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CASE REPORT

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Combined treatment with cyclophosphamide and prednisolone is effective for secondary amyloidosis with SAA1γ/γ genotype in a patient with rheumatoid arthritis

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Abstract A 41-year-old woman, who had been diagnosed with rheumatoid arthritis (RA), was admitted because of proteinuria, and rheumatoid and gastrointestinal symptoms just 1 year after onset. Renal biopsy revealed marked amyloid deposits of AA (amyloid A)-type. Genotyping of serum amyloid A (SAA) showed that she was homozygous for SAA1γ. Combined treatment with cyclophosphamide and prednisolone led to remission of both RA disease activity and proteinuria. Since the renal dysfunction arose from amyloidosis, arrested renal deterioration and a remission of proteinuria would result from a reduction of amyloid deposits. Therefore, early usage of immunosuppresive therapy such as a combined treatment with these two medicines would be useful against systemic amyloidosis secondary to RA, even if the patient has the risky SAA1γ/γ genotype.

Key words Rheumatoid arthritis · SAA1γ/γ amyloidosis · Immunosuppressive therapy

Introduction

In rheumatoid arthritis (RA), approximately 10%–14% of patients in Japan develop secondary systemic amyloidosis.[1] The kidney is the most common and potentially the most serious organ of involvement, and thus renal amyloidosis is the major cause of RA death.[2] Therapy is basically aimed at controlling the inciting RA, and several reports indicate that if such control is achieved, both clinical and pathological resolution of amyloidosis may occur.[3–5] However, in most cases amyloidosis is inexorably progressive and the prognosis for such patients is poor, since the therapeutic tactics cannot help being conservative and have limitations at present.[6]

While the physiological role of serum amyloid A (SAA) is still unclear, its pathological role as the precursor of amyloid A protein, the main constituent of amyloid deposits in secondary systemic amyloidosis, is well known. Chronic inflammatory conditions, such as RA, can result in marked and prolonged increase in plasma levels of acute-phase SAA protein, and this is the major cause of the secondary amyloidosis. However, since not all RA patients who suffer chronically elevated SAA levels develop amyloidosis, it is believed that factors other than high SAA levels in plasma must be involved to induce amyloid deposition. Our recent studies have shown that individuals with a SAA1γ-allele, in particular the SAA1γ/γ-genotype, have a high incidence of amyloidosis, especially in Japanese RA patients, indicating that the SAA1γ is a risk factor for secondary amyloidosis.[7]

Here we report a case of amyloidosis secondary to RA. The patient, who has the SAA1γ/γ-genotype and had complicated severe renal amyloidosis early in the course of RA, has been able to achieve a remission of proteinuria by oral treatment with cyclophosphamide and prednisolone. Thus, we suppose that this combined regimen would be widely effective against RA with secondary amyloidosis irrespective of the SAA1γ-genotype.

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Case report

A 41-year-old woman had been suffering from RA with polyarthralgia and morning stiffness since the age of 40, in 1994. Her disease displayed active inflammation involving the proximal interphalangeal, metacarpophalangeal, wrist, and knee joints. Roentgenographic changes were consistent with early RA with symmetric joint space narrowing,

