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Generation of Polymeric Immunoglobulin Receptor-Deficient Mouse with Marked Reduction of Secretory IgA

Shin-ichi Shimada,* Mariko Kawaguchi-Miyashita,* Akira Kushiro,* Takashi Sato,* Masanobu Nanno,* Tomoyuki Sako,* Yoshiaki Matsuoka,* Katsuko Sudo,† Yoh-ichi Tagawa,† Yoichiro Iwakura,† and Makoto Ohwaki†*

We generated mouse lacking exon 2 of polymeric Ig receptor (pIgR) gene by a gene-targeting strategy (pIgR-deficient mouse; pIgR−/− mouse) to define the physiological role of pIgR in the transcytosis of Igs. pIgR−/− mice were born at the expected ratio from a cross between pIgR+/− mice, indicating that disruption of the pIgR gene in mice is not lethal. pIgR and secretory component proteins were not detected in pIgR−/− mice by Western blot analysis. Moreover, immunohistochemical analysis showed that pIgR protein is not expressed in jejunal and colonic epithelial cells of pIgR−/− mice, whereas IgA+ cells are present in the intestinal mucosa of pIgR+/− mice as well as wild-type littermates. Disruption of the pIgR gene caused a remarkable increase in serum IgA concentration and a slight increment of serum IgG and IgE levels, leaving serum IgM level unaltered. In contrast, IgA was much reduced but not negligible in the bile, feces, and intestinal contents of pIgR−/− mice. Additionally, IgA with a molecular mass of 280 kDa preferentially accumulated in the serum of pIgR−/− mice, suggesting that transepithelial transport of dIgA is severely blocked in pIgR−/− mice. These results demonstrate that dIgA is mainly transported by pIgR on the epithelial cells of intestine and hepatocytes, but a small quantity of IgA may be secreted via other pathways. The Journal of Immunology, 1999, 163: 5367–5373.

Mouse immune system is quite different from systemic one in that the major Ig in exocrine fluids is IgA. It is estimated that the amount of IgA produced every day in human (≈66 mg/kg of body weight/day) is much more than the combined amount of IgG (≈34 mg/kg/day) and IgM (≈8 mg/kg/day) (1). Moreover, the most striking feature of IgA is that it appears in multiple molecular forms (monomer, dimer, and polymer), and dimeric IgA (dIgA)2 and polymeric IgA (pIgA) as well as IgM contain J-chain, a 15-kDa glycoprotein. DlgA is secreted across the epithelial layer, and externally released dIgA (secretory IgA; sIgA) is readily detected in the saliva, bile, intestinal secretion, tear, or breast milk.

It has been proposed that a polymeric Ig receptor (pIgR) plays a critical role for transepithelial transport of dIgA (2). pIgR, after binding dIgA on the basolateral surface, carries it to the apical side and a specific protease cleaves the extracellular region of pIgR, followed by release of sIgA into the lumen. The extracellular region of pIgR is called secretory component (SC), and thus sIgA includes dIgA and SC. Since the pioneering work by Mostov et al. (3) in which cDNA of rabbit pIgR has been cloned, cDNAs and genes of pIgR were isolated from various species one after another and their nucleotide sequences determined (4–11). These results demonstrate that pIgR is a member of Ig superfamily and highly conserved during the evolution.

Many researchers have tried to verify that pIgR is involved in the transepithelial transport of dIgA. Using Madin-Darby canine kidney cell line stably transfected by rabbit pIgR cDNA or human pIgR cDNA, dIgA was shown to be transported through pIgR (12–14). In addition, 125I-labeled human pIgA i.v. given to rats was secreted into the bile, and the incubation of pIgA with anti-J-chain Ab beforehand blocked the biliary transport of pIgA (15). These results suggest that pIgA should bind pIgR via J-chain on epithelial cells of the liver and then sIgA is released into the bile. On the other hand, rat hepatocytes are known to capture human pIgA via asialoglycoprotein receptor and introduce IgA into the degradation process (16). Therefore, it is of great interest to determine the relative significance of pIgR in the transepithelial transport of dIgA and pIgA.

