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ORIGINAL ARTICLE

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Beta 2-microglobulin amyloid deposit in HLA-B27 transgenic rats

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Abstract Beta 2-microglobulin (beta 2-m) amyloid deposition develops serious orthopedic complications in patients with long-term hemodialysis. However, the exact pathogenesis of amyloid deposition in patients with dialysis is unknown. We used transgenic rats with HLA-B27 and beta 2-m to develop an in vivo animal model with beta 2-m amyloid-associated disorders to investigate the mechanism of formation and to report histological findings of beta 2-m amyloid deposition. Transgenic rats were divided into two groups: group 1, no treatment; group 2, arthritis induced by type II collagen. A large number of cells labeled with beta 2-m were observed in the bone marrow and synovium of the knee joint in transgenic rats. In addition, amyloid deposition, identified by Congo red staining, was found only in the knee joints of the transgenic rats with collagen-induced arthritis. Immunostaining with beta 2-m demonstrated the same pattern of tissue distribution as Congo red in serial sections. We hypothesized that elevated serum beta 2-m level-associated local chronic inflammation leads to the development of amyloid deposits and resultant arthropathy.

Key words Amyloid · Beta 2-microglobulin · Hemodialysis · HLA-B27 transgenic rats

Introduction

Hemodialysis has successfully lengthened the life span of chronic renal failure patients. At present, approximately 260 000 patients are undergoing hemodialysis in Japan, and this number is expected to rise considerably when more are diagnosed. However, in patients with long-term hemodialysis, amyloid deposits can cause several serious orthopedic complications, such as carpal tunnel syndrome, arthritis of the hip, knee, and shoulder, destructive spondyloarthropathy, and bone cyst formation, requiring operative treatments. It has been indicated that high levels of beta 2-microglobulin (beta 2-m) may be responsible for amyloid deposits.[1,2] Beta 2-m can be described as a small plasma protein metabolized only by the kidney, which cannot be dialyzed because of the bioincompatibility of the dialysis membrane. However, the exact pathogenesis of amyloid deposition in patients with dialysis is unknown, and there have been no previous animal models established to demonstrate the relationship between amyloidosis and hemodialysis. However, Taurog et al.[3–5] introduced HLA-B27 and human beta 2-m gene in transgenic rats to investigate the role of HLA-B27-associated disorders. These transgenic rats spontaneously developed HLA-B27-associated human disorders such as inflammatory bowel disease, peripheral arthritis, and nail or skin deformity.[3,4] In addition, the rats also expressed human beta 2-m on the cell surface of peripheral blood lymphocyte. Following this, several researchers investigated HLA-B27-associated diseases using these rats.[5,6] However, none have focused on the localization of beta 2-m. We chose to use these existing transgenic rats as an animal model in this study. Although no relationship between amyloid deposits in hemodialysis patients and HLA-B27-associated disorders has been demonstrated clinically, we hypothesized that high serum levels of beta 2-m in transgenic rats may induce the deposition of amyloid within the tissues, thus providing an additional use for this animal model in investigating the pathogenesis of amyloid-associated disorders. The aim of this study was to observe the localization of beta 2-m and to investigate the mechanism of amyloid deposition in these rats.

Materials and methods

Animal models

In this experiment, 14- to 17-week-old female transgenic rats with HLA-B27 and human beta 2-m gene (Taconic, Germantown, NY, USA) were used ($N = 18$). As a negative

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Table 1. Animal model

Group	*N*	Type of rat	Type II collagen-induced arthritis
1	12	Transgenic[a]	–
2	6	Transgenic[a]	+
3	3	Control[b]	–
4	6	Control[b]	+

[a] HLA-B27 transgenic rat
[b] Fisher 344 rat

control, we used Fisher 344 rats (Taconic; $n = 9$). The transgenic rats were divided into two groups: group 1, no treatment ($n = 12$); group 2, arthritis induced by type II collagen ($n = 6$). Type II collagen-induced arthritis was developed following the method previously described by Trentham et al.[7] The Fisher 344 rats were also divided into two groups as follows: group 3, no treatment ($n = 3$); group 4, type II collagen-induced arthritis ($n = 6$). Only animals in which arthritis was successfully induced were used in the study. For all the animals included, the extent of knee arthritis had an arthritis score of greater than 8 points at 4 weeks after immunization.[8] Four weeks after treatment, the beta 2-m level in serum was measured using the latex agglutination method for each rat; then all rats were killed. Histological specimens were obtained from the bilateral knees and prepared for the subsequent analysis (Table 1).