erosive changes in the proximal interphalangeal, metacarpophalangeal, and phalangeal joints, and carpal bone erosion. The disease was classified as stage II and class I. She had been treated with nonsteroidal anti-inflammatory drugs, but the joint pain had been uncontrolled. In April 1995, the patient complained of severe joint stiffness, epigastric pain, body weight loss (6 kg in 3 months), appetite loss, diarrhea, and a low-grade fever. She was transferred to our center. Serological findings were noteworthy with respect to rheumatoid inflammatory activities. Her erythrocyte sedimentation rate was 34 mm/h (normal 3–15), C-reactive protein (CRP) was 3.4 mg/dl (≦0.5), and rheumatoid factor was 93 U/ml (≦35). No other autoantibodies were detected, and serum complement (CH50) was 38 U/ml (30–45). Proteinuria was 1 (+). Occult blood tests were positive in both the urine and stool. Her serum creatinine level was 0.6 mg/dl (0.6–1.5), blood urea nitrogen (BUN) was 6 mg/dl (5–20), urate was 4.8 mg/dl (2.3–5.7), aspartate aminotransferase (AST) was 10 IU/l (5–40), alanine aminotransferase (ALT) was 6 IU/l (≦35), and lactate dehydrogenase (LDH) was 274 IU/l (100–500). Thyroid hormone levels were within the normal limits. The electrocardiogram, chest roentgenogram, and cardiac function were all normal. Upper GI-series showed chronic gastritis, and biopsy of the gastric mucosa revealed amyloid deposition de novo (Fig. 1A). This specimen was positive for Congo-red staining and susceptible to oxidative treatment with potassium permanganate. The green birefringence on polarization microscopy after Congo-red staining of the specimen was recognized

From June 1996, the level of serum creatinine increased gradually and her proteinuria elevated. Antineutrophil cytoplasmic antibody and circulating immune complex were not detected. Percutaneous needle renal biopsy was performed in August 1996. Light microscopic examination of the kidney, which contained 19 glomerular tissues, revealed extensive deposition of periodic acid Schiff reaction and Congo-red-positive substance in the glomeruli, the walls of the small arteries, and the tubular basement membrane (Fig. 1B,C). Vasculitis and proliferation of the mesangial matrix were not found. Immunohistochemical studies revealed deposition of AA amyloid in the glomeruli.