Recently, mice that have a disrupted J-chain gene (J−/− mouse) were established. J−/− mice contained much higher IgA in the serum, leaving IgM and IgG levels unchanged. By contrast, fecal and biliary IgA levels were drastically reduced in J−/− mice, whereas the IgA levels in breast milk, intestinal secretions, and nasal washes were comparable between wild-type and J−/− mice (17, 18). These results indicate that hepatic transport of IgA is severely hindered in the absence of J-chain, although intestinal and nasal secretion of IgA can occur independently of J-chain.

Mouse pIgR gene has been mapped on chromosome 1 and found to be composed of 11 exons (9, 10). As the translation of pIgR protein starts at the initiation codon in exon 2, we tried to inactivate the synthesis of pIgR protein by deleting the exon 2 of pIgR gene. pIgR and SC proteins were not detected in the intestines and bile of pIgR-deficient (pIgR−/−) mice, and both intestinal and hepatic transcytosis of dIgA was severely blocked, resulting in the massive accumulation of dIgA in the serum of pIgR−/− mice. However, a significant amount of IgA was still present in the exocrine fluids of pIgR−/− mice.
Materials and Methods

Mice

BDF1 and C57BL/6J mice were purchased from Japan Clea (Tokyo, Japan).

Gene targeting by homologous recombination

Mouse plgR gene was isolated from genomic library of 129/SvJ mouse, and the complete nucleotide sequence was determined (10). Targeting vector was constructed from MscI fragment including exon 2 of plgR gene. NeoA cassette was inserted into SpeI site of the MscI fragment, and the modified MscI fragment was introduced between NotI and AccI sites of diphtheria toxin A vector. Embryonic stem (ES) cell line, RW4, was purchased from Genomed Systems (St. Louis, MO) and cultured on mitomycin-C-treated embryonic fibroblasts in DMEM supplemented with 15% FCS and leukemia inhibitory factor (Life Technologies, Gaithersburg, MD) at 10^7 U/ml. Targeting vector DNA (50 μg) was transfected by electroporation (500 μF, 250 V; Gene Pulser; Bio-Rad, Hercules, CA) into 1 × 10^7 RW4 ES cells. Cells were selected in the presence of G418 (250 μg/ml; Life Technologies) and 720 colonies picked up. Screening was conducted by PCR using the following primers: sense, 5′-ATCTGCGTGTGACCCATGCGGAGTCG-3′; antisense, 5′-CCTCAGCTTGGATGGCTAGCATT-3′.

Southern blot analysis

Genomic DNA was extracted from mouse tail and digested with HindIII or KpnI. After agarose gel electrophoresis, separated DNA was transferred to nylon membrane (Hybond-N, Amersham, Buckinghamshire, U.K.) at r.t. for 3 h, and then the membrane was incubated at 37°C for 1.5 h. Thereafter, unbound materials were removed and the membrane was rehybridized with probes corresponding to the intron between exons 2 and 3, or the neo gene. Detailed methods of Southern blot analysis were described elsewhere (19).

Northern blot analysis

Northern blot analysis was conducted according to the previous method (19). After total RNA was extracted from various tissues with ISOGEN (Nippongene, Toyama, Japan), 7 μg of RNA was applied into each well and electrophoresed. Separated RNA was transferred to nylon membranes and hybridized with cDNAs corresponding to β-actin mRNA or full-length mouse plgR mRNA.

Preparation of rabbit anti-mouse SC IgG

Recombinant mouse SC fused with GST was synthesized as follows. cDNA encoding mouse SC was prepared from mouse plgR cDNA. SC cDNA inserted into pGEX-5X-3 plasmid (Pharmacia Biotech, Uppsala, Sweden) was transfected in Escherichia coli. GST-SC fusion protein was purified from the inclusion body of transfected E. coli. After rabbit was immunized s.c. with 1 mg of GST-SC fusion protein four times, serum was recovered and IgG fraction purified with Ampure PA kit (Amersham). Ab titer was determined by ELISA by using GST-SC and GST proteins.