Histological examination

Specimens were fixed by immersion in 10% buffered formalin for 24 h at room temperature, then decalcified under agitation for 1 week in Plank–Rychlo's solution (w/v in distilled water containing 7% aluminum chloride, 3.6% hydrochloric acid, and 4.6% formic acid) before being embedded in paraffin. Serial sections, 4 µm in thickness, were made and mounted on gelatin-coated slides. For routine histological examination, the slide was stained with hematoxylin and eosin (HE), and the neighboring section was stained with Congo red staining and immunohistochemical staining.

1. Congo red staining: This was used to detect amyloid deposits. Each section was stained with Congo red using the technique reported by Wright et al.[9] Polarized light was used to observe the amyloid deposits.
2. Immunohistology: Immunohistochemical staining was applied to observe human beta 2-m. Immunolocalization was performed with avidin biotin–peroxidase complex using DAKO LASB Kits (DAKO, Carpinteria, CA, USA). Polyclonal antibody to rabbit anti-human beta 2-m were obtained from DAKO. Antibodies were used at a dilution of 1:500. Rabbit immunoglobulin fraction was used as a negative control, diluted to the same protein concentration as the antibody.

Tissue samples for electron microscopic examination were fixed by immersion for 3 h at 4°C in 2% glutaraldehyde and 4% formaldehyde, and then decalcified for 1 month at 4°C in 5% EDTA in 0.1 M tris buffer (pH 6.9) containing 4% sucrose. Thereafter, the tissue was postfixed in 2% OsO_4, dehydrated in alcohol and embedded in Spurr resin. Ultrathin sections were stained with aqueous uranyl acetate and lead citrate.

Results

Blood examination

Serum beta 2-m levels were high in the transgenic rats. Group 1 data showed 7.05–14.11 µg/ml (average 9.83 µg/ml); group 2, 8.25–16.04 µg/ml (average 10.3 µg/ml). No serum beta 2-m was found in any of the Fisher 344 control rats (groups 3 and 4).

Histological examination

Sagittal sections of synovium in the knee joints in both groups 1 and 3 (transgenic and control rats without induction of arthritis) showed no or few inflammatory cells in routine HE sections. In contrast, hyperplasia of the synovial lining layer and proliferating blood vessels were observed in groups 2 and 4 (both the transgenic rats and control rats with type II collagen-induced arthritis). Neighboring sections of the knee joints in the transgenic rats (groups 1 and 2), immunostained by beta 2-m, showed a large number of cells labeled with beta 2-m in the bone marrow and the superficial layer of the synovium in all specimens (Fig. 1A). Section-labeled control serum showed no staining. No beta 2-m reactivity could be seen in any of the Fisher 344 rats (groups 3 and 4; Fig. 1B, C). On the other hand, amyloid deposition, identified by Congo red staining at the knee joints with positive apple green birefringence in polarized light, was present only in group 2 (transgenic rats with collagen-induced arthritis). This amyloid deposition had a similar distribution to immunolocalization of beta 2-m within the tissue. Specifically, amyloid deposition was localized around blood vessels at the popliteal region in the knee joint (Fig. 2A, B). No amyloid deposition could be seen in any of the non-treated transgenic rats (group 1) or Fisher 344 rats (groups 3 and 4; Fig. 3).

On electron microscopy, samples taken from the popliteal region of the knee joints in group 2 were observed. Amyloid deposits were identified next to the collagenous fibers in the peripheral region of the blood vessels as 5–10 nm fibrils, which were slightly curvilinear and crossed each other (Fig. 4).