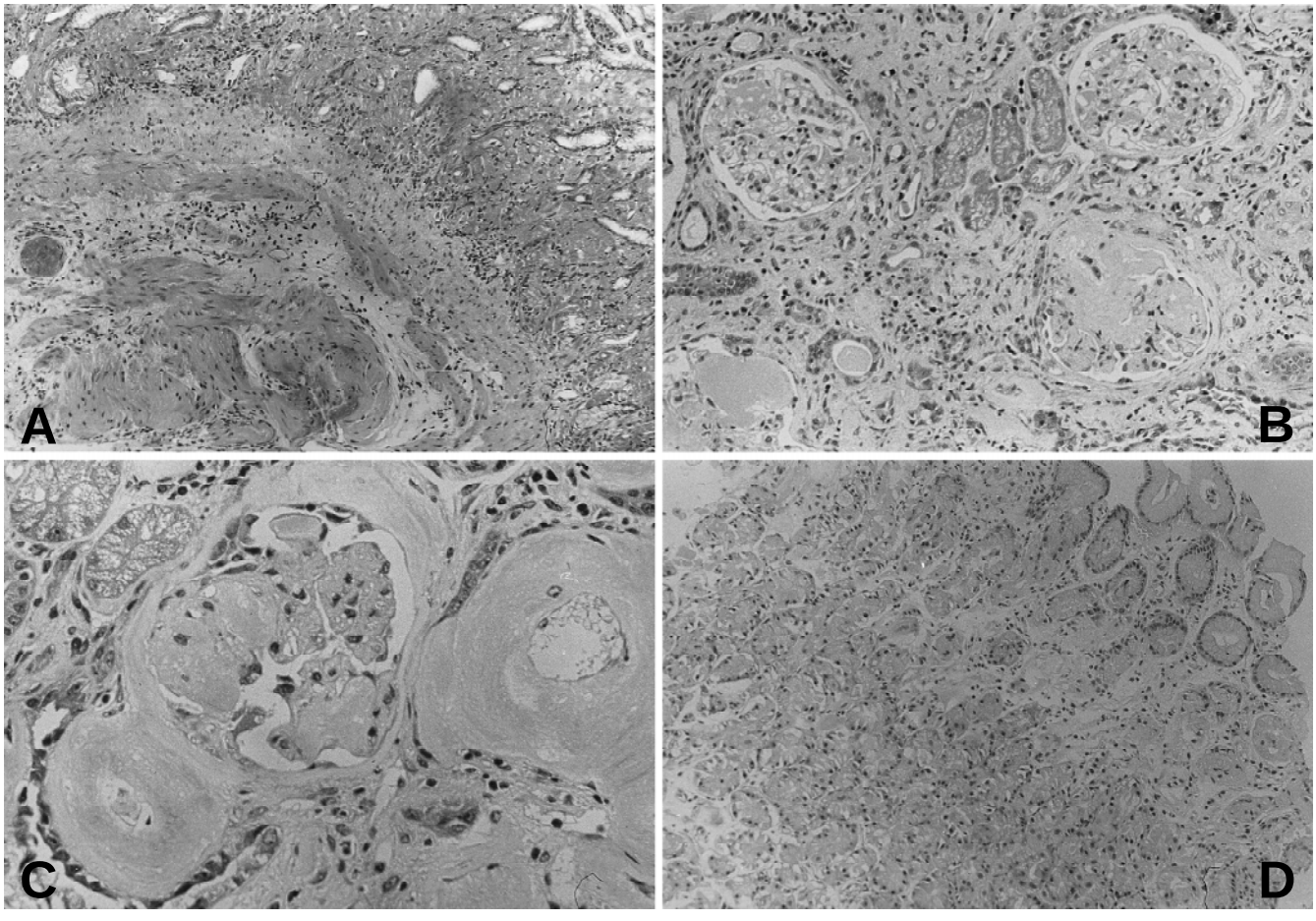

Fig. 1. **A** First gastric biopsy showing a diffuse nonstructural substance which was positive for Congo-red stain revealed as amyloid deposition de novo (Congo-red stain, ×80). **B,C** Renal biopsy showing a remarkable deposition of periodic acid Schiff reaction and Congo-red-positive substance in the glomeruli, the walls of small arteries, and tubular basement membrane (**B**, periodic acid Schiff stain, ×100; **C**, Congo-red stain, ×400). **D** Second gastric biopsy exhibiting rather less deposition of amyloid fibrils when compared with the first one (hematoxylin/eosin stain, ×120)

Immunofluorescent staining was negative for κ-, λ-light chains, immunoglobulins (IgG, IgA, IgM), complement components (C3, C4), and β_2-microglobulin (data not shown). Based on these findings, we diagnosed the patient to have renal amyloidosis associated with RA. Furthermore, the polymerase chain reaction (PCR)-based restriction fragment length polymorphisms (RFLP)[7] revealed that the patient's SAA1 genotype was a SAA1γ/γ homozygote (Fig. 2).

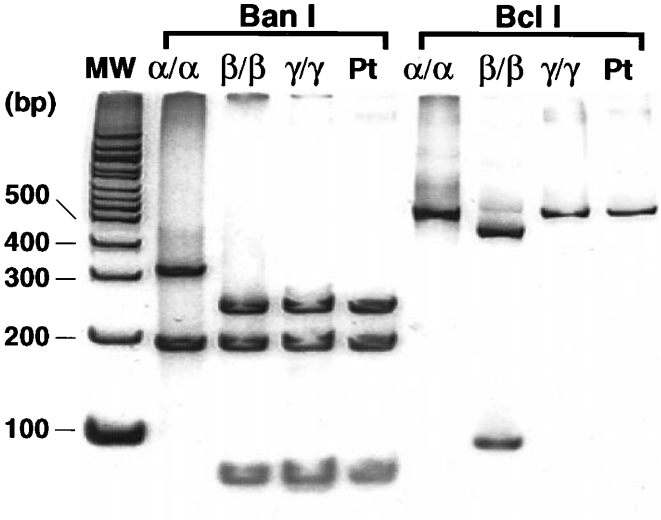

Fig. 2. Genotyping of SAA1 by PCR–RFLP. A polymorphic portion encompassing the exon 3-tail of the SAA1 gene was amplified by PCR. The amplified DNA (530 bp) was digested with the enzymes noted and run on a 10% polyacrylamide gel. The resulting fragments were visualized using silver staining. Homogyzous controls for three alleles (α/α, β/β, γ/γ) and the present case (Pt) are shown. With *Ban* I, the DNA amplified from the α-allele was digested into three fragments (317, 188, and 25 bp), whereas that from the β- and γ-alleles was digested into four fragments (244, 188, 73, and 25 bp). With *Bcl* I, the DNA amplified from the β-allele was digested into two fragments (438 and 92 bp), whereas that from the α- and γ-alleles was not digested