Western blot analysis

Intestinal epithelial cells were prepared by shaking intestinal walls in 50 mM EDTA/25 mM HEPES (pH 7.2)/Ca^2+^- and Mg^2+^-free HBSS at 37°C for 90 min. They were incubated in 1% Triton X-100/50 mM EDTA/25 mM HEPES (pH 7.2)/Ca^2+^- and Mg^2+^-free HBSS including protease inhibitors (1 mM PMSF, 0.2 U/ml aprotinin, 1 μg/ml soybean trypsin inhibitor, 1 μg/ml leupeptin, and 1 μg/ml benzamidine) on ice for 45 min, and soluble materials were collected after centrifuging them at 12,000 rpm for 30 min. Intestinal contents were collected by scraping with forceps after opening the intestinal tract longitudinally. Feces were obtained just after evacuation. Both of them were suspended in PBS including protease inhibitors (1 mM PMSF, 0.2 U/ml aprotinin, 1 μg/ml leupeptin, and 1 μg/ml benzamidine) and electrophoresed. Separated RNA was transferred to nylon membrane (Hybond-N, Amersham) and hybridized with cDNA encoding mouse SC. After total RNA was extracted from various tissues with ISOGEN, 1 μg of RNA was applied into each well and electrophoresed. Separated RNA was transferred to nylon membrane (Hybond-N, Amersham, Buckinghamshire, U.K.) and hybridized with probes corresponding to the intron between exons 2 and 3, or the neo gene. Detailed methods of Southern blot analysis were described elsewhere (19).

Statistical analysis

Significance of the difference between groups was evaluated by Student’s t test.

Results

Disruption of exon 2 of plgR gene by homologous recombination

As translation of plgR protein starts from the initiation codon in exon 2 of plgR gene, we tried to delete the exon 2 for generation of plgR-deficient (plgR−/−) mice. To construct a targeting vector, exon 2 and the surrounding introns in the MscI fragment of plgR gene was replaced by neoA cassette and combined with diphtheria toxin A vector (Fig. 1A). DNA of the targeting vector was introduced by electroporation into RW4 cells, and the cells surviving in the presence of G418 were picked up. Three independent targeted clones were identified among 720 clones, and by aggregating ES cells of one targeted clone and BDF1 eight-cell morulae, a chimeric male mouse having a mutated germine was born. Heterozygous mice were generated and backcrossed with C57BL/6J mice twice (N2). Homozygous mice were produced by intercrossing heterozygous mice. All the experiments were conducted using littermates of N2 generation.

To confirm the disruption of exon 2 of plgR gene in homozygous mice, tail DNAs were obtained from a littermate of a cross between heterozygous mice and genomic Southern blot analysis was done. HindIII-digested DNAs from plgR−/− and plgR+/− mice hybridized to the 3′ probe provided 10-kb and 8.7-kb bands, respectively, and both 10-kb and 8.7-kb bands were detected in DNA from plgR−/− mice. KpnI-digested DNAs from plgR−/− and plgR+/− mice hybridized to neo probe gave 8.4-kb band, but DNA from plgR−/− mice did not show any band when hybridized to neo probe (Fig. 1B). From crosses between heterozygous mice, plgR−/+, plgR−/−, and plgR+/− mice were born at the expected ratio (23%, 49%, and 28%, respectively) and nearly the same number of male and female plgR−/− mice were born (44% and 56%, respectively).
tissues of pIgR from pIgR transcribed from the mutated pIgR gene. Total RNAs were extracted from pIgR 1/2 mice. mRNA hybridized to pIgR cDNA was also detected at the size of 3.9 kb in the livers, small intestines, and large intestines of pIgR 2/2 mice. pIgR mRNA with the size of 3.9 kb was expressed in the livers, though the intensity of pIgR signal was 5- to 10-fold less in pIgR 1/2 mice than in pIgR 2/2 mice. After agarose gel electrophoresis, RNA was hybridized to pIgR or β-actin cDNAs.

