Circulating level of α₂-macroglobulin–β₂-microglobulin complex in hemodialysis patients

YOSHIHIRO MOTOMIYA, YUKIO ANDO, KATSUKI HARAOKA, XUGUO SUN, HISAIKO IWAMOTO, TOMONORI UCHIMURA, and IKURO MARUYAMA

Suiyuikai Clinic, Kashihara, Japan; Department of Laboratory Medicine, Kumamoto University School of Medicine, Kumamoto, Japan; Department of Gastroenterology and Hepatology, Kumamoto University School of Medicine, Kumamoto, Japan; R & D for Diagnostic, A and T Corporation, Fujisawa, Kanagawa, Japan; Department of Laboratory and Molecular Medicine, Kagoshima University School of Medicine, Kagoshima, Japan

Circulating level of α₂-macroglobulin–β₂-microglobulin complex in hemodialysis patients.

Background. The presence of α₂-macroglobulin (α₂M) in amyloid tissue from patients with dialysis-related amyloidosis (DRA) was demonstrated by Argilés et al in 1989. Thereafter, the formation of the complex of β₂-microglobulin (β₂m) with α₂M was confirmed directly by in vitro study. In Alzheimer’s disease, complex formation of amyloid β-peptide and α₂M is considered to play an important role in the pathogenesis by modifying the degradation processes of amyloid protein. Thus, we hypothesized that the α₂M-β₂m complex is an important factor in the pathogenesis of DRA as well. Here, we measured the circulating levels of α₂M-β₂m complex in the maintenance hemodialysis patients and discussed about its clinical significance in DRA.

Methods. One hundred and thirty-seven hemodialysis patients and 11 prehemodialysis chronic renal failure (CRF) patients were included in this study. The affinity of purified α₂M for β₂m was confirmed by a highly sensitive 27 MHz quartz crystal microbalance (QCM). The presence of circulating α₂M-β₂m complex was analyzed by immunoblotting analysis. Furthermore, the serum levels of α₂M-β₂m complex were measured by sandwich enzyme immunoassay.

Results. QCM analysis revealed the high affinity of α₂M for β₂m. The presence of circulating α₂M-β₂m complex was detected in two out of a total 11 prehemodialysis CRF patients and in 95 out of the total of 137 hemodialysis patients. None of the healthy subjects, however, were observed to present with any α₂M-β₂m complex. Serum levels of the α₂M-β₂m complex were correlated to the duration of hemodialysis (P = 0.043). Serum levels of the α₂M-β₂m complex were significantly higher in patients with high DRA score than in patients with negative DRA score (P = 0.018). Moreover, serum levels of the α₂M-β₂m complex showed significantly lower in the hemodiafiltration patients compared to the hemodialysis patients (P = 0.002) and showed a strong correlation with DRA score in hemodialysis patients excluding 11 hemodiafiltration patients (P = 0.0004).

Key words: hemodialysis, α₂-macroglobulin–β₂-microglobulin complex, dialysis-related amyloidosis.

Accepted for publication July 18, 2003

© 2003 by the International Society of Nephrology

Conclusions. This study is the first to demonstrate the presence of circulating α₂M-β₂m complex in hemodialysis patients. Furthermore, we observed the correlation between serum levels of α₂M-β₂m complex and clinical characteristics of DRA. Thus we concluded that a formation of an α₂M-β₂m complex may be implicated in DRA.

α₂-macroglobulin (α₂M) is a major protease inhibitor in vivo [1, 2]. So far, several reports have been published suggesting the presence of protease inhibitors such as α₂M and α₁ antichymotripsin in amyloid fibrils from Alzheimer’s disease or dialysis-related amyloidosis (DRA) patients [3–6]. More recently, the very potent inhibitory action of α₂M on proteases has been interpreted as being indicative of an involvement of α₂M in amyloid-related protein metabolism. In consequence of this, it has been hypothesized that α₂M may modify the degradation processes of amyloid proteins and may play an active role in the pathogenesis of amyloidosis.

In Alzheimer’s disease, amyloid β peptide, a precursor protein of the disease, has now been corroborated to form a complex with α₂M in in vitro studies [7, 8]. Subsequently, β₂-microglobulin (β₂m), a precursor protein of DRA, has been reported to form a complex with α₂M [9]. On the basis of this evidence, the “α₂M hypothesis” has been forwarded by Argilés et al [10] as an interesting mechanism to account for the pathogenesis of DRA. Based on this complex-mediated promotion concept, we established an assay system using the enzyme immunoassay method for α₂M-β₂m complex and measured its serum level in 137 hemodialysis patients as well as 11 prehemodialysis patients.

