Age-dependent decrease of polymeric Ig receptor expression and IgA elevation in ddY mice: a possible cause of IgA nephropathy

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Individual animals in the closed colony population of ddY mice were analyzed to clarify the major cause of age-dependent elevation of serum IgA and the appearance of human IgA nephropathy (IgAN)-like symptoms. Based on the serum IgA levels, the mice were classified into two subgroups. One was a high serum IgA group with some manifestations of IgAN through aging (ddY^{High}), and the other was a normal serum IgA group without IgAN (ddY^{Norm}). The ratio of urinary IgA to serum IgA was significantly reduced in ddY^{High} mice, suggesting an impaired IgA clearance via secretion through the epithelial barrier. The actual clearance rate of the intravenously injected dimeric IgA in ddY^{High} mice was found to be slower than that in ddY^{Norm} mice. Furthermore, we found that the polymeric Ig receptors (pIgRs) that mediate transcytosis of IgA were poorly expressed in the glomeruli as well as in the intestine of ddY^{High} mice, whereas the pIgRs were more abundantly expressed in ddY^{Norm} mice. In addition, the comparative study using polymerase chain reaction showed that decreased pIgR expression occurred at the transcriptional level in the ddY^{High} population. Taken together, these results suggest that a systemic defect in pIgR expression may result in impaired IgA secretion and accumulation of IgA in the serum of ddY^{High} mice. The age-dependent changes of pIgR expression in the dimeric IgA secretion sites of ddY^{High} mice suggest a possible cause for the elevation of serum IgA level and the pathogenesis of IgAN-like disease.

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IgA nephropathy (IgAN) is probably the most common glomerular disease in the world, and is characterized by IgA deposition in the glomeruli, usually with serum IgA elevation.¹ The progression of the disease is indicated by proliferation of mesangial cells and expansion of extracellular matrices. The pathological diagnostic finding of IgAN is determined by the intensity of IgA deposit in the mesangial area, which is usually associated with C3, properdin, factor H, and membrane attack complex (C5b-9), but not with C4.² Thus, the mesangial injury appears to be mediated by IgA-associated activation of complements in an alternate pathway correlating with the elevation of serum IgA,

and the fate of IgA-based immune complexes must be a critical factor in the establishment of IgAN.

However, the actual pathogenesis of IgAN remains poorly understood, although a number of clinical and experimental studies have been reported so far. For example, elevated serum IgA has been shown among those who suffered from IgAN with IgA deposition in the glomeruli, and the major type of IgA in the sera and deposited IgA among these patients have been identified as polymeric IgA (pIgA).^{3,4} This pIgA should instead have been secreted via mucosal barrier to form a complex with secretory components (SCs) produced by epithelial tissues. SCs are the processed portion of polymeric Ig receptor (pIgR) molecules, which should control the secretion of pIgA.^{5,6} Shimada *et al*⁷ have recently established pIgR knockout (pIgR^{-/-}) mice and described that these mice had a high serum pIgA concentration due to the interruption of transepithelial transport of pIgA. Therefore, we focused on the defects of IgA secretion, especially by the pIgR-secretion system, as a cause of serum IgA elevation.

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In the present study, we first quantified serum and urinary IgA concentrations every 4 weeks to identify aged ddY mice showing high serum IgA (HIGA) with relatively low urinary IgA.⁸ We found that we could classify ddY mice into at least two subgroups. One was a HIGA group that exhibited some symptoms of IgAN through aging (ddY^{High}), and the other was a normal serum IgA group without IgAN (ddY^{Norm}). Moreover, taking advantage of dimeric IgA carrying allotypic markers (Igh-2b), we confirmed that the actual clearance rate of the intravenously injected dimeric IgA in ddY^{High} mice was slower than that in ddY^{Norm} mice. Then, we compared the pIgR expression in the kidney as well as the small intestine between those two groups by immunofluorescence staining and reverse transcription polymerase chain reaction (RT-PCR), to clarify the relationship between the expression of pIgR in mucosa and the appearance of IgAN in ddY mice.

