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Experimental transmission of systemic AA amyloidosis in autoimmune disease and type 2 diabetes mellitus model mice

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Abstract: AA amyloidosis is a protein misfolding disease characterized by extracellular deposition of amyloid A (AA) fibrils. AA amyloidosis has been identified in food animals, and it has been postulated that AA amyloidosis may be transmissible to different animal species. Since the precursor protein of AA fibrils is serum amyloid A (SAA), which is an inflammatory acute phase protein, AA amyloidosis is considered to be associated with inflammatory diseases such as rheumatoid arthritis. Chronic diseases such as autoimmune disease and type 2 diabetes mellitus could be potential factors for AA amyloidosis. In this study, to examine the relationship between the induction of AA amyloidosis and chronic abnormalities such as autoimmune disease or type 2 diabetes mellitus, amyloid fibrils from mice, cattle, or chickens were experimentally injected into disease model mice. Wild-type mice were used as controls. The concentrations of SAA, IL-6, and IL-10 in autoimmune disease model mice were higher than those of control mice. However, induction of AA amyloidosis in autoimmune disease and type 2 diabetes mellitus model mice was lower than that in control mice, and the amount of amyloid deposits in the spleens of both mouse models was lower than that of control mice according to Congo red staining and immunohistochemistry. These results suggest that factors other than SAA levels, such as an inflammatory or anti-inflammatory environment in the immune response, may be involved in amyloid deposition.

Key words: AA fibrils, amyloidosis, autoimmune disease, interspecies transmission, type 2 diabetes mellitus

Introduction

Amyloid A (AA) amyloidosis is a protein misfolding disease characterized by the extracellular deposition of insoluble amyloid fibrils with β -sheet-rich structures in several organs, such as the spleen, liver, and kidney, that induces functional impairment [12]. Amyloidosis is classified into approximately 30 types according to the pre-

cursor protein and clinical symptoms [28]. AA amyloidosis is a common form of fatal systemic amyloidosis. Since the precursor protein of AA is serum amyloid A (SAA), which is an inflammatory acute phase protein [8], AA amyloidosis is considered to be associated with inflammatory diseases such as rheumatoid arthritis (RA) and familial Mediterranean fever (FMF). SAA is primarily synthesized in the liver and increases by about 1,000-

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fold over normal levels in the plasma during the inflammatory acute-phase response [26].

In chronic inflammatory conditions such as RA and FMF, SAA can incidentally aggregate as amyloid deposits in several organs, primarily in the spleen, liver, and kidney [32]. Indeed, a high incidence of AA amyloidosis among RA patients has been reported, and in Japan, 90% of AA amyloidosis cases in people occurs secondary to RA [23]. Therefore, other inflammatory diseases, such as other autoimmune diseases or type 2 diabetes mellitus, may be potential factors for AA amyloidosis. In tissues of patients with autoimmune diseases, the deposition of antibody-antigen immune complexes causes chronic inflammation [11]. In the blood of people with diabetes mellitus, SAA levels are relatively high compared with those of healthy people [2].

AA amyloidosis in mice can be experimentally induced by injection of AgNO₃, casein, or lipopolysaccharide as an inflammatory stimulant, which causes a significant increase in SAA levels in the plasma, and the time to disease onset can be markedly shortened by injection of amyloid fibrils extracted from amyloid-containing tissue [19, 21]. Furthermore, possible transmission of AA amyloidosis from humans [27], mice [9, 31], cattle [4], fowls [10], and cheetahs [34] to mice has been reported. In addition, induction of AA amyloidosis in rabbits using bovine amyloid fibrils has been reported [7, 20]. AA amyloid deposits have been observed in food animals including slaughtered cattle [30, 33]. Therefore, it is considered that amyloid fibrils in food animals such as cattle and chickens may have potential to induce AA amyloidosis in humans with chronic disease.

In this study, to investigate the relationship between the induction of AA amyloidosis and chronic disease, amyloid fibrils were experimentally injected into two kinds of model mice, C57BL *lpr/lpr* and C57BL Ham *ob/ob* mice. C57BL *lpr/lpr* mice are a model for autoimmune disease, which show systemic autoimmunity [3], while C57BL Ham *ob/ob* mice are a model for type 2 diabetes mellitus [2]. The transmission of AA amyloidosis was examined by inoculation of murine, bovine, and chicken amyloid fibrils into both model mouse strains. Our results revealed that the transmission of AA amyloidosis to these model mice occurred to a lesser extent than in the controls, contrary to expectations.