Discussion

Recently, the number of patients with long-term hemodialysis has increased, and a corresponding rise in clinical syndromes associated with amyloid formations, which derive from beta 2-m is being observed. From an orthopedic viewpoint, these syndromes present clinical problems such as destructive spondyloarthropathy in the cervical spine, amyloid arthritis of the knee and hip joint, and pathological

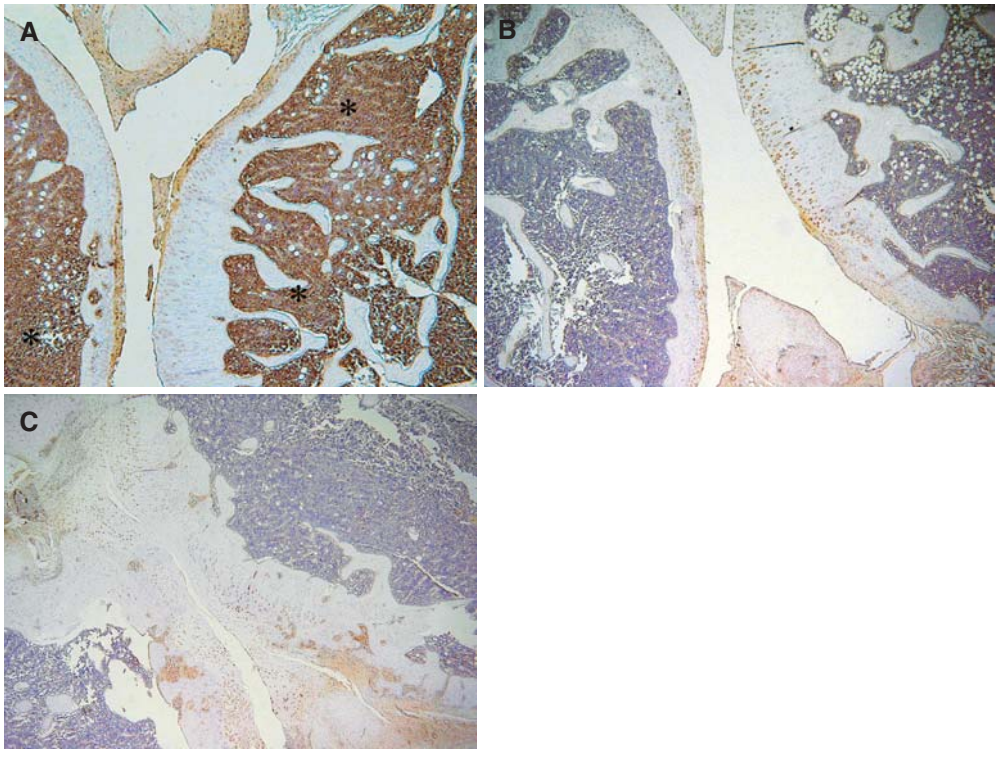

Fig. 1. Immunostaining with anti-beta 2-microglobulin (beta 2-m) antibody. **A** Group 1 (no treatment transgenic rats): Reactivity is seen as a brown reaction. A large number of cells labeled with beta 2-m are observed in bone marrow cells in all specimens (*asterisks*; **A,** ×40). **B** Group 3 (no treatment Fisher 344 rat): No beta 2-m reactivity (**B**, ×40). **C** Group 4 (type II collagen-induced arthritis Fisher 344 rat): No beta 2-m reactivity (**C**, ×40)