Materials and methods

Mice

In all, 10 female BALB/c mice, C57BL/6 and 26 female ddY mice at 11 weeks old were purchased from Japan SLC (Shizuoka, Japan). The female ddY mice were selectively used because of their long-lived nature compared to male mice, and the phenotype of IgA elevation through aging is independent of the sex. The mice were maintained in specific pathogen-free conditions and fed autoclaved food and water. All mice were cared for according to the guidelines of the NIH Guide for the care and use of laboratory animals.

Enzyme-Linked Immunosorbent Assay for Serum, Urinary, and Fecal IgA

Blood, urine, and feces of each mouse were collected at 4-week intervals between 12 and 44 weeks of age. Blood samples were obtained from the tail vein, and the serum was prepared, frozen immediately, and stored at -80°C until the analysis. The urine samples were collected directly to sample tubes by tenderly pushing their abdomen. They were centrifuged at 400 g and the supernatant was stocked at -80°C until the analysis. Four to five fecal pellets from each mouse were collected into preweighed Eppendorf tubes. Fecal weight was calculated by subtracting the preweight values. PBS (1 ml) was added per 100 mg of feces. Samples were vortexed until all materials were dispersed, settled at room temperature for 15 min, and then centrifuged at 13 000 rpm for 15 min. The supernatant was removed and stored at -80°C for analysis. Serum, urinary, and fecal IgA concentrations were measured by modified sandwich Enzyme-linked immunosorbent assay (ELISA), as described previously.⁹ Briefly, polystyrene micro-

titer plates (Nunc-Immuno Plate; Nalge Nunc International, Rochester, NY, USA) were coated with goat anti-mouse IgA antibody (Cappel Laboratories, Durham, NC, USA). After washing with 1% BSA-PBS three times, the sera, urine, or fecal samples were added at 1:100, 1:20, or 1:40 dilution. The bound IgA molecules were detected with biotin-conjugated rat anti-mouse IgA antibodies (BD Pharmingen, San Diego, CA, USA), followed by incubation with horseradish peroxidase-conjugated avidin D. The substrate, 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (Sigma-Aldrich, St Louis, MO, USA), was used for color development, and the absorbance at 415 nm was measured for quantification. The net quantities of IgA were determined using the standard curve obtained from the mouse IgA proteins (MOPC315, Cappel).

Purification and Molecular Analysis of Serum IgA

The composition and qualitative differences of serum IgA molecules were analyzed by reducing and nonreducing sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) on 2/15% gradient polyacrylamide gels (Multigel; Daiichi Pure Chemicals Co., Ltd, Tokyo, Japan). The serum IgA was purified with anti-mouse IgA antibody (Cappel)-conjugated CNBr-activated Sepharose 4B beads and Sephadex G25 column described in affinity chromatography (Amersham Pharmacia Biotech, Uppsala, Sweden). The location of monomeric and dimeric IgA molecules were reconfirmed by Western blotting using biotin-labeled rat anti-IgA antibodies and peroxidase-labeled avidin D. Silver Staining Kit (Amersham Pharmacia Biotech) was used for staining of proteins.

The dimeric IgA molecules of Igh-2b allotype derived from C57BL/6 mouse sera were prepared from the affinity-purified IgA monomer and dimer mixtures as follows. First, the dimers were separated by nonreducing 2/15% gradient PAGE. Second, the gel regions containing dimeric IgA were cut off and the dimer molecules were electro-eluted and renatured by dialyzing against PBS at 4°C for 3 days.