METHODS

Subjects

One hundred and thirty-seven hemodialysis patients, 11 prehemodialysis patients with chronic renal failure...
(CRF) (control subjects, prehemodialysis CRF patients), and 15 normal persons were enrolled in this study after obtaining their oral consent.

All patients were non-diabetic. Their average ages were 58 years in the hemodialysis patients, 61 years in the control subjects, and 33 years in the normal persons. Gender breakdown was 77 males and 60 females of the hemodialysis patients, nine males and two females of the prehemodialysis CRF patients, and 12 males and three females of normal persons. Duration of hemodialysis varied from 2 to 290 months, 123 months in average. All hemodialysis patients underwent hemodialysis or hemodiafiltration with bicarbonate dialysate twice or three times a week. Several kinds of dialyzers were used at the time of the study, including polysulfone (N = 45), polyacrylonitrile (N = 36), ethylenepoxyalcohol (N = 31), cellulose triacetate (N = 20), and others (N = 5).

The clinical diagnosis of DRA was evaluated as previously reported [11]. In brief, all clinical signs were scored by the criteria of Gejyo et al [12], which ranks the clinical features of DRA from 0 to 7 according to joint pain, bone cyst on x-ray film, and carpal tunnel syndrome. The DRA scores were negative in 84 cases (0 in 58 cases, 1 in 26 cases), mild in 33 cases (2 in 21 cases, 3 in 12 cases), and high in 20 cases (4 in ten cases, 5 in five cases, 6 in three cases, and 7 in two cases).

The operation for carpal tunnel syndrome had been done in 20 patients, the amyloid bone cyst in either carpal, shoulder, or hip bone was confirmed in 70 patients and 47 patients showed symptoms of multiple joint pain. Eleven patients who had a moderate or high DRA score had been undergoing hemodiafiltration for 6 months or more.

As far as we could survey, there were no reports concerning the complex of α2M-β2m even in the patients with the systemic AA amyloidosis. Therefore, age-matched prehemodialysis CRF patients served as control in this study.

**Laboratory characteristics**

Serum creatinine values were 12.0 ± 0.3 mg/dL in the hemodialysis patients and 5.4 ± 2.3 mg/dL in the prehemodialysis CRF patients. Serum values of β2m were 35.7 ± 8.5 mg/L in the hemodialysis patients and 10.7 ± 6.0 mg/L in the prehemodialysis CRF patients. Values of serum total protein, albumin, C-reactive protein, and concentration of hemoglobin were 6.5 ± 0.4 g/dL, 3.8 ± 0.3 g/dL, 0.24 ± 0.31 mg/dL, and 10.0 ± 1.2 g/dL, respectively, in hemodialysis patients. All data in hemodialysis patients were values before hemodialysis.

**Measurement of a circulating α2M-β2m complex**

The affinity of β2m for α2M. The affinity of β2m for α2M was examined by using a highly sensitive 27 MHz quartz crystal microbalance (QCM) (Affinity Q) (Inishimura Co., Tokyo, Japan) as described earlier [13, 14]. Briefly, 2 μL of 200 μg/mL α2M was directly immobilized on a QCM plate, and the plate was soaked in 6 mL of 10% dimethyl sulfoxide (DMSO) at 25°C. The resonance frequency of the QCM was defined as the 0 position after equilibrium. The stability and drift of the 27 MHz QCM frequency in the solution were ± 5 Hz for 12 hours at 25°C.

**Preparation of standard α2M-β2m complex.** A α2M-β2m complex was prepared as described previously [9]. Other chemicals were of analytical grade. In outline, 1 mL of 0.1 mg/mL β2m (Sigma Chemical Co., St. Louis, MO, USA) was incubated with 1 mL of 1 mg/mL α2M (Sigma Chemical Co.) for 36 hours in 66.6 mmol/L phosphate buffer at room temperature. Specificity of the standard complex was studied by Western blotting as shown in Figure 1. The complex was used as a sample for Western blotting or a standard for enzyme immunoassay.

