Advanced glycation end products and \( \beta_2 \)-microglobulin. The story unfolds

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Introduction

Dialysis-related amyloidosis (DRA) is a serious complication occurring in most patients undergoing long-term haemodialysis [1]. Amyloid fibrils consisting of \( \beta_2 \)-microglobulin (\( \beta_2 \)M) are deposited preferentially in the osteoarticular tissues and then, over the years, lead to bone and joint destruction. To develop an effective treatment strategy and to prevent this debilitating complication, elucidation of its molecular pathogenesis is an issue requiring urgent resolution. Although recent histological studies have shown the accumulation of monocytes/macrophages around amyloid deposits [1,2], the factor(s) causing their infiltration and pathological involvement remain poorly understood. Since \( \beta_2 \)M is a major constituent of amyloid fibrils, it has been the target molecule for many studies attempting to elucidate the pathogenesis of DRA. Intact \( \beta_2 \)M per se has seemed an unlikely contributor to the pathogenesis, because no difference in the plasma levels of intact \( \beta_2 \)M has yet been found between haemodialysis patients with and those without this complication. Some investigators, therefore, have focused on the modification of this molecule, and have found several modifications in \( \beta_2 \)M such as polymerization, truncation by lysine-specific cleavage, deamidation, and advanced glycation [1]. This review focuses on the modification of \( \beta_2 \)M with advanced glycation end-products (AGEs), especially on their structure and pathological role in DRA.

Presence of AGE-modified \( \beta_2 \)M in amyloid fibrils

The electrophoretically acidic isoform of \( \beta_2 \)M is a minor isoform in the serum and urine, but a major one in amyloid fibril proteins isolated from patients [3]. The acidic \( \beta_2 \)M purified from urine of long-term haemodialysis patients was found to have the characteristic physicochemical triad of AGE-protein, i.e. brown colour, fluorescence, and polymerization tendency, and showed positive immunoreactivity to anti-AGE antibody [3,4]. Amyloid fibril \( \beta_2 \)M isolated from patients with DRA also reacted with these antibodies [3,4]. Table 1 summarizes evidence thus far obtained for the presence of AGEs in acidic \( \beta_2 \)M and/or amyloid fibril \( \beta_2 \)M.

AGEs are formed, over the months, by a non-enzymatic reaction between aldoses and protein amino groups, called the Maillard reaction [5]. Since the AGE modification occurs in long-lived proteins in vivo and amyloid fibril proteins have a long life due to their extreme insolubility and resistance to proteases, it is likely that the AGE modification takes place directly in situ in long-lived amyloid fibril \( \beta_2 \)M. It currently remains unclear whether AGE-modified \( \beta_2 \)M formed in the circulation is taken up into amyloid deposits due to intermolecular cross-linking with amyloid fibrils or through engagement with the AGE receptor [6] on macrophages.

Structure of AGE-modified \( \beta_2 \)M

The \( \alpha \)-amino group of the amino terminal isoleucine has been determined to be the primary Maillard site in human \( \beta_2 \)M [7]. Computer graphics analysis of the three-dimensional structure of human \( \beta_2 \)M suggests that the imidazole group of histidine-31 is uniquely positioned very close to the \( \alpha \)-amino group of isoleucine-1 [7].

The structures of Amadori products, the early Maillard products in acidic \( \beta_2 \)M were identified as Na-(1-deoxyfructosyl)-isoleucine (major structure) and Nc-(1-deoxyfructosyl)-lysine (minor one) [8]. Elucidation of the AGE structure(s) in amyloid fibrils is an issue of particular interest. AGEs have been
thought to constitute a heterogeneous class of structures. Two AGE structures have been identified so far in acidic β2M and amyloid fibril β2M [4,9]. One structure is pentosidine, which involves a lysine and an arginine residue combined in an imidazo-(4,5b)-pyridinium ring; the other is carboxymethyl lysine, which was recently determined to be the epitope structure for anti-AGE antibody [9]. Since formation of pentosidine and carboxymethyl lysine is known to be related to oxidative stress (recently termed 'glycoxidation'), not only glycation but oxidative stress might play a role in the development of DRA.

Pathological role of AGE-modified β2M

Several lines of evidence have suggested that AGE proteins play a role in normal tissue remodelling, i.e. the removal and replacement of senescent extracellular matrix components [5]. However, under pathological conditions such as diabetes, renal failure, and ageing, the accumulation of AGE proteins might lead to tissue damage through a variety of mechanisms: through an alteration of the structure and function of tissue proteins, by the stimulation of cellular responses via receptors specific for AGE proteins, or by the generation of reactive oxygen intermediates. AGEs have thus been implicated in the pathogenesis of atherosclerosis, diabetic nephropathy, senile cataract, and Alzheimer's disease.