Materials and Methods

Animals

Female C57BL *lpr/lpr* mice, C57BL mice as a control for C57BL *lpr/lpr* mice, C57BL Ham *ob/ob* mice, and C57BL Ham^{+/+} mice as a control for C57BL Ham *ob/ob* mice were purchased from Japan SLC (Shizuoka, Japan). C57BL *lpr/lpr* mice are model animals for autoimmune disease and show deposition of immune complexes in several organs that causes inflammation [3]. C57BL *lpr/lpr* mice were used in experiments after confirming the development of systemic lupus erythematosus-like disease by examining urine proteins using Albuwell M (Exocell, Philadelphia, PA, USA). C57BL Ham *ob/ob* mice are model animals for type 2 diabetes mellitus [2]. C57BL Ham *ob/ob* mice were used in experiments after confirming the development of type 2 diabetes mellitus by examining urinary glucose using a Glucose Colorimetric/Fluorometric Assay Kit (BioVision, San Francisco, CA, USA). Animals were kept five mice per cage with *ad libitum* access to feed and water. For adaptation to the laboratory environment, animals were kept for four weeks before experiments. All procedures used in the following experiments were approved by the Animal Care Committee at Gifu University (approval number 11104).

Extraction and purification of amyloid fibrils from murine, bovine, and chicken AA amyloidosis

Amyloid fibrils were extracted from the spleens of AA amyloidosis-affected C57BL mice, the livers of AA amyloidosis-affected cattle, and the livers of avian AA amyloidosis-affected chickens, which were previously ascertained to have amyloid deposits by Congo red staining and immunohistochemistry (IHC) as described in previous reports [18, 22]. Extraction of AA fibrils was performed according to the method described by Pras *et al.* [24]. Briefly, 2 g of the spleens from mice or 8 g of the livers of cattle or chickens were homogenized in 80 ml of 0.15 M NaCl by a microhomogenizer (Microtec, Chiba, Japan) and centrifuged at 40,000 × *g* for 20 min at 4°C with an MLA-55 rotor using an Optima MAX-XP ultracentrifuge (Beckman Coulter, Brea, CA, USA), and the supernatant was discarded. The tissue pellet was suspended with 80 ml of 0.15 M NaCl and centrifuged again at 40,000 × *g* for 20 min at 4°C. This process was repeated five times. Then the pellet was homogenized with 80 ml of distilled water and centrifuged again, and

the supernatant was collected. The pooled supernatants were centrifuged at $100,000 \times g$ for 1 h at 4°C . Resulting pellets of amyloid fibrils were dissolved in 8 ml of distilled water and stored at -30°C until use. The concentration of protein was measured by a spectrophotometer (GeneQuant 100, GE Healthcare, Buckinghamshire, UK) using a DC Protein Assay kit (Bio-Rad Laboratories, Hercules, CA, USA).

Coomassie Brilliant Blue staining and Western blot analysis of amyloid fibrils

Amyloid fibrils were electrophoresed on 12% sodium dodecyl sulfate polyacrylamide gels. After electrophoresis, gels were stained with Coomassie Brilliant Blue (CBB). Another set of electrophoresed gels was transferred onto PVDF membranes (Millipore, Billerica, MA, USA) and blocked with 5% skim milk in phosphate buffered saline (PBS) supplemented with 0.1% Tween 20 (PBST). The membranes were incubated with anti-mouse SAA1 goat antibody (1:5,000) (AF2948, R&D Systems, Minneapolis, MN, USA), anti-bovine SAA1 monoclonal antibody, 25BF12 (1:1,600) [29], which was purified from the culture supernatant of hybridoma cells by protein A-sepharose (GE Healthcare), or anti-chicken SAA1 mouse antibody (1:1,600) [22] in 1% skim milk in PBST at room temperature for 1 h. Membranes were washed three times with PBST and incubated with anti-goat IgG donkey antibody F (ab')₂ conjugated with horseradish peroxidase (HRP) (1:1,000) (SC3851, Santa Cruz Biotechnology, Dallas, TX, USA), anti-mouse IgG F (ab')₂ conjugated with HRP (1:1,600) (NA9310V, GE Healthcare, Buckinghamshire, UK) in 1% skim milk in PBST at room temperature for 45 min. The membranes were washed four times with PBST. The band of amyloid fibrils was detected by an ECL Western Blotting Detection System (GE Healthcare), and bands in Western blot analysis were visualized by an LAS 4000 mini (Fujifilm, Tokyo, Japan).

Induction of AA amyloidosis by injection with different amyloid fibrils and AgNO₃

As shown in Table 1, all mice in three experiments were sorted into four groups, with five mice in each group according to inoculation with different amyloid fibrils at different doses. Mice in the groups were injected with murine, bovine, or chicken amyloid fibrils by intraperitoneal route. All mice were also injected subcutaneously with 0.5 ml of a 3% solution of AgNO₃.