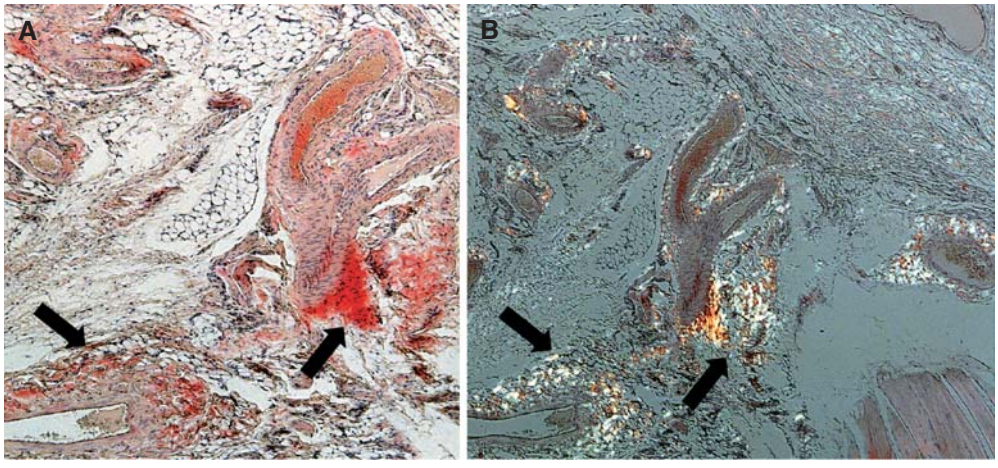

Fig. 2. Group 2 histological findings (transgenic rats treated with type II collagen). Amyloid deposition, identified by red staining (*arrow*; **A**, ×100) and apple green birefringence (*arrow*; **B**, ×100) by Congo red staining, is present around blood vessels at posterior capsule in the knee joint

fractures derived from amyloid bone cysts. However, the pathogenesis of these complications is not clearly understood, providing increasing challenges for orthopedic surgeons.[10–16] In clinical samples taken from patients with total joint arthroplasty, laminoplasty, and carpal tunnel syndrome, large numbers of macrophages surrounding amyloid deposits have been shown to be immunoreactive with beta 2-m.[17,18] Moreover, the serum beta 2-m levels are extremely high in patients with long-term hemodialysis. Thus, it has been suggested that the accumulation of excessive beta 2-m might be responsible for amyloid deposits.[1,2] However, not all these patients develop amyloid deposits and beta 2-m amyloid-associated disorders, suggesting the existence of a mechanism that triggers the development of the deposits. At our institution, we performed 48 total joint arthroplasties for 35 amyloid arthritis patients with long-term hemodialysis. Among these, a considerable number of patients (10 of 35, 28%) were complicated with rheumatoid arthritis. This clinical observation seems to support the hypothesis that the presence of arthritis might be one of the factors triggering amyloid deposit**s** in patients with long-term hemodialysis. On the other hand, the pathogenesis of amyloid-associated disorders has been described as beta 2-m isolated from amyloid deposits in patients with dialysis-related amyloidosis that has been modified by advanced glycation end products (AGEs).[19,20] AGEs are pigmented and fluorescent adducts that are formed by the non-enzymatic reaction between sugar and matrix collagens. This process is called the Maillard reaction.[19,20] Especially, in patients with chronic renal failure, activation of the oxidative and carbonyl stress may induce the progression of the AGEs modification. Miyata et al.[20] described that beta

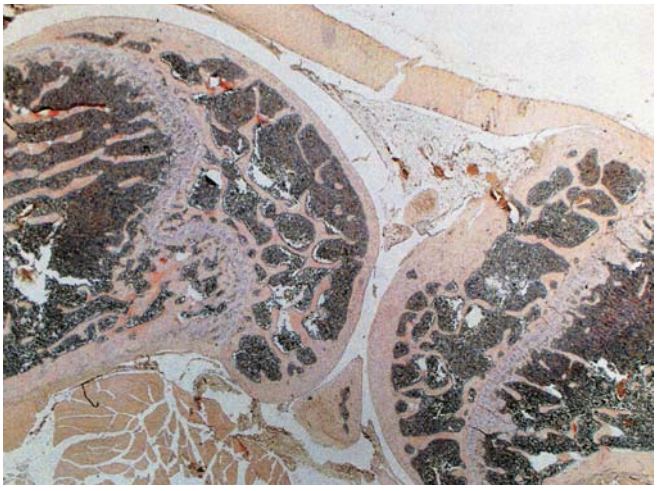

Fig. 3. Congo red staining in group 1. Congo red staining is absent in bone marrow or synovium (×25)

