used in scintigraphic studies with 123I-labeled SAP, first in a mouse model of systemic amyloidosis and then in various types of human amyloidosis (60).

In a cross-sectional study of 38 long-term HD patients, 123I-labeled SAP accumulated at all sites of histologically proven Aβ2m and at many sites of clinically suspected Aβ2m (61). Wrist uptake was predominant. Surprisingly, however, splenic uptake was observed in 12 of the 38 long-term HD patients, although histologic Aβ2m is highly uncommon at that site and, when present, minimal. In contrast, hip uptake was infrequent and shoulder uptake was not detected in seven of 19 cases with clinical manifestations. Splenic uptake is probably an artefact, whereas the lack of hip or shoulder uptake reflects methodologic problems. Thus, the specificity and the sensitivity of the method seem limited. A more recent study of SAP scintigraphy in AL amyloidosis supports a cautious evaluation of the diagnostic potential of SAP scintigraphy (62).

The specificity of SAP scintigraphy is further limited by the fact that SAP binds all types of amyloid and, thus, does not distinguish Aβ2m from the other amyloidoses, including that associated with age. The ability of SAP scintigraphy to quantify deposited amyloid is as yet not supported by convincing histologic evidence (63).

β2m Scintigraphy. β2m labeled by 131I has also been used in scintigraphic studies. Sensitivity for the detection of large amyloid deposits and specificity appear excellent in 42 long-term HD patients (64).

Scintigraphic images are reproducible and more sensitive in the detection of Aβ2m than clinical or radiologic methods (64). The diagnostic value of 131I-labeled β2m scintigraphy for small, incipient amyloid deposits remains to be documented. The method cannot be used in patients with a significant

Figure 3. (A) Multiple bone cysts in the left wrist and distal radius (arrowheads) at the time of renal transplantation. (B) Unchanged aspect 113 mo later. Reprinted with permission. From reference 143.
residual renal function, because $\beta_2m$ is readily excreted by the kidney.

Unfortunately, $^{131}$I $\beta_2m$ scintigraphy entails a substantial radiation exposure. The recently developed $^{111}$indium-diethyl-entriamine penta-acetic acid $\beta_2m$ scintigraphy lowers radiation exposure and provides better optical resolution and stable images (65). It still requires histologic validation.

**Risk Factors for $\alpha\beta_2m$**

The clinical and histologic identification of $\alpha\beta_2m$ in cohorts of patients on dialysis has helped identify several risk factors for the development of this complication.

**Dialysis Modality**

$\alpha\beta_2m$ develops in patients undergoing all forms of renal replacement therapy: HD, continuous ambulatory peritoneal dialysis (CAPD) (66), or hemofiltration (67). The prevalence of histologic $\alpha\beta_2m$ in joints is slightly but not significantly lower in patients treated by CAPD than in patients treated by HD, matched for age and duration of dialysis treatment (68).

**Duration of Dialysis**

Duration of dialysis has rapidly emerged as a critical factor in the clinical manifestation of $\alpha\beta_2m$. As pointed out earlier, the prevalence of CTS requiring surgery (22), of radiologic signs of $\alpha\beta_2m$ (55), and of histologic joint $\alpha\beta_2m$ (at autopsy) rises dramatically with dialysis duration (25,53).

**Age at the Onset of Dialysis**

Independent of dialysis duration, age at the onset of dialysis has been identified as a significant independent risk factor (25,55,61).

**HD Membrane**

Although $\alpha\beta_2m$ has been reported in a few patients with chronic, longstanding renal failure (69) before renal replacement therapy, the fact that the highest prevalence of $\alpha\beta_2m$ is observed in patients on long-term HD raises the question of a potential role of HD membrane type as a risk factor for $\alpha\beta_2m$ (70). Several studies relying on different clinical end points have thus been performed. Current evidence suggests that, indeed, high-flux biocompatible (i.e., with low complement activation) membranes such as polysulphone (AN69) significantly delay $\alpha\beta_2m$ development compared with low-flux, complement-activating membranes such as Cuprophane.