**Western blotting.** Prepared α2M-β2m complex, commercial β2m, and commercial α2M were run by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) at 18 mA for 1 1/2 hours with a 4% to 20% gradient gel (Tefco, Tokyo, Japan). Prepared α2M-β2m complex was pretreated at 100°C for 5 minutes by mixing with 2-mercaptoethanol of 5% final concentration. After transfer to a polyvinylidene difluoride (PVDF) membrane (Millipore, Bedford, MA, USA) for 1 hour at 20 V, the membrane was incubated with Block Ace (Dainippon Pharmaceutical, Osaka, Japan) for 1 hour.
followed by three washes with 20 mmol/L phosphate-buffered saline (PBS) (pH 7.4) containing 0.05% Tween 20 (washing buffer). The membrane was then incubated with biotin-labeled anti-α₂M antibody or anti-β₂m antibody at room temperature for 1 hour. After the reaction, the membrane was washed three times with wash buffer and reacted with biotin-labeled anti-α₂M antibody solution at room temperature for 20 minutes. The HRP antibody solution was then diluted 1000 times and added to the antibody solution. After complete mixing, the solution was incubated at room temperature for 2 hours. After adding 0.1 mL of sodium borohydride (4 mg/mL in water) (Wako, Osaka, Japan) to the solution, the membrane was washed three times repeatedly with the wash buffer and reacted with nitrotetrazolium blue solution containing 0.02% hydrogen peroxide and the reduced form of nicotinamide adenine dinucleotide (NADH).

### Biotin labeling of anti-α₂M antibody

Biotinylation of anti-α₂M polyclonal antibody (Rockland) (Gilbertsvilles, PA, USA) was performed using sulfosuccinimidyl D-biotin (Biotin Sulfo-OSu) (Dojindo, Kumamoto, Japan). First, 1 mL of the antibody solution (0.7 mg/mL) in 0.01 mol/L hepes buffer (pH 8.5) was prepared. Next, 0.1 mL of 1 mmol/L Biotin Sulfo-OSu in distilled water was prepared and then added to the antibody solution. After complete mixing, the solution was incubated at room temperature for 4 hours. The biotinylated antibody solution was loaded into the desalting column (R1).

### A coupling anti-β₂m antibody to horseradish peroxidase.

Five milligrams of horseradish peroxidase (HRP) (Toyobo, Osaka, Japan) was suspended in 1.2 mL of water and added to 0.3 mL of a freshly prepared 0.1 mol/L sodium periodate solution in 10 mmol/L sodium phosphate (pH 7.0). After the HRP solution had been incubated at room temperature for 20 minutes, it was dialyzed versus 1 mmol/L sodium acetate (pH 4.0) at 4°C with several changes overnight. The HRP solution was then incubated for 2 minutes at 45°C in tube. Then, 50 μL of the sample were mixed and preincubated for 5 minutes at 45°C in tube. Then, 50 μL of the R1 sample mixture was transferred to the reaction cup. Twenty microliters of HRP-coupled anti-β₂m antibody (R2), containing 1% BSA, 1% mouse serum, and 0.03% Microcide I (Amresco, Solon, OH, USA) in 20 mmol/L PBS, and 50 μL of the sample were mixed and preincubated for 5 minutes at 45°C in tube. Then, 50 μL of the R1 sample mixture was transferred to the reaction cup. Twenty microliters of HRP-coupled anti-β₂m antibody (R2), containing 1% BSA, 1% mouse serum, and 0.03% Microcide I (Amresco) in 20 mmol/L PBS, was added to the reaction cup (second step). The reaction cup contents were then incubated for 2 minutes at 45°C and washed two times with 0.05% Tween 20 solution. After the addition of 30 μL of chromogen (A & T) which consists of 3,3′,5,5′-tetramethylbenzidine and hydrogen peroxide, changes of laser reflectance were measured.

The α₂M-β₂m complex, prepared in vitro, was used as the standard for the changes of laser reflectance. The sera of normal persons, hemodialysis, and prehemodialysis CRF patients were used as samples. The complex concentration of the samples was calculated with an MI01 (A & T) by fitting the standard curve using a spline function. The reference line showed a good linearity from 0 to 1.0 U/mL (Fig. 2). The intra-assay coefficient of variation was 4.67%.

### Statistical analysis

Results are expressed as mean ± SD. Statistical analysis was performed using the software package, Stat View version 5.0 for Windows. Differences between the groups were compared using the Mann-Whitney U test for nonnormally distributed variables, and for normally distributed variables, compared using unpaired t test. To evaluate the relation between variables, Pearson’s correlation coefficient in normally distribution, and Spearman’s ranked correlation coefficient in nonnormally distributed variables.
distribution were used. A $P$ value less than 0.05 were considered as significant.