It was demonstrated that AGE-modified β2M enhances chemotaxis and chemokinesis of monocytes, but normal β2M does not enhance any migratory activity of monocytes [8,10]. This might explain the preferential localization of monocytes/macrophages around amyloid deposits. AGE-modified β2M, but not normal β2M, also stimulates monocyte-derived macrophages to secrete interleukin-1β (IL-1β), tumour necrosis factor-α (TNF-α) and interleukin-6 [8,10,11], all of which are potent bone-resorbing cytokines. These findings are in good agreement with a previous histological observation that amyloid deposits of long-term haemodialysis patients were surrounded by a number of monocytes/macrophages immunohistochemically which stained positive for IL-1β and TNF-α [2]. Furthermore, the amount of cytokines secreted from macrophages by the action of AGE-modified β2M is sufficient to stimulate collagenase synthesis in cultured human synovial cells [10]. Our collaborative study with Stern's group revealed that the biological effects of AGE-modified β2M on macrophages are mediated by the receptor for AGEs [12].

Recently the effect of AGE-modified β2M on osteoclast-induced bone resorption was evaluated by pit formation assay using an unfractionated bone-cell culture system containing mature osteoclasts from the femur and tibia of newborn mice [13]. When the cells were cultured on dentin slices, AGE-modified β2M increased the number of resorption pits formed by osteoclasts, but normal β2M did not. This AGE-induced bone resorption was effectively inhibited by calcitonin and iriprilavone, both of which are inhibitors of bone resorption and used for the treatment of osteoporosis. These findings suggest that AGE-modified β2M accelerates bone resorption of osteoclasts. This contention was also supported by the observation that AGE-modified β2M enhanced net calcium efflux from cultured neonatal mouse calvariae to a much greater extent than normal β2M (S. Sprague and T. Miyata, unpublished observation).

Taking these biological effects of AGE-modified β2M together, the mechanism of bone resorption and joint destruction in DRA could be hypothesized as follows. AGE-modified β2M is present in long-lived amyloid deposits. AGE-modified β2M induces monocyte chemotaxis and recruits them into amyloid deposits from the circulation, where monocytes accumulate through engagement with the AGE receptor. Then AGE-modified β2M stimulates monocyte-derived macrophages in situ to secrete potent bone-resorbing cytokines due to interaction with the AGE receptor. These cytokines stimulate synovial cells to produce collagenase, leading to matrix degradation. Furthermore AGE-modified β2M enhances osteoclast-induced bone resorption, probably in concert with osteoblasts.

The incidence of DRA has been shown to increase with the duration of dialytic therapy and the age of the patient [1]. It is of note that the AGEs level in the plasma and tissue proteins is known to increase with these parameters. Although we have not yet compared the β2M-linked AGE levels between diabetic and nondiabetic haemodialysis patients, we found similar levels of albumin-linked pentosidine between the two groups [14], strongly suggesting that the β2M-linked AGE levels may be also similar between diabetics and nondiabetics since the AGEs precursor is thought to be identical in albumin and β2M. This agrees with the finding that diabetic haemodialysis patients do not seem more prone to develop DRA (C. van Ypersele, unpublished observation).

The future problem

Several lines of evidence suggest a potential link of AGE-modified β2M in long-lived amyloid fibrils to bone and joint destruction due to interaction with monocytes/macrophages, synovial cells, and osteoclasts/osteoblasts. Thus AGE-mediated tissue destruction might be the combined result of excessive accumulation of AGEs in amyloid deposits linked to a heightened cellular response to these deposits. However, it should be emphasized that it remains important to further investigate whether the AGE-modification of β2M actively plays a role in the pathogenesis of DRA or is merely a result of long-term accumulation in amyloid fibrils. In addition, the mechanism of amyloid formation of β2M (amyloidogenesis) and predisposition of amyloid to deposit on osteoarticular structures remain poorly understood. Further study will undoubtedly be necessary to elucidate the
pathogenesis of DRA in order to develop an effective treatment strategy.

References
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Glomerular involvement in type II diabetes—is it all diabetic glomerulosclerosis?

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Introduction
The proportion of diabetic patients entering renal replacement therapy programs is increasing. It is evident that the percentage of type II (non insulin dependent) diabetes continues to increase whereas the proportion of type I diabetes remains stable. It is strange that the overall renal prognosis and survival is the same in patients with type I and type II diabetes [1] even though the former are often older by two decades than the latter.

Amongst possible explanations for this paradox one can discuss the nature of the renal disease: as a matter of fact, prognosis of diabetes strongly depends on the presence or absence of associated microangiopathic and macroangiopathic complications, particularly those affecting the renal and cardiac vasculature.

It has been known for a long time that the presence of renal signs and symptoms in a diabetic patient does not necessarily mean that he suffers from typical diffuse or nodular diabetic glomerulosclerosis. This possibility was first entertained more as an academic curiosity, but one has recently come to appreciate that it may potentially be of great prognostic importance: For the patient to have non-diabetic nephropathy implies at least theoretically that he or she might be free of microvascular or macrovascular disease. Should such non-diabetic nephropathy progress, the pace will be the one of the specific disease, i.e. different from that of diabetic nephropathy, and the patient would have a much lower risk of associated blindness, lower limb amputation, stroke and myocardial infarction [2].

Glomerulonephritis in type II diabetes
It has been claimed by Amoah that non-diabetic nephropathy was clearly more common in type II than in type I diabetes [3]. This observation was confirmed by subsequent investigators [4–10]. The spectrum of nephropathies was wide and varied from minimal change nephrotic syndrome to extra-capillary glomerulonephritis. The prevalence of glomerulonephritis (either isolated or superimposed upon diabetic glom-