Before injection of amyloid fibrils, solutions containing the fibrils were treated with three cycles of ultrasonication for 30 and quiescence of 1 min by an ultrasonic processor (model 7500, Seiko I&E, Tokyo, Japan). After inoculation at 10 days, all mice were sacrificed, and then spleens, livers, kidneys, lungs, hearts, and small intestines were collected, fixed in 10% neutral buffered formalin, and embedded in paraffin.

Detection of amyloid deposits and immunohistochemical examination

Deparaffinized tissue sections were stained with hematoxylin and eosin or Congo red [25] and evaluated for amyloid depositions by a polarized light microscope (BX43, Olympus, Tokyo, Japan). To confirm amyloid deposits, sections with Congo red staining were checked for emerald-green birefringence under polarized light. Another set of deparaffinized sections was processed for IHC using steam/heat treatment by autoclave for antigen retrieval. The sections were washed with PBS and treated with 3% H₂O₂ and then incubated with anti-mouse SAA1 goat antibody (1:200) (AF2948, R&D Systems) as a primary antibody in PBS for 2 h at 37°C . The sections were incubated with SC3851 as a secondary antibody in PBS at room temperature for 30 min, and color development with 3,3-diaminobenzidine-4HCl (DAB) was performed for approximately 40s. Immunostained sections were observed with a BX43 light microscope. The intensity of amyloid deposits was scored using the NIH ImageJ software [1].

Measurement of concentrations of SAA and cytokines in serum

Blood samples were collected from mice by retro-orbital bleeding under inhalant anesthesia before injection and 1 day after injection. Collected blood samples were centrifuged at $7,000 \times g$ for 10 min at 4°C in a TMA-29 rotor using an MX-150 centrifuge (Tomy, Tokyo, Japan), and collected sera were stored at -30°C until use. Concentrations of SAA in sera were measured using a Mouse Serum Amyloid A ELISA Kit (Life Diagnostics, West Chester, PA, USA) according to the manufacturer's instructions. Concentrations of cytokines (interleukin 6 [IL-6] and IL-10) in sera were measured using a mouse T helper type 1 (Th1)/Th2/Th17 cytokine cytometric bead array (CBA) kit (BD Biosciences, Franklin Lakes, NJ, USA) according to the manufacturer's instructions. Data were measured by a BD FAC-

Table 1. Amyloid deposition in C57BL, C57BL *lpr/lpr*, C57BL Ham +/+, and C57BL Ham *ob/ob* mice inoculated with several AA fibrils

Mice	Injection (+ 3% AgNO ₃)	Experiment 1			Experiment 2			Experiment 3		
		Age (weeks)	Injected amount of protein (μ g)	Number of mice affected/ examined	Age (weeks)	Injected amount of protein (μ g)	Number of mice affected/ examined	Age (weeks)	Injected amount of protein (μ g)	Number of mice affected/ examined
C57BL	Murine AA fibrils	18	300	5/5	21	300	5/5	NT	NT	NT
	Bovine AA fibrils		300	0/5		300	0/5			
	Chicken AA fibrils		300	1/5		300	0/5			
	None		-	0/5		-	0/5			
C57BL <i>lpr/lpr</i>	Murine AA fibrils	18	300	4/5	21	300	3/5	21	300	4/5
	Bovine AA fibrils		300	0/10		300	0/5		1,500	0/5
	Chicken AA fibrils		300	0/10		300	0/5		300	0/5
	None		-	0/5		-	0/5		-	0/5
C57BL Ham +/+	Murine AA fibrils	18	300	5/5	30	300	5/5	NT	NT	NT
	Bovine AA fibrils		300	0/5		1,500	2/5			
	Chicken AA fibrils		300	2/5		300	0/5			
	None		-	0/5		-	0/0			
C57BL Ham <i>ob/ob</i>	Murine AA fibrils	18	300	5/5	30	300	4/5	NT	NT	NT
	Bovine AA fibrils		300	0/5		1,500	0/5			
	Chicken AA fibrils		300	0/5		300	0/5			
	None		-	0/5		-	0/5			

NT, not tested.

SCanto II flow cytometer (BD Biosciences) and analyzed with the FACSDiva software (BD Biosciences).

Statistical analysis

Data were collected from at least three independent experiments, expressed as means \pm SD, and analyzed for statistical significance by unpaired *t*-test.