**RESULTS**

**The affinity of $\beta_2m$ for $\alpha_2M$**

The time course of the change in frequency of the QCM responding to 10 $\mu$L of 1 mg/mL $\alpha_2M$ was recorded (Fig. 3A). Association constants ($K_a$) and the maximum binding amount ($\Delta M_{\text{max}}$) were calculated form Eadie-Hofstee plots ($K_a$ 443.27 nmol/L, $\Delta M_{\text{max}}$ 232.93 Hz) (Fig. 3B).

$\beta_2m$ exhibited a very strong affinity for $\alpha_2M$ in a dose-dependent manner ($K_a$ 443.27 nmol/L, $\Delta M_{\text{max}}$ 232.93 Hz). The frequency of the QCM responding to $\alpha_2M$ decreased over time, which indicated that $\alpha_2M$ had significant affinity for $\beta_2m$ in a dose-dependent manner.

**Serum levels of $\alpha_2M$**

Serum levels of $\alpha_2M$ were 151.5 ± 43.9 mg/dL in hemodialysis patients and 157.8 ± 50.3 mg/dL in pre-hemodialysis CRF patients. An increased level of serum $\alpha_2M$ greater than maximum reference value (200 mg/dL for males and 250 mg/dL for females) could be found only 14 out of 137 cases (10.2%). Serum $\alpha_2M$ values in the hemodialysis patients failed to correlate neither with the serum $\beta_2m$ values or with the $\alpha_2M$-$\beta_2m$ complex (Fig. 4).

 Serum $\beta_2m$ values showed also no correlation with the $\alpha_2M$-$\beta_2m$ complex (data not shown).

**Serum levels of $\alpha_2M$-$\beta_2m$ complex**

Serum levels of $\alpha_2M$-$\beta_2m$ complex could not be detected in serum from 15 normal persons, but could be detected in two out of 11 sera from the prehemodialysis CRF patients (18%), whose levels varied from 0 to 0.8 U/mL (0.09 ± 0.20 U/mL) (Fig. 5). In the hemodialysis patients, the complex could be detected in 95 out of 137 sera (69.3%), which varied from 0 to 1.0 U/mL (0.20 ± 0.24 U/mL). The complex level in the hemodialysis patients was significantly higher than that in the prehemodialysis CRF patients ($P = 0.013$).

**Correlation between the serum $\alpha_2M$-$\beta_2m$ complex levels and clinical characteristics related to hemodialysis**

Serum levels of $\alpha_2M$-$\beta_2m$ complex showed a correlation with the duration of hemodialysis ($r = 0.257, P = 0.043$) (Fig. 6) and an average value of the complex was significantly higher in the patients with long-term history of 12 years or more ($N = 57$) (0.28 ± 0.28 U/mL, 0 to 1.0 U/mL) than that in the patients with a hemodialysis history less than 12 years ($N = 80$) (0.15 ± 0.19 U/mL, 0 to 0.9 U/mL) ($P = 0.009$) (Fig. 7).

Furthermore, no correlation could be found between serum levels of $\alpha_2M$-$\beta_2m$ complex with the DRA scores in all hemodialysis patients, but serum complex levels were significantly higher in patients with high DRA score than that in patients with negative DRA scores (0.33 ± 0.32 U/mL vs. 0.16 ± 0.21 U/mL, $P = 0.018$) (Fig. 8).

Even so, however, the time on hemodialysis was also significantly longer in the former than in the later, 218.7 months vs. 81.3 months ($P < 0.0001$).

Serum complex levels in patients who underwent an operation of carpel tunnel syndrome were 0.32 ± 0.32 U/mL, which was slightly, but not significantly, higher than 0.19 ± 0.22 U/mL in patients who had not undergone an operation ($P = 0.065$) (data not shown).

**An effect of hemodiafiltration on serum levels of the $\alpha_2M$-$\beta_2m$ complex**

Serum levels of the $\alpha_2M$-$\beta_2m$ complex in 11 patients who had undergone hemodiafiltration for more than 6 months with the high-performance membrane (hemodiafiltration group) was compared with 34 patients who had no history of hemodiafiltration with the high-performance membrane (nonhemodiafiltration...
Fig. 4. Correlation between serum α₂-macroglobulin (α₂M) and β₂-microglobulin (β₂m) (A) and α₂M-β₂m complex (B) in hemodialysis patients. No significant correlation was found both of them (N = 137, r = 0.024, NS; N = 137, r = 0.013, NS).