Chanard et al. relied on CTS operation as a marker of $\alpha\beta_2m$. In a longitudinal study of 85 patients given HD for more than 5 yr on the same membrane, actuarial survival without CTS operation was higher ($P < 0.012$) in the AN69 than in the Cuprophane group (71).

van Ypersele et al. (55) relied on radiologic evidence of $\alpha\beta_2m$. In a longitudinal multicenter study, the onset of typical amyloid bone cysts, as well as CTS surgery, was evaluated in 221 patients treated exclusively either with Cuprophane or with AN69 HD for $\geq 5$ yr. Actuarial survival curves without CTS operation was not significantly different between Cuprophane and AN69-treated patients. In contrast, the actuarial risk of developing amyloid bone cysts was 5.5 times higher in Cuprophane than in AN69 patients. Both membrane type ($P < 0.004$) and age at the onset of therapy ($P < 0.007$) emerged as independent risk factors (55). More recently, Kückle et al. (72) demonstrated that high-flux polysulphone HD is associated with a lower risk of DRA than Cuprophane HD. These conclusions have been validated in several other cross-sectional studies (73).

**Pathophysiology**

**Role of $\beta_2m$ Retention**

Long-term $\beta_2m$ retention is a prerequisite for $\alpha\beta_2m$ development, although a critical threshold has not been demonstrated. Serum $\beta_2m$ levels might be taken as a marker for $\beta_2m$ retention in dialyzed patients. They are influenced by several factors.

**$\beta_2m$ Production.** As pointed out earlier, $\beta_2m$ production, unrelated to HD, increases markedly in a variety of immunoinflammatory states. As yet, however, clinical evidence does not suggest that these conditions enhance $\alpha\beta_2m$ development.

**Influence of Residual Renal Function.** Because $\beta_2m$ is normally catabolized by the kidney, it is not surprising that even a minimal residual renal function is associated with lower $\beta_2m$ levels (74). Serum $\beta_2m$ levels are twice as high in HD patients with a GFR $< 1 \text{ ml/min}$ than in those with a GFR of 4 to 5 ml/min (75).

**Patients’ Age and Duration of HD.** Patients’ age is negatively correlated with serum $\beta_2m$ levels, independent of residual renal function (74). By contrast, duration of hemodialysis, which has been considered a significant determinant of serum $\beta_2m$ levels in a large cross-sectional analysis (22), does not emerge as an independent determinant when age and residual renal function are considered in a stepwise multiple regression analysis (74).

**Metabolic Acidosis.** Metabolic acidosis might be a stimulus for $\beta_2m$ production. In vitro, lowering the pH of the medium from 7.34 to 5.1 reduces $\beta_2m$ cellular expression of cultured human myeloid cells and increases $\beta_2m$ release in the supernatant. In vivo serum $\beta_2m$ level is inversely correlated with serum bicarbonate concentration both in advanced renal failure. Shifting from acetate to bicarbonate dialysate raises blood pH and lowers serum $\beta_2m$ level (76).

**Dialysis Modality Including Membrane Type.** The dialysis modality including membrane type also influences serum $\beta_2m$ levels. Available evidence demonstrates that serum $\beta_2m$ levels are 30% lower in patients treated by CAPD or HD with high-flux biocompatible membranes such as AN69 or polysulphone than in patients maintained on low-flux bioincompatible Cuprophane dialysis (77,78). This difference reflects the ability of both high-flux and peritoneal membranes to clear $\beta_2m$ more effectively, as well as the better preservation of residual renal function in patients given CAPD (79).

Serum $\beta_2m$ levels are determined more by the porosity than by the biocompatibility of the membrane (80). Some studies, however, have suggested that complement-activating membranes such as Cuprophane stimulate $\beta_2m$ synthesis and/or release. Available in vitro data remain conflicting. Increased
release by activated blood mononuclear cells, demonstrated in some studies (81), is of marginal significance when compared with daily β2m synthesis. In vivo studies of β2m turnover have failed to detect any significant effect of membrane type (80). However, the number of such studies, as well as that of the included patients, is small, interindividual variations are substantial, and the assumptions underlying the mathematical models are multiple and often unsubstantiated. Still, should some membrane types enhance β2m production, the relevance of this phenomenon would seem limited when compared with the substantial clearance of β2m by some HD membranes (78–80).

Whatever the ability of dialysis membranes to remove β2m, it always fails to meet daily β2m production whether normal (3 mg/kg per d) or enhanced (4.35 mg/kg per d). Indeed, as HD is intensified, predialysis serum β2m level decreases, thus reducing β2m mass removal. Even daily hemofiltration with AN69 removes less than 1000 mg per week (82). It has been calculated that a patient dialyzed with Cuprophane accumulates 400 g of β2m within 4 yr (assuming an increased β2m production) or 8 yr (assuming a normal β2m production). Under the same condition, a patient dialyzed with AN69, a membrane with a high clearing capacity for β2m, retains 400 g after 8 and 14 yr, respectively, according to the assumed β2m production (80).