Results

Extraction and confirmation of amyloid fibrils from murine, bovine, and chicken amyloidosis

Extracted murine, bovine, and chicken amyloid fibrils were examined by CBB staining and Western blot analysis (Fig. 1). The molecular weights of the extracted amyloid fibrils were approximately 7–9 kDa, and the expected sized bands were detected by both CBB staining (Fig. 1, lanes 1, 3, 5, and 7) and Western blot analysis (Fig. 1, lanes 2, 4, 6, and 8). As AA amyloidosis could not be induced by the first bovine amyloid fibrils in mice (Fig. 1, lanes 3 and 4), bovine amyloid fibrils were extracted again (Fig. 1, lanes 5 and 6). The former bovine amyloid fibrils (Fig. 1, lanes 3 and 4) were injected into C57BL, C57BL *lpr/lpr*, C57BL Ham +/+, and C57BL Ham *ob/ob* mice at 18 weeks in experiment 1 (Table 1). The latter bovine amyloid fibrils (Fig. 1, lanes 5 and 6) were injected into C57BL and C57BL *lpr/lpr* mice at 21

weeks in experiments 2 and 3 and into C57BL Ham +/+ and C57BL Ham *ob/ob* mice at 30 weeks in experiment 2 (Table 1). Extractions of murine and chicken amyloid fibrils were done once (Fig. 1, lanes 1, 2, 7, and 8), and the lots were used in all experiments.

Induction of AA amyloidosis by murine, bovine, and chicken amyloid fibrils

To investigate the induction of AA amyloidosis, mice were inoculated with AgNO₃ alone or with murine, bovine, or chicken amyloid fibrils with AgNO₃. C57BL *lpr/lpr* mice and C57BL Ham *ob/ob* mice were inoculated with AgNO₃ and amyloid fibrils after confirming that the level of urine protein or urine glucose was significantly higher than that of control mice, respectively. The results of the C57BL mice and C57BL *lpr/lpr* mice groups in experiments 1, 2, and 3 are shown in Table 1. Murine AA fibrils caused AA amyloidosis in all C57BL mice in experiments 1 and 2. In contrast, among C57BL *lpr/lpr* mice inoculated with murine amyloid fibrils, AA amyloidosis was observed in 11 out of 15 mice in experiments 1, 2, and 3. Bovine AA fibrils did not cause AA amyloidosis in either C57BL mice or C57BL *lpr/lpr* mice in experiments 1, 2, or 3, even with 1,500 μ g of bovine amyloid fibrils. Among C57BL mice inoculated with chicken amyloid fibrils, AA amyloidosis was observed in 1 out of 10 mice in experiments 1 and 2. Injection of

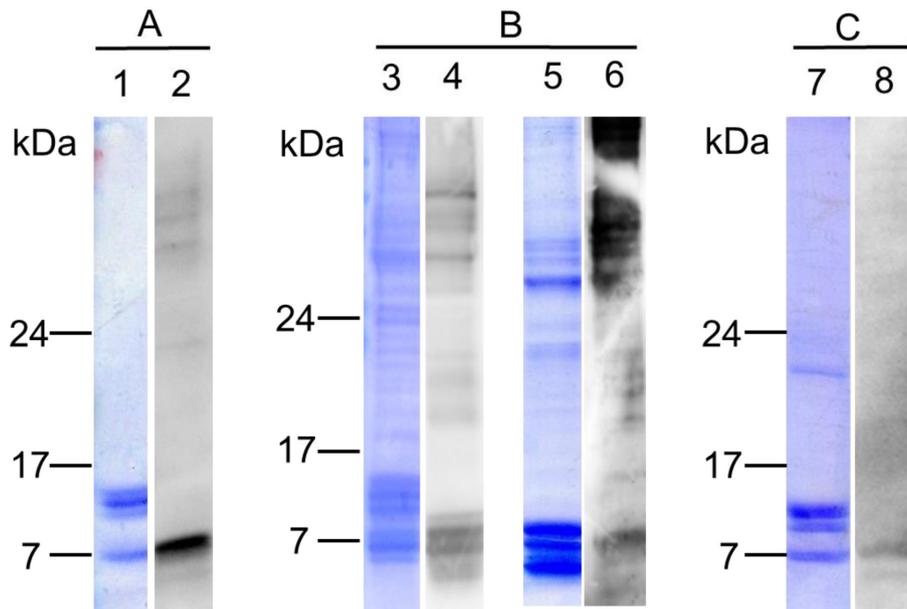


Fig. 1. Coomassie Brilliant Blue (CBB) staining and Western blot analysis of amyloid fibrils extracted from the murine spleen, bovine liver, and chicken liver. A, murine amyloid fibrils (1, 2); B, bovine amyloid fibrils (3, 4, 5, and 6); C, chicken amyloid fibrils (7, 8). Amyloid fibrils with CBB staining (1, 3, 5, and 7) and amyloid fibrils in Western blot analysis (2, 4, 6, and 8). Bovine amyloid fibrils were extracted twice. The former were used in experiment 1, and the latter were used in experiments 2 and 3.