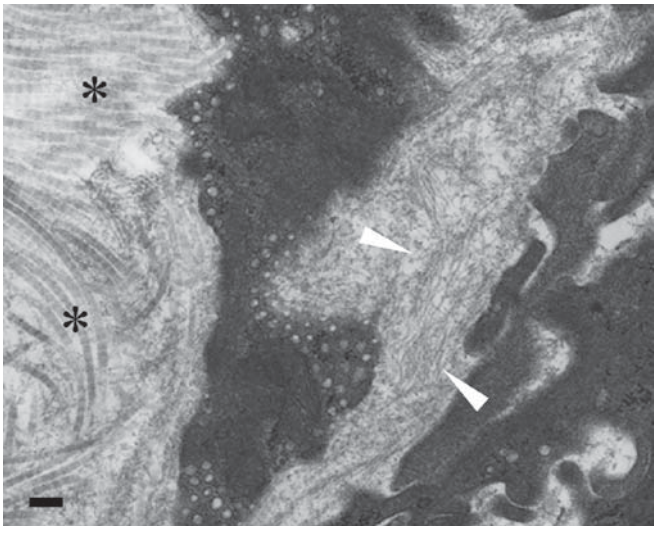

Fig. 4. Electron microscopy finding with group 2, samples taken from the popliteal region of the knee joints. Amyloid (*arrow*) is identified next to the collagenous fibers (*asterisks*) around the blood vessels as 5–10 nm fibrils (*bar* 0.5 μm)

2-m modified with AGEs (AGEs beta 2-m) can initiate a variety of biological response, mediating receptors/cell surface binding proteins on monocyte/macrophages, inducing interleukin (IL)-1, IL-6, and tumor necrosis factor-α from human macrophages, subsequently leading to the development of amyloidosis.[21,22] They also described that AGEs beta 2-m enhances osteoclast-induced bone resorption in vitro and in vivo,[23] and further hypothesized that AGEs beta 2-m activates osteoclasts or alters microenvironments favorable for bone resorption by osteoclasts. In this study, transgenic rats expressed an elevated level of beta 2-m in serum and the knee joints. However, the knee joints in non-treated transgenic rats, showed no or few inflammatory cells in synovium, and amyloid deposition was recognized only in group 2, the transgenic rats with type II collagen-induced arthritis. Thus, our study emphasized chronic inflammation as a major trigger for beta 2-m amyloid deposition in the joint. We subsequently speculated the mechanism of beta 2-m amyloid formation in those joints as follows. Type II collagen-induced arthritis as chronic inflammation affects native beta 2-m, inducing a transform action to bigger molecules. Big molecule beta 2-m, such as AGEs beta 2-m, has a tendency to make amyloid fibrils. This sequence may explain the process of amyloid deposition in the arthritic knee as shown in this study. However, whether the amyloid deposits are produced directly from beta 2-m or develop with AGEs beta 2-m modification has not been clarified. In our clinical situation, we have recognized a high dosage of beta 2-m in long-term hemodialysis patients with chronic arthritis such as osteoarthritis or rheumatoid arthritis, leads to AGEs beta 2-m modification and amyloid deposits in the joint, combined with the activation of macrophages or osteoclasts. Once amyloid deposits accumulate in the joint with the arthritis, the process of cartilage degradation and osteoclastic bony resorption may follow. Moreover, these patients' conditions are also complicated by renal osteodystrophy, and thus osteoclastic activity is increased by elevated serum parathyroid hormone level. Therefore, it is speculated that these combined pathologies act to make a vicious circle that can cause progressive joint destruction in this group of patients.

This animal model has some limitations in that it cannot completely reproduce the clinical situation of the amyloid arthritis because we can only see the amyloid deposition in the joint without the presentation of progressive joint destruction. Further studies are required to create a model more closely simulating the actual disease process by adding other factors such as chronic renal dysfunction. However, this animal model could be a valuable in vivo amyloid arthritis model for investigating the mechanism of arthritis and reporting histological findings of beta 2-m amyloid deposition. The model may also provide a vehicle for investigating therapeutic and prophylactic regimens for beta 2-m amyloid-associated disorders.

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