FIGURE 1. Targeting of pIgR gene and genomic Southern analysis of mutant mice. A. Construct of targeting vector, which carries diphtheria toxin A (DT-A) gene at 3’-end (top). Structure of wild-type pIgR gene (middle). Structure of disrupted pIgR gene. Probes used for hybridization are shown in the figure (bottom). The predicted DNA fragments hybridized to these probes were presented under the double-arrowed lines. M, MscI; H, HindIII; K, KpnI. B. Genomic Southern blots of tail DNAs of a littermate from a cross between pIgR 1/2 and pIgR 2/2 mice. After polyacrylamide gel electrophoresis, the membrane was blotted with anti-mouse SC IgG and normal rabbit IgG (data not shown), we ascertained as a doublet of 80 kDa and 68 kDa in the intestinal secretion of pIgR 2/+ and pIgR 2/- mice (Fig. 3). Because a band at 52 kDa in the intestinal secretions was detected by both rabbit anti-mouse SC IgG and normal rabbit IgG (data not shown), we regarded the band as nonspecific staining. It is noteworthy that the amount of pIgR or SC proteins in pIgR 2/- mice was consistently less than that in pIgR 2/+ mice.

Expression of truncated pIgR mRNA in pIgR 2/- mice

We checked by Northern blot analysis whether aberrant mRNA is transcribed from the mutated pIgR gene. Total RNAs were extracted from spleens, livers, small intestines, and large intestines from pIgR 2/+, pIgR 2/-, and pIgR 2/- mice, separated by agarose gel electrophoresis, and hybridized to pIgR or β-actin cDNAs. pIgR mRNA with the size of 3.9 kb was expressed in the livers, small intestines, and large intestines of pIgR 2/+ and pIgR 2/- mice. mRNA hybridized to pIgR cDNA was also detected at the size of ~3.9 kb in the liver and intestines of pIgR 2/- mice, although the intensity of pIgR signal was 5- to 10-fold less in pIgR 2/- mice than pIgR 2/+ mice. In contrast, RNAs in the spleens did not contain pIgR mRNA irrespective of mouse genotypes. On the other hand, β-actin mRNA was detected in the above tissues of pIgR 2/+, pIgR 2/-, and pIgR 2/- mice to the same extent (Fig. 2). These results demonstrate that replacement of exon 2 of pIgR protein with the neo gene results in either decreased transcription or stability of pIgR mRNA, as a small amount of truncated pIgR mRNA is present in pIgR 2/- mice.

pIgR and SC proteins are undetectable in pIgR 2/- mice

We examined by Western blot analysis whether pIgR protein is absent in pIgR 2/- mice. pIgR protein with a molecular mass of 120 kDa and 100 kDa was detected in the extracts of intestinal epithelial cells (IEC) of pIgR 2/+ and pIgR 2/- mice, but the extract of IEC from pIgR 2/- mice did not contain pIgR protein at the detectable level. Moreover, SC protein was detected as a single band at 94 kDa in the bile samples from pIgR 2/+ and pIgR 2/- mice, but SC protein was absent in the bile of pIgR 2/- mice. Likewise, SC protein was absent in the bile of pIgR 2/- mice. However, circulating pIgR of pIgR 2/- mice was consistently less than that in pIgR 2/+ mice.

By immunohistochemical analysis with rabbit anti-mouse SC IgG, we tried to investigate the expression of pIgR protein in IEC. Although pIgR protein was readily detected on the apical surface and in the cytoplasm of IEC in IEC in jejunum and colon of pIgR 2/+ and pIgR 2/- mice, anti-mouse SC Ab did not stain IEC of pIgR 2/- mice, demonstrating again that pIgR protein is undetectable in pIgR 2/- mice. In contrast, IgA-producing cells existed in the intestinal lamina propria of pIgR 2/- mice as well as pIgR 2/+ and pIgR 2/- mice. Furthermore, staining with anti-IgA Ab of the jejunum showed that there is a drastic accumulation of IgA in the lamina propria of pIgR 2/- mice (Fig. 4). These results suggest that inactivation of synthesis of pIgR protein does not interrupt the development of IgA-producing cells and support that decreased or absent expression of pIgR would result in the accumulation of IgA in the lamina propria.