AgNO₃ alone did not cause AA amyloidosis in any C57BL mice or any C57BL *lpr/lpr* mice (Table 1).

The results of the C57BL Ham *+/+* mice and C57BL Ham *ob/ob* mice groups in experiments 1 and 2 are shown in Table 1. Murine amyloid fibrils induced AA amyloidosis in all C57BL Ham *+/+* mice. On the other hand, among C57BL Ham *ob/ob* mice inoculated with murine amyloid fibrils, 9 out of 10 mice developed AA amyloidosis in experiments 1 and 2. Among C57BL Ham *+/+* mice inoculated with bovine amyloid fibrils, 2 out of 10 mice developed AA amyloidosis, but AA amyloidosis was not observed in Ham *ob/ob* mice (Table 1). Among C57BL Ham *+/+* mice inoculated with chicken amyloid fibrils, 2 of the 10 mice developed AA amyloidosis in experiments 1 and 2, but no AA amyloidosis was observed in C57BL Ham *ob/ob* mice. Injection of AgNO₃ alone did not cause AA amyloidosis in any C57BL Ham *+/+* mice or any C57BL Ham *ob/ob* mice (Table 1). The occurrence of AA amyloidosis in either the C57BL *lpr/lpr* or C57BL Ham *ob/ob* mouse strain was not influenced by age.

Amyloid deposits in murine spleens were examined by Congo red staining and IHC (Fig. 2). Amyloid deposits were seen around spleen follicles by staining with Congo

red and were also observed as green birefringence under polarized light in all groups (Fig. 2, upper panel). Moreover, these deposits reacted with anti-mouse SAA antibody in IHC experiments, irrespective of the originating species of amyloid fibrils used for induction of AA amyloidosis (Fig. 2, lower panel). Amyloid deposits were not observed in kidneys, lungs, hearts, and small intestines.

Intensity scoring of amyloid deposits around spleen follicles

To estimate the severity of AA amyloidosis, the intensity of amyloid deposits around spleen follicles from all samples was measured in IHC sections by using the ImageJ software (Fig. 3). The respective scores of amyloid deposits in each tissues section of mice inoculated with murine, chicken, or bovine amyloid fibrils are shown at the bottom of Fig. 3.

The relative mean values of ImageJ scores for the four groups of mice inoculated with AgNO₃ alone or murine amyloid fibrils, bovine amyloid fibrils, or chicken amyloid fibrils with AgNO₃ are shown in Fig. 4. The scores of C57BL *lpr/lpr* mice inoculated with murine amyloid fibrils were significantly lower than those of C57BL mice inoculated with murine amyloid fibrils. The significant

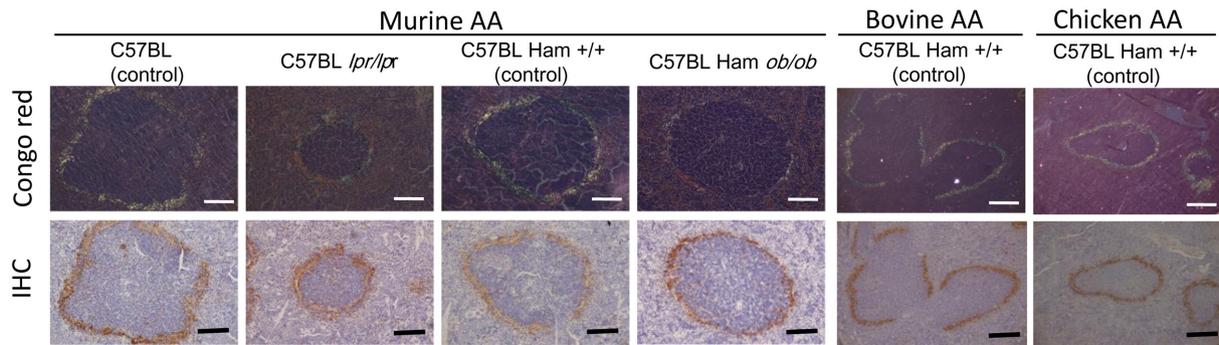


Fig. 2. Amyloid deposits in spleens of several mice. Amyloid deposits were stained with Congo red and observed as green birefringence under polarized light. Amyloid fibrils in spleens of 18-week-old mice (C57BL, C57BL *lpr/lpr*, C57BL Ham +/+, and C57BL *ob/ob*) inoculated with murine amyloid fibrils were reacted with AF2948 antibody in IHC. Also, amyloid fibrils in spleens of 18- and 30-week-old C57BL Ham +/+ mice inoculated with bovine amyloid fibrils and chicken amyloid fibrils, respectively, were reacted with AF2948 antibody in IHC. Bar=100 μ m.