We started a combined treatment with cyclophosphamide and prednisolone from January 1997. As shown in Fig. 3, serum creatinine level declined and proteinuria disappeared. Because of a little gastrointestinal discomfort, a second biopsy of gastric mucosa was performed in February 1998 and showed less deposition of amyloid fibrils (Fig. 1D) when compared with the first one (Fig. 1A). Control of rheumatoid disease activity and satisfaction with the activity of daily living have been obtained after the combined treatment with cyclophosphamide and prednisolone. Follow-up renal biopsy is required to confirm the disappearance of Congo-red-positive amyloid substance.

Discussion

It is well known that secondary amyloidosis is one of the complications associated with RA. The prognosis for such patients is poor, and the renal disturbance due to amyloidosis progresses chronically. Most of the cases develop nephrotic syndrome, and gradually progress to chronic renal failure. It is likely that renal amyloidosis contributes to the rapid progression of renal failure, and that the renal amyloidosis is an important cause of death in RA patients. Effective treatments have not been reported, although immunosuppressive and cytotoxic agents, e.g., azathioprine, methotrexate, cyclophosphamide, and chlorambucil appear to be promising medicines in patients with chronic rheumatic diseases.[3–5,8,9] Berglund et al.[3,10] reported that alkylating cytostatic drugs, cyclophosphamide, and chlorambucil had an ameliorating effect on the course of the renal process in patients with secondary amyloidosis of rheumatic inflammatory diseases. They used

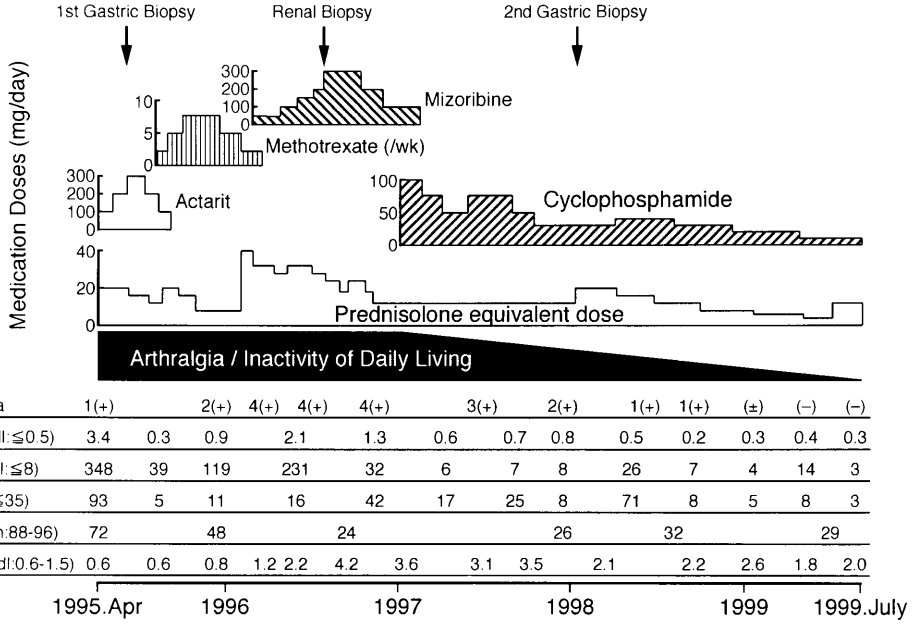

Fig. 3. The patient's clinical course over 4 years, showing remission of proteinuria and rheumatoid activity correlated with the combined treatment with cyclophosphamide and prednisolone. *Arrows* indicate the times of the three biopsies, with the results shown microscopically in Fig. 1. CRP, SAA, RF, and Creat denote C-reactive protein, serum amyoid protein A, rheumatoid factor, and creatinine, respectively. The values in parentheses are normal values