Intravenous Administration of Dimeric IgA and Analysis of the Clearance by ELISA

A total of 20 μg of purified dimeric IgA of the b allotype (Igh-2b) in 0.5 ml of PBS was intravenously injected into ddY^{High} and ddY^{Norm} mice, which do not have the genotype of Igh-2b and whose IgAs do not react to monoclonal anti-Igh-2b antibodies (data not shown). Blood samples of 20–50 μl of blood was collected from the tail vein of each mouse at 1, 6, 12, 24, 36, 48, 60, 72, 84, and 96 h after the intravenous administration of Igh-2b at 44 weeks old of age. The amount of Igh-2b protein was selectively measured by utilizing the specific reactivity of the monoclonal anti-Igh-2b antibody (HIS-M2, BD Pharmingen) to

the allotypic determinants of IgA molecules. The details of the measurement have been described in the ELISA section above, except that the purified monoclonal anti-Igh-2b antibodies (10 μ g/ml in PBS) were used to coat the plates in the first step. This system allows us to examine the fate of administered IgA (Igh-2b) molecules selectively at high sensitivity without the need for radiolabeled or chemically modified IgA proteins. The statistical significance was scored according to Turkey's Student's *t*-test. The predetermined upper limit of probability for statistical significance was $P < 0.01$.

Histological and Immunofluorescent Analysis

All mice were killed at 44 weeks of age, and the kidneys and the small intestines were removed and trimmed off the fat and connective tissues. Some of them were treated by standard paraformaldehyde fixation protocol for histological staining, and the others were placed in an embedding compound (TISSUE MOUNT; Chiba Medical, Saitama, Japan), and snap-frozen on dry ice and ethanol for immunofluorescent analysis. Tissue injury was assessed by hematoxylin-eosin (HE) staining and periodic acid-Schiff (PAS) staining. To detect pIgR expression and IgA deposition, each frozen section (6 mm thickness) was fixed with cold acetone for 10 min, incubated with 1% BSA and 10% goat serum at 4°C overnight and with Avidin/Biotin Blocking Kit (Vector Laboratories, Burlingame, CA, USA). Subsequently, the specimens were labeled with rabbit anti-mouse SC IgG⁷ (5 μ g/ml) for the kidney or rabbit anti-mouse SC serum¹⁰ (1:150) for the small intestine, or rat anti-mouse IgA antibody (1 μ g/ml) (BD Pharmingen) for 1 h at 37°C. Finally, fluorescein isothiocyanate (FITC)-conjugated anti-rabbit Ig (1:200) (BD Pharmingen), or biotin-conjugated anti-rat IgG (1:200) (Cedarlane Laboratories, Hornby, Ontario, Canada) was added for 30 min at 37°C and FITC-conjugated streptavidin (1:2000) (BD Pharmingen) was added for 10 min at room temperature. The staining pattern was analyzed by using Confocal Laser Scope (LSM510; Carl Zeiss, Jena, Germany).

RT-PCR for pIgR mRNA in the Kidney and the Small Intestine

Total RNA was extracted from the renal cortex and the small intestine of ddY mice at 44 weeks old, from mouse pIgR transfectant cell (2S9.1), and from mock transfectant cells (3N.1)¹⁰ using the RNeasy Protect Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instruction. RNA (2 μ g) was reverse transcribed with oligo-(dT)18 (Perkin Elmer, Wellesley, USA) priming and Superscript III (Invitrogen, Sandiego, USA) reverse transcriptase in a 20 μ l reaction mixture at 50°C for 60 min. A measure of 1 μ l (equal to about 200 ng) of

the cDNA product was then subjected to 40 cycles of PCR amplification consisting of 1 min at 95°C, 2 min at 55°C, and 2 min at 72°C with a 7 min final extension at 72°C, with a thermocycler (PCR Express; Hybaid, Teddington, Middlesex, UK). The amplification was performed in a reaction volume of 20 μ l with LA PCR buffer (Takara, Shiga, Japan), 2.5 mM MgCl₂, 0.3 mM each deoxynucleotide triphosphate, 2.5 mM of each primer, and 1 U of LA Taq polymerase (Takara). The following oligonucleotide primers for pIgR and GAPDH are designed from published cDNA sequence:⁷ pIgR senseprimer (5'-TTCCTGAGTTGCCGAGTGACA-3', the beginning of the exon 4) and antisenseprimer (5'-CTAGGC TTCCTGGGGACCATC-3', the end of the exon 11); GAPDH senseprimer (5'-GCCTCAAGATCATCAGCAATGC-3') and antisenseprimer (5'-ATGCCAGT GAGCTTCCCGTTC-3').