Role of β2m Modification

Because DRA deposits consist of β2m fibrils, the factors responsible for β2m fibril formation have been actively sought. Some researchers (83,84) provided evidence that β2m amyloid fibrils can be formed in vitro from intact β2m, but the experimental conditions were stringent and very different from those prevailing in human joints. Investigators have thus considered the possibility that β2m had to be modified to precipitate as fibrils.

Linke et al. (85) isolated from Aβ2m deposits not only native β2m but also truncated peptides with a shortened aminoterminal sequence. They propose that the proteolytic transformation of a hydrophilic precursor molecule into a more hydrophobic amyloidogenic peptide is a prerequisite for Aβ2m, as suggested for other types of amyloid. These observations, however, were not confirmed by Campistol et al. (86) and Argilés et al. (87).

An isoform of β2m with a more acidic isoelectric point (acidic β2m) has been subsequently recognized in the serum, ultrafiltrate, and amyloid deposits from HD patients (88, 89). Deamidation at amino acid position 17 was suggested as the main factor contributing to a more acidic isoelectric point (89). Its relevance to Aβ2m genesis, however, was questioned because acidic β2m is not specific to long-term HD patients or amyloid deposits (87). More recently, it has been demonstrated that a part of acidic β2m was due to progressive glycation and oxidation ("glycoxidation") of the molecule through the non-enzymatic Maillard reaction (90,91). Approximately 10% of acidic β2m consists of Amadori products and less than 1% of irreversible advanced glycation end products (AGE). The latter AGE-modified β2m has unique cross-linking and chemotactic powers and has thus attracted considerable interest despite its minimal concentration in uremic patients.

AGE Modification of Proteins

Over a period of several months, carbohydrate-derived carbonyl groups link with protein amino group to produce Schiff base, and, through reversible molecular rearrangements, Amadori products. Eventually, they are transformed into irreversible AGE (92). This nonenzymatic process is called the Maillard reaction. AGE levels increase slowly with age in a variety of collagenous structures as well as in the serum (93,94). These AGE-modified proteins are recognized by specific receptors (95) present in cells such as macrophages (96,97) and are broken down so as to allow appropriate tissue remodeling.

Sustained hyperglycemia markedly enhances AGE transformation (92). AGE levels are elevated in the tissues and serum of diabetic patients (92,93) and correlated with that of fructoselysine, a biomarker of hyperglycemia (93). They are also correlated with the severity of diabetic complications (98,99), suggesting that the accumulation of AGE-modified proteins is instrumental in the development of the vascular, ocular, and renal complications associated with diabetes.

The subsequent discovery that AGE accumulate also in uremic patients came as a great surprise. Observed serum AGE levels are more than 10-fold above those of diabetic patients and, more importantly, appear unrelated to elevated glucose levels (100–102). In contrast to diabetes, they are not correlated with that of fructoselysine.

AGE are heterogeneous structures containing a variety of specific epitopes. Fortunately, specific methodologies have recently allowed the recognition of various AGE structures in uremic sera and β2m amyloid deposits, e.g., pentosidine (103), Nε-carboxymethyllysine (CML) (104), and imidazolone (105). These substances are markedly elevated in uremic sera regardless of the presence of diabetes, and have been specifically identified in β2m amyloid deposits (Figure 4) (106–110).

Increased AGE Genesis in Uremia

The accumulation of AGE in nondiabetic uremic patients is not attributable to hyperglycemia in the absence of any correlation between pentosidine or CML and fructoselysine levels. Serum pentosidine and CML are mainly linked to circulating albumin (101 and unpublished observation), so that their elevated serum levels cannot be attributed to a decreased urinary removal. Obviously, uremic sera contain either unknown precursors and/or catalysts of the Maillard reaction.

Recently, gathered evidence (111 and unpublished observations) suggests that AGE production is enhanced in uremia as the result of the accumulation of carbonyl compounds, derived from the autoxidation of both carbohydrates and lipids. These compounds eventually modify proteins by glycoxidation. AGE production is closely linked to "oxidation" processes (112). CML was originally identified as a product formed by oxidative cleavage of a glucose-derived Amadori compound (104). Glyoxal and dehydroascorbate, formed as the results of autoxidation of glucose, ascorbate, and polyunsaturated fatty acids, are also precursors of CML (109,113,114). Pentosidine origi-
nates from a reaction of protein amino groups with ribose (103), but also from arabinose, another antioxidative product of glucose and ascorbate (109,113,115). AGE are thus products of the combined process of glycation and oxidation. This contention is supported by evidence that in vitro formation of CML and pentosidine (104,115) is prevented by the removal of oxygen from the incubation medium.