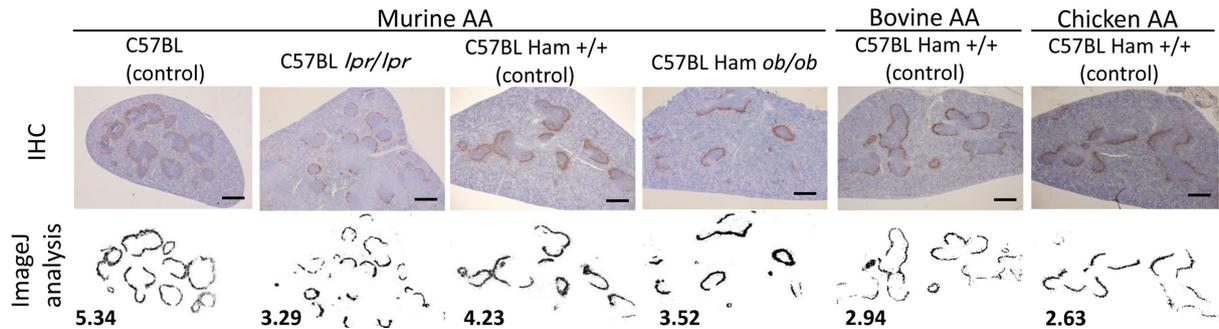


Fig. 3. Intensity scoring of amyloid deposits in the spleen using ImageJ. Amyloid fibrils in spleens of mice inoculated with murine, bovine, or chicken amyloid fibrils were reacted with AF2948 antibody in IHC. Images of IHC indicate amyloid deposits around spleen follicles. ImageJ was used to measure the intensity of amyloid deposits. ImageJ intensity scores are shown at the bottom. Bar=400 μ m.

difference in the scores of amyloid deposits between C57BL *lpr/lpr* and C57BL mice was statistically confirmed ($P<0.001$) (Fig. 4). Moreover, the ImageJ scores of C57BL Ham *ob/ob* mice inoculated with murine AA fibrils were also significantly lower than those of C57BL Ham +/+ mice inoculated with murine amyloid fibrils. The significant difference in the scores of amyloid deposits between C57BL Ham *ob/ob* and C57BL Ham +/+ mice was statistically confirmed ($P=0.003$) (Fig. 4). Amyloid deposits in groups inoculated with murine AA fibrils were more severe than those in groups inoculated with bovine or chicken amyloid fibrils (Figs. 3 and 4).

Concentrations of SAA in serum

Concentrations of SAA were measured before injection and 1 day after injection of AgNO₃ and murine amyloid fibrils (Fig. 5). The concentrations of SAA in mice inoculated with bovine or chicken amyloid fibrils

with AgNO₃ were the same as those in mice inoculated with murine amyloid fibrils and AgNO₃ (data not shown). At day 0, the relative concentration of SAA in C57BL *lpr/lpr* mice was significantly higher than that in C57BL control mice, but the concentration of SAA in C57BL Ham *ob/ob* mice at day 0 was the same as that in C57BL Ham +/+ mice. The significant difference in SAA concentration between C57BL *lpr/lpr* mice and control mice was statistically confirmed ($P=0.004$) (Fig. 5). On the other hand, concentrations of SAA were markedly raised at day 1 in response to inflammatory stimuli, but were the same in all mice.

Concentrations of cytokines in serum

The relative concentrations of cytokines IL-6 and IL-10 were measured before injection and 1 day after injection of AgNO₃ and murine amyloid fibrils (Fig. 6). The concentrations of cytokines in mice inoculated with

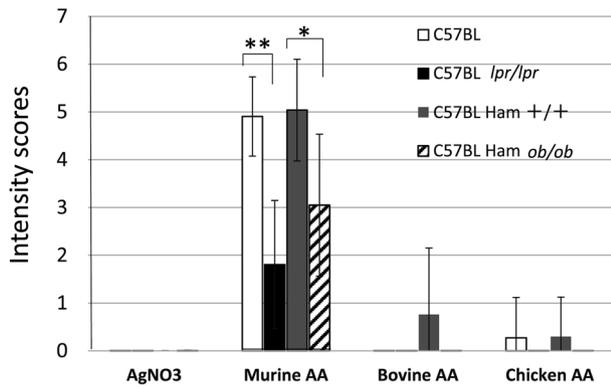


Fig. 4. Relative means of ImageJ intensity scores for amyloid deposits in spleens. Relative ImageJ intensity scores of amyloid deposits were calculated from all mice inoculated with amyloid fibril samples in each group. Mice were inoculated with AgNO₃, murine amyloid fibrils, bovine amyloid fibrils, or chicken amyloid fibrils intraperitoneally and examined for amyloid deposits in spleens. Results are expressed as means \pm SD. ** P <0.001 vs. C57BL. * P <0.01 vs. C57BL Ham +/+.