Results

Age-Dependent Changes of Serum and Urinary IgA Levels

We examined the age-dependent changes of serum IgA concentration among ddY and BALB/c mice. Although the serum IgA levels of BALB/c mice did not increase and stayed close to the mean of 13 μ g/ml until 44 weeks of age (Figure 1A), the ddY mice population appeared to be segregated into at least two groups based on the serum IgA concentration. One was the ddY^{High} mice population that showed serum IgA concentrations two standard deviations (s.d.s) beyond the mean of control BALB/c mice. The other was the ddY^{Norm} mice population with the IgA level within the mean $\pm$ 2 s.d.s of BALB/c mice throughout the whole course of the experiment. This result was consistent with the genetic segregation analysis observed in the closed-colony ddY population carried out by Imai *et al.*⁸

Next, we studied the age-dependent changes of urinary IgA concentration in ddY mice. Although we could also observe the elevation of IgA in urine among ddY^{High} mice to some extent, the magnitude of age-dependent IgA increase in urine was very low. Thus, we plotted the scatter diagram between urine and serum IgA levels at 24 weeks old (Figure 1B; a and b) and 44 weeks old (Figure 1B; c and d) of those two segregated ddY mice. As indicated in Figure 1B, while the slopes of the regression curve in ddY^{Norm} mice at 24 and 44 weeks old were 0.172 and 0.160 (a and c), those in ddY^{High} mice at correspondent weeks were 0.110 and 0.059 (b and d), respectively. These results show that the urine/serum IgA ratios in ddY^{High} mice are lower than that in ddY^{Norm} mice at 24 weeks of age and that they further decrease through aging. The relatively low level of urine IgA in ddY^{High} mice may suggest the decrease in the efficacy of serum IgA transportation to the urinary tracts in an age-dependent manner.

Figure 1 Serum and urinary IgA. (A) Serum IgA concentrations of BALB/c mice at 44 weeks old. The maximal level of the serum IgA in BALB/c mice was 15.2 $\mu\text{g/ml}$. We referred the mean ($13.0 \mu\text{g/ml}$) $\pm$ two standard deviations (s.d.s) as the normal level of serum IgA for further experiments. (B) Correlation between urinary and serum IgA level of ddY^{High} and ddY^{Norm} mice at 24 weeks old (a and b) and 44 weeks old (c and d), respectively. The slopes of regression curve of ddY^{Norm} mice at 24 and 44 weeks old were almost unchanged as 0.172 and 0.160 (a and c), while those of ddY^{High} mice declined as 0.110 and 0.059 (b and d), respectively. Consequently, ddY mice were classified into two populations according to their serum IgA level, and we confirmed that the rate of urinary to serum IgA level of ddY^{High} mice decreased through aging compared with that of ddY^{Norm} mice.

Since the small intestine was known to be one of the major IgA-secreting organs, we also examined the amount of IgA in the feces of ddY^{High} mice by plotting the scatter diagram between feces and serum IgA levels. The results were similar to that found in urine (data not shown). These data suggest that ddY^{High} mice may have an age-dependent deficiency of systemic IgA secretion through mucosa.

pIgR Expression in the Kidney and the Small Intestine

To gain an insight into the defect of IgA secretion observed in ddY^{High} mice, the pIgR expression was compared between ddY^{High} and ddY^{Norm} mice. The specimens from ddY^{High} and ddY^{Norm} kidneys were stained with FITC-labeled anti-pIgR polyclonal antibodies. Figure 2a and b shows the pIgR expression by FITC, and Figure 2c and d show the localization of the glomeruli in PAS staining. While both the glomeruli and the tubules of ddY^{Norm} mice expressed pIgR (Figure 2a and c), the glomeruli in ddY^{High} mice did not express a detectable level of pIgR and only a marginal expression was observed in the tubules (Figure 2b and d). The results suggest that the elevation of serum IgA may correlate with the decrease of pIgR in the kidney.