Several lines of evidence suggest that chronic uremia is a state of increased oxidative stress: augmented lipid peroxidation (116,117), increased ratio of oxidized to reduced serum glutathione (118,119), increased ratio of oxidized to reduced form of serum albumin (120), depressed activity of serum glutathione-dependent enzymes (119), elevated serum levels of "advanced oxidation protein products" (121), and increased ratio of oxidized to reduced form of serum ascorbate (109).

The contribution of an increased oxidative stress in the generation of AGE is supported by the demonstration that in uremic serum, levels of pentosidine are correlated with those of independent oxidative stress markers such as oxidized ascorbate and advanced oxidation protein products (109,121). In uremic plasma, levels of CML are also correlated with those of malondialdehyde, a lipid peroxidation product taken as an independent marker of oxidative stress (unpublished observation). Pentosidine levels are also correlated with the degree of monocyte activation, a condition known to augment oxidative stress (122).

**Role of AGE in Aβ2m**

As mentioned above, two stages may be considered in the development of Aβ2m. The first stage is asymptomatic and diagnosed mainly by pathology. It precedes by several years the onset of clinical and radiologic signs (25,53). Neither macrophages nor bone destruction is detectable in the vicinity of amyloid deposits (Figure 2). The second stage is symptomatic and accompanied by an inflammatory reaction. Macrophages surround Aβ2m deposits, and cysts develop within bones (26,53) (Figure 2). The mechanism transforming silent early deposits into clinically manifest bone and joint destruction remains to be elucidated.

The demonstration that AGE-modified β2m is endowed with chemotactic properties capable of attracting monocytes and stimulating macrophages to release proinflammatory cytokines (123–125) is of interest in this context. The released cytokines are also able to stimulate the synthesis of collagenase in cultured human synovial cells (123) and might contribute to progressive bone loss and the formation of bone cysts.
AGE-modified β2m also stimulates osteoclast-induced bone resorption and induces a net calcium efflux from cultured neonatal mouse calvaria much larger than that provoked by normal β2m (126). In the model of mouse unfractionated bone cells cultured on dentin slices, AGE-modified β2m increases the number of resorption pits formed by osteoclasts, without an increase in the number of newly formed osteoclasts (127). Apparently, AGE either activate osteoclasts or alter their microenvironment so as to promote bone resorption.

Among the various forms of acidic β2m, only AGE-modified β2m has these biological properties. Truncated β2m, deamidated β2m, and early Amadori β2m do not share this capacity (125). The clinical manifestations of Aβ2m thus might result from the progressive AGE transformation of long-lived amyloid deposits linked to a heightened cellular response of monocyte/macrophage, synovial cell, and osteoclast/osteoblast to AGE-modified β2m.

In this framework, AGE protein alterations induce a clinically manifest local inflammatory reaction to preexisting amyloid deposits. Whether AGE-modified β2m also plays a role in amyloid formation remains unknown. It is of interest that pentosidine is able to cross-link proteins, a characteristic possibly relevant to the formation of amyloid fibrils.

The preferential location of AGE-modified β2m in joints has not been fully explained. Hyperparathyroidism, aluminum, and iron overload do not appear to be significant factors (73). The biochemical composition of the osteoarticular structure provides a more plausible explanation. In various types of amyloid deposits, fibrils are closely associated with some extracellular matrix components such as glycosaminoglycans. Athanasou et al. (128) used various histochemical and immunohistochemical approaches and found a close anatomical association between highly sulfated glycosaminoglycans (especially keratan sulfate) and both senile and β2m amyloid deposits observed in cartilage, synovium, and heart. More recently, Hou et al. (129) demonstrated that AGE-modified collagen binds significantly larger amounts of human β2m than unmodified collagen, a finding that is of interest because AGE-modified collagen is increased in dialysis patients (100).

Although several lines of evidence suggest a potential link of AGE-modified β2m to bone and joint destruction, the role of advanced glycoxidation in the formation of Aβ2m has not been definitively demonstrated.

Prevention and Treatment of Aβ2m

Prevention

Aβ2m has been reported in patients treated with all forms of dialysis, including CAPD, hemofiltration, and HD. The only large-scale comparison of the prevalence of Aβ2m in HD and CAPD patients at autopsy failed to detect a significant difference (68).

Role of HD Membrane Type. High-flux membranes such as AN69 and probably polysulfone delay the development of Aβ2m. They should thus be advocated in patients who are not suitable candidates for renal transplantation (TP), e.g., older patients (especially because age increases the risk of Aβ2m and those likely to wait for many years on dialysis for a successful graft (e.g., hyperimmunized patients or patients who have no or little access to TP). Because the “membrane effect” appears mainly linked to the ability to clear β2m, this characteristic should be considered in membrane prescription. β2m removal is achieved by convection (highest for high-flux polysulfone and AN69) and by adsorption (highest for AN69 and polymethylmethacrylate). Both parameters have to be included in the evaluation of membranes (80).