Preferential accumulation of IgA in serum and reduction of IgA in bile and intestinal secretion of pIgR 2/- mice

The above results show that pIgR 2/- mice do not express pIgR protein in the intestinal and hepatic tissues. Therefore, if pIgR-mediated transcytosis is the predominant pathway for the transport of dIgA, it is expected that dIgA produced by plasma cells in the intestinal lamina propria or present in serum is not secreted and circulates in the blood. This is consistent with our previous results (Fig. 2). Because IgA is synthesised in the intestinal lamina propria, we anticipated that pIgR 2/- mice would have a reduced IgA content in serum and bile. In fact, the IgA content of pIgR 2/- mice was consistently less than that in pIgR 2/+ mice.

SC protein was absent in the bile of pIgR 2/- mice. After polyacrylamide gel electrophoresis, the membrane was blotted with anti-mouse SC IgG Ab. Ordinate, molecular masses of marker proteins (in kDa).
measured Ig concentration in the sera of pIgR+/+, pIgR+-, and pIgR-- mice.

Concentration of serum IgA was 40,370 μg/ml in pIgR+/+ mice and much higher in pIgR-- mice (4360 ± 560 μg/ml at an age of 9–10 wk; 6360 ± 2830 μg/ml at an age of 19 wk). Serum IgA level of pIgR-- mice was the intermediate between those of age-matched pIgR+/+ and pIgR-- mice. In contrast, serum IgM level was comparable irrespective of genotypes. The amount of serum IgG was almost comparable among pIgR+/+, pIgR+-, and pIgR-- mice, although serum IgG in pIgR-- mice at an age of 19 wk was slightly more than age-matched pIgR+/+ mice. Likewise, serum IgE levels of pIgR+/+, pIgR+-, and pIgR-- mice were not significantly different, but the averaged value of serum IgE level in older pIgR-- mice (19-wk-old) was slightly higher than that of age-matched pIgR+/+ mice (Fig. 5).

The range of biliary IgA covered 50–1,000 μg/ml in pIgR+/+ mice. By contrast, the concentration of biliary IgA was much lower in pIgR-- mice (20–80 μg/ml). Although IgA was detected in the feces of pIgR+/+ and pIgR-- mice in the range of 40–170 μg/g, a lower level of IgA was detected in the feces of pIgR-- mice (<10 μg/g). In addition, intestinal contents were collected from small and large intestines of each mouse and their Ig level was determined. IgA concentration of intestinal contents of pIgR-- mice was markedly lower than that of pIgR+/+ mice, whereas IgG level in small intestinal contents of pIgR-- mice was significantly higher than that of pIgR+/+ mice and IgM levels were comparable as with serum contents.

FIGURE 4. Immunohistochemical analysis of intestines. Jejunum and colon were removed from a littermate of a cross between pIgR+/+ mice. Each section was stained by anti-mouse SC IgG or anti-mouse IgA Abs.
were comparable among pIgR<sup>1</sup>/1, pIgR<sup>1</sup>/2, and pIgR<sup>2</sup>/2 mice (Fig. 6).

Because a significant amount of IgA was detected in the exocrine fluids of pIgR<sup>2</sup>/2 mice, we made certain that our ELISA system could specifically detect IgA. To address this issue, we measured IgA concentration of sera, bile, fecal extract, and intestinal contents of RAG-2<sup>2</sup>/2 mice (20), and found that IgA was not detected in RAG-2<sup>2</sup>/2 mice (data not shown). Therefore, it is considered that a low but significant level of IgA is present in the bile and intestinal contents of pIgR<sup>2</sup>/2 mice.