We also investigated pIgR expression in the guts of ddY^{High} and ddY^{Norm} mice. Decreased pIgR expression in the intestinal epithelial cells at the tip of the villi was noted in ddY^{High} mice (Figure 3b), as compared with ddY^{Norm} mice (Figure 3a). This

Figure 2 pIgR expression in the kidney. (a and b) The pIgR expression stained by FITC. (c and d) The localization of the glomeruli in PAS staining. The upper figures show the kidney of ddY^{Norm} mice at 44 weeks, and the bottom figures show those of ddY^{High} mice. The magnification is $200\times$. We could observe pIgR expression in the glomeruli in ddY^{Norm} mice, but not in ddY^{High} mice.

observation in the gut was parallel to the decreased expression of pIgR in ddY^{High} kidney. It was likely that the decrease of pIgR expression in ddY^{High} mice occurred systemically.

Figure 3 pIgR expression in the small intestine. (a and b) The pIgR expression with FITC and surface IgA positive B cells with Texas-Red. (b) It shows decreased pIgR expression in the intestinal epithelial cells at the tip of the villi in ddY^{High} mice compared with ddY^{Norm} mice (a). This observation is consistent with the decreased pIgR expression in the ddY^{High} kidney. Thus, the decrease of pIgR expression in ddY^{High} mice may be the systemic symptom.

Figure 4 RT-PCR analysis of pIgR mRNA expression in the kidney (a) and the small intestine (b). The mRNA was prepared and analyzed as described in section Materials and methods. mRNA expression (a) in the kidney and (b) in the small intestine. The lane 2S9.1 represents amplified pIgR mRNA of pIgR-transfected cell as a positive control, and the lane 3N1 shows that of mock-transfected cells as a negative control. The GAPDH product was shown at counter part of each figure as an internal control. The lane ddY^{High} and ddY^{Norm} showed the amplified pIgR mRNA of each kidney and small intestine. The pIgR mRNA expression of ddY^{High} mice was lower than that of ddY^{Norm} mice compared with the density of GAPDH.

pIgR mRNA Expression in the Kidney

To further support the difference in pIgR protein expression in ddY^{High} and in ddY^{Norm} mice, pIgR mRNA transcription was also compared using semiquantitative RT-PCR analysis. The expression of pIgR mRNA in both the kidney and the small intestine of ddY^{High} mice were lower than in those of ddY^{Norm} mice (Figure 4a and b) compared with an internal control mRNA (GAPDH). Thus, the pIgR expression was confirmed to decrease in the kidney as well as in the small intestine of ddY^{High} mice. These results are consistent with the lower expression assessed by immunofluorescent staining.

Composition and Qualitative Differences of IgA Molecules in Sera

To examine the qualitative differences of serum IgA molecules between ddY^{High} and ddY^{Norm} mice,

affinity-purified serum IgA proteins were run on 2/15% gradient SDS-PAGE under nonreducing conditions, followed by silver staining. The apparent molecular differences were not detected in terms of the ratio of monomeric IgA to dimeric IgA in ddY^{High} mice at 12 and 44 weeks of age (Figure 5). Also, molecular abnormalities of heavy and light chains of serum IgA molecules in ddY^{High} and ddY^{Norm} mice were not detected by SDS-PAGE analysis under reducing conditions (data not shown). These results ruled out the possibility that the structural abnormality of IgAs in the sera of ddY^{High} mice influences the efficacy of IgA clearance in mucosa.