Duration of Dialysis Session. Just as for most middle molecules, β2m removal per dialysis is time-dependent (130). Longer duration of each dialysis session thus might delay Aβ2m.

Treatment of Established Aβ2m

Medical Treatment. Chronic arthralgias are best treated by paracetamol/dextropropoxyphene. Nonsteroidal antiinflammatory drugs entail a substantial risk of gastrointestinal tract complications in such fragile patients.

Intra-articular steroids are helpful especially when a single joint is very painful. However, their transient effectiveness and the risk of infectious complications limit their long-term usefulness.

If first-line therapies fail, low-dose oral prednisone (0.1 mg/kg) may prove effective. In a prospective open trial in 27 HD patients with severe, symptomatic Aβ2m, a dramatic reduction in the number of painful joints was observed shortly after initiation of prednisone. Pain recurred within 24 h after drug interruption (21).

Surgical Treatment. Early surgery is recommended after CTS diagnosis in HD patients because the relentless course of Aβ2m may otherwise lead to serious, irreversible neuromuscular impairment. CTS surgery may be performed either classically or endoscopically (131), although the experience with the latter procedure in HD patients is limited. Recurrences of CTS have been reported (27) but should be rare if the surgeon is experienced and meticulously removes the tissue compressing the nerve. Pathologic femoral fractures or spinal cord compression are obvious indications for joint prosthesis or vertebral fusion, respectively (38,132).

Severe shoulder arthralgias may benefit from various endoscopic or surgical procedures. Endoscopic resection of the coracacromial ligament in 29 long-term HD patients with intolerable shoulder pain resulted in a prompt relief of pain, which lasted throughout the follow-up of 10 to 25 mo (133). Arthroscopic synovectomy performed in eight patients with shoulder pain led to a transient relief (≤12 mo) in most patients (134). Open surgery, performed by the same group in five patients with humeral cysts, including curettage of cysts and ceramic implantation, together with resection of hypertrophied synovium and masses, relieved pain throughout the follow-up period of 12 mo. More information on the long-term follow-up of such interventions is needed.

Dialytic Treatment. Small, uncontrolled, and up to now unconfirmed studies have reported a subjective improvement of arthralgias in a few patients switched from Cuprophane HD to high-flux HD or CAPD (135,136). Similarly, Nakazawa et al. (137) have used a selective β2m adsorbent for hemoperfu-
tion connected in series with an AN69 dialyzer in an effort to reduce predialysis serum β2m level. Serum β2m level fell by 20% within a few weeks during the use of the device. Articular symptoms improved in two of three treated patients but recurred after interruption of the treatment. Here again, controlled studies are required. Whatever the benefit, the cost of the device is likely to preclude its widespread use.

Renal Transplantation

Renal transplantation (TP) should be urgently considered in all patients with AB2m who are suitable candidates. A striking, almost immediate improvement of AB2m joint symptoms and signs has been observed after a successful TP (138–140). Although this beneficial short-term effect has been ascribed to the high doses of steroids, the effect lasts despite their reduction and, sometimes, the interruption of steroid treatment. In addition, the progression of AB2m deposits is halted as demonstrated by the stability of typical amyloid bone cysts after a successful TP (138–141) (Figure 3).

More controversial is the issue of the potential regression of AB2m deposits after a successful TP. Bone cysts do not regress (138,139), as result of the actual persistence of the AB2m deposits (142) or of a very slow turnover of bone amyloid deposits. Persistent AB2m deposits have been demonstrated up to 10 yr after a successful TP (141,142). In the absence of sequential quantitative biopsies, however, the possibility of partial regression of AB2m cannot be excluded. The unusually rapid recurrence of signs of AB2m after resumption of HD in graft failure confirms that regression is very limited at best (139).

Tan et al. (140) recently reported that labeled SAP uptake fell 3 to 5 yr after successful transplantation, whereas it increased in similar patients maintained on HD for the same duration. These data are taken as evidence of significant AB2m regression after TP. Such a conclusion hinges both on the specificity of the method and on its ability to quantify amyloid deposits, neither of which has been conclusively demonstrated (63). Overall, both the clinicoradiologic and the scintigraphic approaches provide interesting information, apparently contradictory at this stage. Their reconciliation will probably require more accurate and sensitive methods to detect AB2m regression.

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