**Accumulation of dimeric IgA in the blood circulation of pIgR<sup>2</sup>/2 mice**

The above results demonstrate that hepatic and intestinal transcytosis of IgA is severely blocked in pIgR<sup>2</sup>/2 mice. As a majority of IgA in the intestinal lamina propria is dIgA, Western blot analysis of serum IgA was conducted under nonreducing condition to see the origin of serum IgA in pIgR<sup>2</sup>/2 mice. Comparing that monomeric IgA (mIgA; 130 kDa) and dIgA (280 kDa) were comparably detected in the serum of pIgR<sup>1</sup>/1 mice, the serum of pIgR<sup>2</sup>/2 mice contained a huge amount of dIgA (280 kDa) and an additional IgA molecule with a molecular mass of 350 kDa. Interestingly, the amount of dIgA in the serum of pIgR<sup>1</sup>/2 mice was between those of pIgR<sup>1</sup>/1 and pIgR<sup>2</sup>/2 mice.

Under reducing condition, IgA heavy chains with a molecular mass of #77 kDa were detected in all the serum samples from pIgR<sup>1</sup>/1, pIgR<sup>1</sup>/2, and pIgR<sup>2</sup>/2 mice. The amount and composition of serum proteins in pIgR<sup>1</sup>/1, pIgR<sup>1</sup>/2, and pIgR<sup>2</sup>/2 mice were almost comparable when polyacrylamide gel was stained with Coomassie brilliant blue (data not shown). On the other hand, mIgA (130 kDa), dIgA (280 kDa), and sIgA (350 kDa) were readily detected in the bile of pIgR<sup>1</sup>/1 and pIgR<sup>2</sup>/2 mice, but the bile of pIgR<sup>2</sup>/2 mice included only a small amount of IgA. Again, the amount of biliary dIgA in pIgR<sup>2</sup>/2 mice was the intermediate between those of pIgR<sup>1</sup>/1 and pIgR<sup>2</sup>/2 mice (Fig. 7).

**Discussion**

In this paper, we described the generation of mice lacking exon 2 of plgR gene and found that dlgA accumulates in the serum of...
plgR−/− mice due to the interruption of plgR-mediated transepithelial transport. Our results support the earlier evidence that plgR plays a critical role in transepithelial transport of dIgA. Both in vitro and in vivo studies showed that plgR selectively transports dIgA across the epithelial layer (12–15). These results demonstrate the significance of plgR in the transcytosis of dIgA, but the relative importance of plgR for the transcytosis of dIgA has not been evaluated. Our investigation about plgR−/− mice clearly presented that plgR-mediated transcytosis is the main pathway in hepatic and intestinal transport of dIgA in mice.

In contrast to the remarkable reduction of sIgA in plgR−/− mice, IgM levels in sera and external secretion were not different between plgR−/− and plgR+/− mice. Together with the previous finding that SC binds to IgA but does poorly to IgM in rat (21), our results suggest that plgR-mediated transcytosis may not be critical for the secretion of IgM.

Northern blot analysis of RNA from the intestines revealed the expression of mRNA of ~3.9 kb hybridized to plgR cDNA in plgR+/− mice. As our targeting strategy was aimed at delezing exon 2 of plgR gene, exon 1 and the region from exons 3 to 11 remain intact in plgR−/− mice. Using RT-PCR analysis, the truncated plgR mRNA including exon 1 and the downstream from exon 3 but excluding exon 2 was detected in plgR−/− mice (data not shown). Although the size of this truncated plgR mRNA was estimated to be 3764 bp, it cannot be separated from the intact plgR mRNA (3863 bp) in the agarose gel electrophoresis. Expression of aberrant mRNA in gene-knockout mice has been already noted, although those aberrant mRNAs did not encode functional proteins (22, 23). In plgR−/− mice, the truncated plgR mRNA may be translated into plgR protein lacking the exon 2-encoded peptide. If such an aberrant plgR protein is produced in plgR−/− mice, the molecule is thought to lack the signal peptide and start at the initiation codon, Met 45 (6). However, we could not detect plgR protein in plgR−/− mice by Western blot and immunohistochemical analyses. Nevertheless, the bile and intestinal secretion of plgR−/− mice contained a significant amount of IgA. These results support that other pathways than plgR-mediated transcytosis such as asialoglycoprotein receptor-mediated endocytosis (16), CD89 (Fcc receptor)-mediated binding (24), or intercellular diffusion may work for the transport of dIgA. However, we cannot exclude the possibility that the amount of an aberrant plgR protein produced in plgR−/− mice is too small to be detected by Western blot and immunohistochemical analyses but sufficient for the transport of dIgA.