cyclophosphamide chiefly in six consecutive patients, four with RA and two with ankylosing spondylitis, who all had secondary amyloidosis. They used eight separate treatment periods varying in duration between 6 and 30 months. Renal function improved in six treatment periods, renal deterioration was arrested in one period, and the rate of functional decline slowed down in one period. They concluded that the survival of renal function might be substantially prolonged when cyclophosphamide was appropriately administered at signs of kidney deterioration due to active disease. In the present case, clinical remission of proteinuria was achieved by treatment with cyclophosphamide and prednisolone for about 1 year (Fig. 3). Although a second renal biopsy is not recommended because of the risk to the patient, it may be possible to estimate that the degree of renal amyloid deposits would decrease, as well as the histological result of the gastric specimen (Fig. 1D). It is suggested that the mesangial amyloid substance was degraded to granular material, and that the subepithelial amyloid deposits were resolved by mechanisms similar to those involved in the resolution of subepithelial immune complex deposit, i.e., through slow washing out and incorporation into the basement membrane.[4]

The pathogenesis and natural course of renal amyloidosis remain controversial. One hypothesis regarding the source of the amyloid is derived from the precursor of an amyloid protein called SAA, which is often present in high concentration in the circulation of RA patients.[11,12] It is the amyloid fibrils and deposits that induce glomerular dysfunction. Therefore, if the production of SAA can be suppressed by effective treatments, the damaged glomeruli might recover by washing out or degradation of amyloid fibrils.[13] Histological evidence for the decrease of renal deposits of amyloid fibrils has been obtained in a few cases of amyloidosis secondary to RA treated with cyclophosphamide.[14] In the present case, we observed that as CRP normalized by the combined treatment with cyclophosphamide and prednisolone, there was a reduction of disease activity of RA which correlated with the suppression of SAA production. The level of serum SAA paralleled that of CRP (Fig. 3). The result suggested an ameliorating effect of the combined therapy on the course of the renal process. Because the proteinuria fell along with the reduction in SAA levels, it is likely that without a supplement of new material, the amyloid fibrils in the kidney would be washed out and the reduction in amyloid deposition might contribute to improved renal function. Since the therapeutic effect of cyclophosphamide in RA is limited because of the versatile action of the medicine, prospective studies using cyclophosphamide should be considered for renal or systemic amyloidosis complicated with RA.

Generally speaking, it has been known that amyloidosis tends to be a complication with RA patients with a long disease duration of more than 10 years. Nevertheless, the majority of RA patients do not show any symptoms due to amyloidosis in spite of active rheumatoid inflammation. Thus, inflammatory conditions are necessary, but not sufficient, to induce amyloid deposits. Other factors must be involved.

We have reported that SAA1γ, a variant form of human SAA1, remains a strong risk factor for amyloidosis in RA.[7] In brief, the human SAA1 protein has three major allelic variants, i.e., SAA1α, β, and γ, and we have found that their gene frequencies differ among ethnic populations, and show a marked association between SAA1γ isoprotein and Japanese secondary amyloidosis in RA, but not RA onset itself. Moreover, preliminary data suggest that in cases of SAA1γ/γ homozygosity, the duration of amyloidosis complications after the onset of RA is about 3 years earlier than in those of other genotypes. The SAA1γ/γ homozygosity is a strong risk factor for the development of amyloidosis in RA. Although all SAA isoforms have an amyloidogenesis, there are differences in the degree of amyloid pathogenesis among them that has still not been elucidated.[15] Since the patient was SAA1γ/γ-homozygous (Fig. 2) and secondary amyloidosis developed briefly after the onset of RA, we speculate that SAA1γ contributed to the onset and development of amyloidosis in the present case. Further studies correlating SAA1 genotypes with the occurrence of amyloidosis in RA will reveal why some RA patients are predisposed to developing amyloidosis.

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