Fig. 5. Comparative study of serum levels of α₂-macroglobulin (α₂M)-β₂-microglobulin (β₂m) complex in the nonhemodialysis patients with chronic renal failure (CRF) (prehemodialysis CRF) (N = 11) and the hemodialysis (HD) patients (N = 137). In the hemodialysis patients, serum level of α₂M-β₂m complex was significantly higher than the prehemodialysis CRF patients (control subjects) (0.20 ± 0.24 U/mL vs. 0.09 ± 0.20 U/mL, P = 0.013).

In the nonhemodialfiltration group, the duration of hemodialysis was matched with the hemodialfiltration group. Duration of hemodialysis was 203.8 ± 51.6 months in the hemodialfiltration group and 205.1 ± 32.8 months in the nonhemodialfiltration group (P = 0.920). Age was 54.5 ± 6.6 years in the hemodialfiltration group and 54.4 ± 8.7 years in the nonhemodialfiltration group, respectively (P = 0.955). Serum values of β₂m were 32.8 ± 7.7 mg/L in the hemodialfiltration group and 35.4 ± 6.1 mg/L in the nonhemodialfiltration group (P = 0.252) and serum levels of α₂M were 156.0 ± 59.4 mg/dL in the hemodialfiltration group and 152.2 ± 36.4 mg/dL in the nonhemodialfiltration group (P = 0.818). Serum values of α₂M-β₂m complex were from 0 to 0.3 U/mL (0.05 ± 0.09 U/mL) in the hemodialfiltration group, which were significantly lower than not only those in the nonhemodialfiltration group (from 0 to 1.0 U/mL, 0.31 ± 0.29 U/mL) (P = 0.002) (Fig. 9), but also those in all hemodialysis patients included in this study (0.20 ± 0.24 U/mL) (P = 0.009). Taking into account this significant lower value of serum complex in the hemodialfiltration patients, a correlation between serum complex levels and DRA score was further studied in a patient subgroup excluding 11 hemodialfiltration patients, which
showed a significant correlation as shown in Figure 10 ($\rho = 0.316, P = 0.0004$).

**DISCUSSION**

The presence of $\alpha_2M$ in amyloid tissue from patients with DRA was first demonstrated by Argilés et al in 1989 [3] and later corroborated by Campistol et al [4]. Thereafter, the complex formation of $\beta_2m$ with $\alpha_2M$ was confirmed directly in an in vitro setting [9] and the $\alpha_2M$ hypothesis for the pathogenesis of DRA was proposed [10]. Thus far, however, no report concerning a circulating complex of $\alpha_2M$-$\beta_2m$ in hemodialysis patients has been published.

At present study, we could confirm that $\alpha_2M$ showed high affinity for $\beta_2m$ in vitro by QCM analysis, a new method for binding assay [13, 14] and detected $\beta_2m$ associated with $\alpha_2M$ ($\alpha_2M$-$\beta_2m$ complex) in serum from 95 out of 137 hemodialysis patients (69.3%). Circulating levels of $\alpha_2M$ were reported to be significantly increased and had a correlation with the serum levels of $\beta_2m$ in hemodialysis patients [15, 16]. Interestingly, a significant correlation between serum levels of $\alpha_2M$ and $\beta_2m$ was, however, limited to the patients with DRA in the study by Argilés et al [16]. By contrast to that study by Argilés et al, this study failed to show such correlation despite almost similar patient’s background such as duration of hemodialysis. Two distinct differences could be pointed out between these two studies, one with serum levels of $\alpha_2M$ and another with proportion of patients with DRA. Our study involved apparently fewer patients with increase of serum $\alpha_2M$ as well as with DRA than the study of Argilés et al [16]. Not only was there no increase of serum $\alpha_2M$ but also no correlation between serum levels
of $\alpha_2$M and $\beta_2$m in patients free of DRA in their study similar to our study. On the other hand, Curatola et al [15] reported merely a significant increase of serum $\alpha_2$M in hemodialysis patients. Recently, polymorphism of $\alpha_2$M gene was reported [17], but to what extent it might be accountable for the difference in serum levels of $\alpha_2$M on hemodialysis remains unclear.

It is generally acknowledged that $\beta_2$m is the key substance in DRA and that the development of DRA is strongly dependent on the length of time on hemodialysis, which is about 15 years on average in Japan [18]. In our study, a significant correlation was confirmed between the serum $\alpha_2$M-$\beta_2$m complex levels and the duration of hemodialysis. However, we failed to find a correlation between the serum value of the $\alpha_2$M-$\beta_2$m complex and the DRA score. In addition, patients involved in this study were extremely overbalanced by so many patients with a negative DRA score, the lower value of the serum $\alpha_2$M-$\beta_2$m complex in the hemodiafiltration group was lower than the hemodialysis group (0.05 ± 0.09 U/mL vs. 0.31 ± 0.29 U/mL, $P = 0.002$).