Clearance of Dimeric IgA Molecules Following the Intravenous Administration to ddY^{High} and ddY^{Norm} Mice

A total of 20 μ g of purified dimeric IgA of the b allotype (Igh-2b) was injected intravenously into the tail vein of aged ddY^{High} and ddY^{Norm} mice having the different genotype of IgA from Igh-2b. After administration, serum Igh-2b concentrations were determined using ELISA at the intervals shown in the horizontal axis (Figure 6). Although the clearance rate of administrated dimeric IgA (Igh-2b) among ddY^{High} and ddY^{Norm} did not change at the early phase of clearance, a significant slow clearance rate was observed in ddY^{High} compared to ddY^{Norm} ($P < 0.01$) at the later phase (more than 24 h after dimeric Igh-2b IgA administration). The finding that the IgA secretion equilibrium in ddY^{High} was shifted to the lower rate suggests the systemic decrease of pIgR expression in the mucosal surface.

Histological and Immunofluorescent Analysis

The tissue sections of the kidneys from ddY^{High} and ddY^{Norm} mice were stained by HE staining (data not shown) and PAS staining (Figure 7a and b). Figure 7a shows a representative PAS staining of one of ddY^{High} mice. It shows the diffuse mesangial hypercellularity, mesangial PAS-positive deposits,

Figure 5 Qualitative differences of IgA molecules in sera. Affinity-purified serum IgA samples were applied to 2/15% gradient SDS-PAGE under nonreducing conditions, followed by silver staining. Serum IgA of BALB/c, ddY^{Norm} and ddY^{High} mice at 12 and 44 weeks old were applied. The apparent molecular and structural differences were not observed in terms of the proportion of monomeric IgA vs dimeric IgA in ddY^{High} mice when compared with control murine IgA (MOPC315 clone). Thus, the possibility that the molecular abnormality of IgA in the sera was ruled out.

and matrix proliferation, which were typical appearances for IgAN. In contrast, as shown in Figure 7b, ddY^{Norm} mice showed no apparent abnormality in the kidneys. The HE staining revealed compatible results as detected by PAS staining (data not shown).

Figure 8a and b show IgA deposition in the glomeruli of ddY^{High} mice and ddY^{Norm} mice, respectively. The IgA deposition pattern in the glomeruli of ddY^{High} mice was granular, which is compatible with that of IgAN. On the other hand, the IgA deposition in the glomeruli of ddY^{Norm} mice was not predominant. These histological and immunofluorescent findings show that the decrease of pIgR might be responsible for IgA deposition in the glomeruli of ddY^{High} mice.

Discussion

By measuring the age-dependent changes of serum IgA level and IgA excretion profiles of ddY mice, we could classify the closed-colony population of ddY mice into at least two groups. In the ddY^{High} population, the age-dependent elevation of serum IgA and the relatively lower excretion of secretory IgA into urinary fluid and feces were observed. The ddY^{Norm} population exhibited a steady level of serum IgA and relatively higher excretion of secretory IgA into urine and feces. Imai *et al*⁸ reported the genetic segregation of the ddY population into two groups with different criteria of IgA elevations. By selective breeding, Miyawaki *et al*¹¹ established the HIGA mouse strain, which has the HIGA level and the IgAN-like manifestations. The ddY^{High} popula-

Figure 6 Clearance of dimeric IgA molecules following the intravenous administration to ddY^{High} and ddY^{Norm} mice. A total of 20 μ g of purified dimeric IgA of the b allotype (Igh-2b) was intravenously injected into the tail veins of ddY^{High} and ddY^{Norm} mice having the different genotype of IgA from Igh-2b. After the administration, the serum Igh-2b concentration was determined using ELISA at the intervals shown in the horizontal axis. The line plotted by dot open circles and by filled circles show the profiles of ddY^{High} and ddY^{Norm} mice respectively. The results represent the mean $\pm$ standard error of three independent experiments ($n=3$ at each experiment). The statistical significance was scored according to Turkey's Student's *t*-test. A significant difference ($P<0.01$) between ddY^{High} and ddY^{Norm} was detected at 24, 60, 72, 84, and 96 h after the administration.