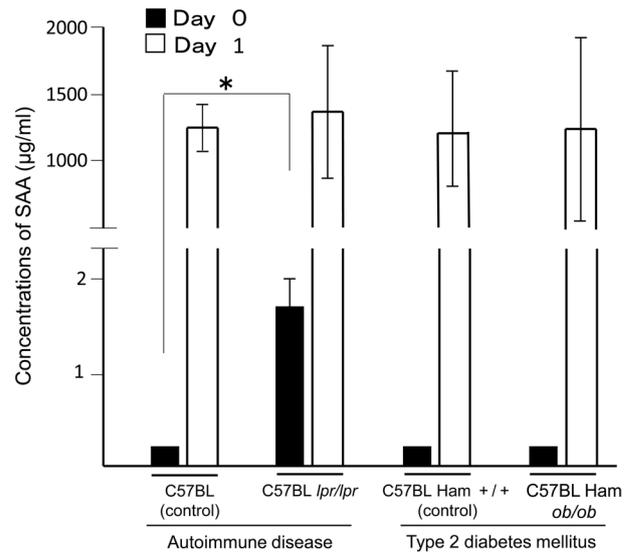


Fig. 5. Concentrations of SAA in serum from different model mice inoculated with murine amyloid fibrils. Concentrations of SAA in serum at days 0 and 1 were measured by ELISA. Results are expressed as means \pm SD. * P <0.01 vs. C57BL.

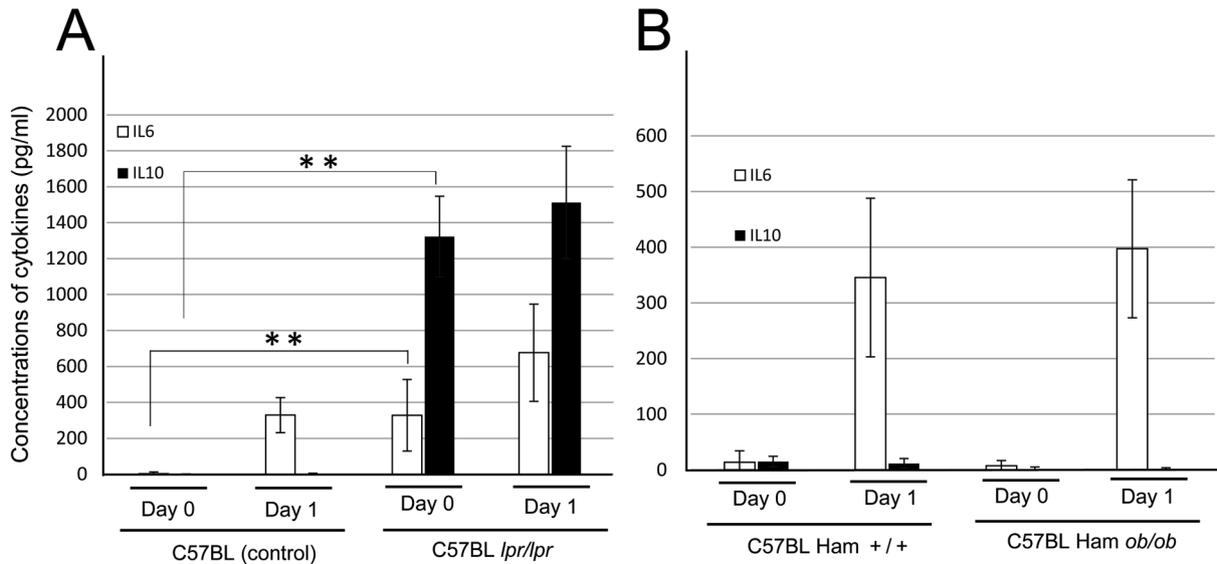


Fig. 6. Concentrations of cytokines in serum from different model mice inoculated with murine amyloid fibrils. Concentrations of IL-6 and IL-10 in serum at days 0 and 1 were measured with a CBA kit. Results are expressed as means \pm SD. ** P <0.001 vs. C57BL.

bovine or chicken amyloid fibrils with AgNO₃ were mostly the same as those in mice inoculated with murine amyloid fibrils and AgNO₃ (data not shown). The concentrations of IL-6 and IL-10 in C57BL *lpr/lpr* mice were significantly higher than those in C57BL control mice at day 0, and the difference was statistically significant (P <0.001) (Fig. 6A); in particular, the concentra-

tion of IL-10 was 1,000 times that of control mice. On the other hand, the concentrations of these cytokines were roughly the same in C57BL Ham +/+ mice and C57BL Ham *ob/ob* mice at day 0 (Fig. 6B). The concentrations of IL-6 were raised at day 1 in response to inflammatory stimuli in C57BL, C57BL *lpr/lpr*, C57BL Ham +/+, and C57BL Ham *ob/ob* mice.