plgR−/− mice exhibited conspicuously high IgA level in serum accompanied with the significant reduction of biliary and fecal IgA (17), plgR−/− mice also showed a 40-fold higher IgA level in the serum than plgR+/− mice, and biliary and fecal IgA were much reduced in plgR−/− mice compared with plgR+/− mice. These results are consistent with the earlier observation that hepatic transport of dIgA is mediated by covalent binding of J-chain and plgR. However, IgA levels in intestinal surfaces obtained using absorbent wicks were comparable between plgR−/− and plgR+/− mice (18), whereas intestinal contents of plgR−/− mice contained much fewer amount of IgA than plgR+/− mice. This discrepancy may be explained by the differences of methodology. Another explanation is that J-chain-containing dIgA is mainly transported by plgR but J-chain-deficient IgA may be secreted in a plgR-independent pathway in the intestinal epithelia. Because the molecular form of IgA in the serum and milk of J−/− mice is mainly monomer, it is likely that different pathways in the intestine transport J-chain-deficient mlgA and J-chain-containing dIgA.

It is known that the expression of hepatic and intestinal plgR is strictly regulated during the ontogeny in rat (25, 26). These findings demonstrate that the production of plgR protein in hepatocytes and IEC gradually increases after birth. We analyzed plgR expression in mice from gestational day 14 to adult age and found that plgR mRNA and protein are already expressed in the intestine at gestational day 18 when IgA-producing cells do not colonize the intestinal mucosa yet (data not shown). This result suggests that the production of plgR protein in IEC precedes the development of intestinal IgA-producing cells in mice and dIgA does not induce the expression of plgR. On the other hand, as the amount of dIgA in the intestinal contents was roughly correlated with those of plgR protein in IEC of plgR+/+, plgR−/−, and plgR−/+ mice, it is likely that the amount of plgR expressed in IEC may determine the amount of dIgA transcytosed across the epithelial layer.

IgA nephropathy (IgAN) is caused by the massive deposition of Ag-IgA immune complex to glomerular mesangium. The concentration of serum IgA of patients with IgAN is frequently higher than age-matched normal controls (27), and some of the patients have abnormally few IgA in the intestinal secretion (28). On the basis of these findings, primary abnormalities of IgAN may be ascribed to the impaired plgR-mediated transport of IgA. This hypothesis is supported by the fact that some patients with IgAN have deficiency in the production of J-chain (28). plgR−/− mice have increased level of IgA in the serum due to the interruption of transepithelial transport of dIgA. It has been already reported that HIGA mice established from breeding of dY mice show high serum IgA level (~1000 µg/ml) and IgA deposition in the mesangial area of a glomerulus at an age of 25 wk (29). Therefore, it is of great importance to see whether plgR−/− mice suffer from IgAN in association with aging.

Physiological significance of slgA is now been reevaluated. IgA−/− mice can protect against vaginal infection of herpes simplex virus-type 2 and intranasal infection of influenza virus when previously immunized by those viral vaccines (30, 31). These results suggest that slgA may not be necessary for Ag-induced immunity in the mucosal tissues. On the other hand, Kaetzel et al. (32) have shown using plgR CDNA-transfected Madin-Darby canine kidney cells that the immune complex of dIgA and Ag is transported via plgR-mediated pathway. This result proposes that one of the important roles for plgR is to eliminate dIgA complexed with the exogenous or self Ags outside the body. Furthermore, IgA-Ab is able to neutralize virus intracellularly, giving IgA a role in host protection that has been reserved for cell-mediated immunity (33, 34).

From this viewpoint, plgR−/− mice should be a valuable animal model for analysis of Ag specificity and physiological function of slgA.

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