![Fig. 8. Serum $\alpha_2$-macroglobulin ($\alpha_2$M)-$\beta_2$-microglobulin ($\beta_2$m) complex levels in patients with negative DRA score and patients with high dialysis-related amyloidosis (DRA) score. In the patient with high DRA score, $\alpha_2$M-$\beta_2$m complex level was significantly higher than negative DRA score ($P = 0.018$).](image)

![Fig. 9. Comparative study of serum levels of the $\alpha_2$-macroglobulin ($\alpha_2$M)-$\beta_2$-microglobulin ($\beta_2$m) complex in the hemodialysis (HD) group ($N = 34$) and treated the hemodiafiltration (HDF) group ($N = 11$). Serum level of the $\alpha_2$M-$\beta_2$m complex in the hemodiafiltration group was lower than the hemodialysis group (0.05 ± 0.09 U/mL vs. 0.31 ± 0.29 U/mL, $P = 0.002$).](image)
Although any direct inhibitory effect on protease by $\alpha_2M$ must still be substantiated in DRA amyloid tissue [19], Argilés et al [10] have given a clear indication that the $\alpha_2M$-$\beta_2m$ complex has great pathophysiologic significance in DRA and stressed the importance of an impaired catabolism of extravasated $\beta_2m$ in amyloid tissue by protection from protease [10].

A kinetic study of $\beta_2m$ using $^{125}$I-$\beta_2m$ suggested a progressive accumulation of $\beta_2m$ in the extravascular space along with the time on hemodialysis [20, 21], while the serum $\beta_2m$ concentrations tended to level off [22].

Because the binding of the $\alpha_2M$-$\beta_2m$ complex was reported to be extremely tight, as indicated by an estimated kD of $10^{-9}$ mol/L [10], which was almost similar value in this study, a preformed complex is, therefore, assumed unlikely to be dissociated in the physiologic condition.

A molecular size of the complex is unlikely to be removed even by hemodiafiltration. Under such circumstance, circulating levels of the complex might be dependent on the extent by which the complex is generated.

Thus, it makes sense to assume that a lower value of serum complex in the hemodiafiltration group compared with the nonhemodiafiltration group may be due to lower $\beta_2m$ value in those patients, albeit not significant, by high shunting effect across the vascular wall of convection flow.

A recent report by Narita et al [23] suggested the possibility of a different process from the one involving the protection from protease, which is mediated via a low-density lipoprotein receptor-related protein (LRP). They have shown that the amyloid $\beta$ peptide-$\alpha_2M$ complex is degraded by glioblastoma cells and fibroblast via LRP. Although it is not clear to what extent this process contributes to $\alpha_2M$-$\beta_2m$ catabolism, it is well-known that the involvement of activated macrophages would be ubiquitous in the late stages of DRA [24–26]. $\alpha_2M$ is also reported to bind to LRP on macrophages. Accordingly, an analogous possibility could be suggested in catabolism of $\alpha_2M$-$\beta_2m$ complex.

As shown in a statement by Glabe [27] concerning the up-to-date report by Iwata et al [28], the overall evidence from an investigation on the catabolic breakdown of amyloid proteins can be anticipated to provide new insights into the pathogenesis of the amyloidosis.

Of greater interest, the binding site of $\beta_2m$ to $\alpha_2M$ was reported to be folded in the physiologic state [9]. The complex, therefore, may indicate the presence of an unfolded $\beta_2m$, which suggests a conformational change in this molecule, misfolding, in vivo [29].

Finally, as stated in a paragraph compared with the study by Argilés et al, this study involved many patients free of clinical evidences of DRA, which might enable us to undertake further study on prognostic value of $\alpha_2M$-$\beta_2m$ complex in progression of DRA.

Reprint requests to Yoshihiro Motomiya, M.D., Suiyukai Clinic, 676-1 Kazumoto-cho, Kashihara, Nara 634-0007, Japan.
E-mail: motomiya@silver.ocn.ne.jp

REFERENCES

2. BORTH W: $\alpha_2$-macroglobulin, a multifunctional binding protein with targeting characteristics. FASEB J 6:3345–3353, 1992
Motomiya et al: Circulating levels of α2M-β2m complex in hemodialysis patients