Discussion

Experimental induction of AA amyloidosis by murine, bovine, or chicken amyloid fibrils in C57BL *lpr/lpr* mice as a model for autoimmune disease occurred less often than in control mice. In addition, the amount of amyloid deposits in autoimmune disease mice was less than that in control mice. Clinically, systemic AA amyloidosis has rarely been described in patients with systemic lupus erythematosus (SLE) [6], which the C57BL *lpr/lpr* mice were a model of in this study. In contrast to SLE, patients with type 2 diabetes mellitus have a higher prevalence of renal amyloidosis [5]. We used C57BL Ham *ob/ob* mice as a type 2 diabetes mellitus model in this study. However, experimental induction of AA amyloidosis in C57BL Ham *ob/ob* mice occurred less often, and the amounts of amyloid deposits in them were less than those in control mice. Therefore, AA amyloidosis was transmitted to two chronic disease mouse models less frequently than to control mice over the short experimental period. In practice, these diseases progress over the course of long periods of time in patients and incidentally have the potential for SAA elevation similar to that seen in acute infection. The elevation of SAA spontaneously produces some small aggregation of SAA particles known as “seeds” that play a role as templates for further SAA aggregation to form amyloid deposits [17]. However, the seeds may not have been sufficient in these disease model mice over the short term of this study. In patients with these diseases, the seeds may be formed by spontaneous aggregation of SAA through the rise and decline of SAA levels over the long disease progression.

Bovine and chicken amyloid fibrils showed different specificities with respect to the cause of AA amyloidosis in mice and amyloid deposits in the spleen compared with murine amyloid fibrils (Table 1 and Fig. 4), which is consistent with previous studies showing that it is difficult to reproduce the transmission of AA amyloidosis between different animal species [7, 19, 21, 24]. The difference in specific cause of AA amyloidosis is probably due to the primary amino acid structure of the SAA molecule [10]. Nucleotide and amino acid identities between murine SAA and bovine SAA and between murine SAA and chicken SAA (GenBank accession nos.: mouse, NM_009117; bovine, XM_005226741; chicken, GU929209) are estimated to be 78.3% and 68.6% (murine and bovine) and 60.4% and 61.2% (murine and chicken), respectively.

Since SAA is the precursor protein of amyloid fibrils [8], its high serum concentration is considered to be involved directly in the induction of AA amyloidosis. Indeed, there is a report showing that suppression of SAA with medication reduces amyloid deposits in patients with AA amyloidosis [16]. However, the amounts of amyloid deposits in C57BL *lpr/lpr* mice were markedly less than those in control mice, despite higher starting serum concentrations of SAA in the former, contrary to expectations. In addition, the amounts of amyloid deposits in C57BL Ham *ob/ob* mice were less than those in control mice, although the concentrations of SAA were the same between them. These results suggest that higher concentration of SAA is not the only factor that determines incidence and intensity of amyloid deposits in mice. It has not yet been explained why these model mice (C57BL *lpr/lpr* and C57BL Ham *ob/ob*) are unsusceptible to the transmission of AA amyloidosis. However, use of these model mice in the experimental transmission of AA amyloidosis might be a useful approach to estimate the transmission and pathogenesis of AA amyloidosis between animals.

In this study, inflammatory stimuli increased IL-6 in the sera of all mice. IL-6 is a proinflammatory cytokine that facilitates the production of SAA and has an important role in amyloid deposition [15]. IL-10 is an anti-inflammatory cytokine and inhibits the production of IL-6 [13], and it was found at high levels in C57BL *lpr/lpr* mice in this study. The role of IL-10 in amyloid deposition remains unclear. However, it is known that a proinflammatory or anti-inflammatory environment may modify the function of macrophages in the formation or degradation of amyloids [14]. Thus, the anti-inflammatory environment in C57BL *lpr/lpr* mice probably inhibited amyloid deposition.

As described previously, AA amyloidosis is reported to be transmissible to different animal species [19, 21]. If AA amyloidosis can be transmitted between different species, the presence of AA amyloids in food animals might be a potential risk to public health. However, the underlying pathogenic mechanism in AA amyloidosis and its prevention remain unclear [21]. Our findings demonstrate that inflammatory or anti-inflammatory conditions are involved in amyloid deposition, in addition to the concentration of SAA. Further investigations are necessary to identify the factors that stimulate or inhibit the formation of amyloid deposits in disease model mice to further understand the pathogenic mechanism of AA amyloidosis and its prevention.

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