Figure 7 Pathological changes of the glomeruli of old ddY mice. The glomeruli of ddY^{High} (a) and ddY^{Norm} (b) mice with PAS staining are shown. (a) The typical feature of IgAN, diffuse mesangial hypercellularity, mesangial PAS-positive deposits (indicated with arrows), and matrix proliferation, was observed. (b) Unchanged normal feature in the glomeruli of ddY^{Norm} mice was observed.

tion in this study is thought to have the same phenotype as that of the HIGA. However, the genetic basis that accounts for the elevation of serum IgA is still unknown.

As shown in Figure 5, we could not observe any qualitative differences in the structural properties of serum IgA between old ddY^{High} and ddY^{Norm} populations. Also, molecular abnormalities of heavy

Figure 8 IgA deposition in the glomeruli of old ddY mice. The IgA deposition in the glomeruli of ddY^{High} (a) and ddY^{Norm} (b) mice was analyzed by using confocal laser microscope after immunofluorescent staining (FITC) for IgA. (a) The granular pattern of IgA deposition was observed in the glomeruli of ddY^{High} mice, which showed a decrease of pIgR expression in the kidney. (b) In contrast, few IgA deposition was detected in the glomeruli of ddY^{Norm} mice, which had the normal expression of pIgR in the kidney.

and light chains of serum IgA molecules from ddY^{High} and ddY^{Norm} mice were not detected. Thus, the molecular alterations of IgA in the sera of ddY^{High} mice would not affect IgA clearance in mucosa. However, as demonstrated in Figure 6, the clearance rate of dimeric IgA in the serum was found to be slower in ddY^{High} than ddY^{Norm} mice. Moreover, both histological immunofluorescence analysis and semiquantitative RT-PCR examination revealed a decrease of pIgR expression in the epithelial cells of the kidney and the intestine in ddY^{High}, but not ddY^{Norm}, mice. Therefore, the age-dependent changes in the pIgR expression but not in the IgA structure seem to be a possible cause for the elevation of serum IgA.

The reason for the decrease of pIgR expression in the epithelial cells in mucosa of ddY^{High} is still unknown. In rabbits, two forms of the SC (a high molecular weight form and a low molecular weight form) were reported to arise from differential RNA splicing.¹² It is still unclear whether this type of mRNA splicing variation is one of the causes of decreased expression in mucosa of ddY^{High} mice. Nonetheless, it is likely that the age-dependent decrease of pIgR in mucosa and the anti-parallel increase of serum IgA in ddY^{High} mice may be associated directly with IgA deposition and with IgAN-like manifestations in ddY^{High} mice.

Kaetzel *et al*¹³ have reported on active transport of immune complexes containing pIgA (pIgA-IC) across the epithelial cells by the same pIgR-dependent mechanism that normally applies to free pIgA. Several investigators^{14–18} have emphasized the importance of the pIgR-dependent system for transport of pIgA-IC, rather than the mononuclear phagocyte system, which mediates a major clearance pathway of immune complexes in circulation. Thus, the decreased pIgR expression observed in ddY^{High} mice may potentially not only result in serum pIgA

elevation but also serum pIgA-IC accumulation. Moreover, the fact that IgA accumulation in lamina propria of the intestinal mucosa has been shown in pIgR^{-/-} mice indicates that the impaired clearance of pIgA-IC and IgA deposition may occur in the pIgR-deficient glomeruli, as observed in ddY^{High} mice.

In the case of human IgAN, the defective clearance of dimeric IgA and/or IgA-IC has been demonstrated.^{19–21} In addition, Yasumori²² has represented the cases of decreased pIgR expression in the duodenum and impaired secretory-IgA-secretion in extra fluid in two of the 10 IgAN patients after duodenal biopsy. Therefore, decreased pIgR expression should also be found in some IgAN patients like ddY^{High} mice.

Taken together, the experiments in the present study suggest that systemic defects in pIgR expression may be a critical cause of inducing an accumulation of pIgA in the serum, followed by IgAN-like manifestation in ddY^{High} mice. Identification and segregation of a highly disease-associated population in phenotypically heterogeneous ddY mice will allow us to carry out precise investigation for the pathogenesis of IgAN in mice and perhaps determine the mechanism of high concentration of serum pIgA in IgAN patients.

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