Evaluation of a receptor gene responsible for maternal blood IgY transfer into egg yolks using bursectomized IgY-depleted chickens

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ABSTRACT In avian species, maternal immunoglobulin Y (**IgY**) is transferred from the blood to the yolks of maturing oocytes; however, the mechanism underlying this transfer is unknown. To gain insight into the mechanisms of maternal IgY transfer into egg yolks, IgY-depleted chickens were generated by removing the bursa of Fabricius (bursectomy) during egg incubation, and their egg production and IgY transport ability into egg yolks were determined. After hatching, blood IgY concentrations of the bursectomized chickens decreased gradually until sexual maturity, whereas those of IgA remained low from an early stage of growth (from at least 2 wk of age). Chickens identified as depleted in IgY through screening of blood IgY and IgA concentrations were raised to sexual maturity. At 20 wk of age, both blood and egg yolk IgY concentrations in the IgY-depleted group were 600-fold lower than those of the control group, whereas egg production did not differ between the groups. Intravenously injected, digoxigenin-labeled IgY uptake into the egg yolk was approximately 2-fold higher in the IgY-depleted chickens than in the controls, suggesting that IgY depletion may enhance IgY uptake in maturing oocytes. DNA microarray analysis of the germinal disc, including the oocyte nucleus, revealed that the expression levels of 73 genes were upregulated more than 1.5-fold in the IgY-depleted group, although we could not identify a convincing candidate gene for the IgY receptor. In conclusion, we successfully raised IgY-depleted chickens presenting a marked reduction in egg yolk IgY. The enhanced uptake of injected IgY into the egg yolks of the IgY-depleted chickens supports the existence of a selective IgY transport mechanism in maturing oocytes and ovarian follicles in avian species.

Key words: bursectomy, IgY depletion, maternal IgY transfer, egg yolk, chicken

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INTRODUCTION

Immunoglobulin Y (**IgY**), the functional equivalent of mammalian immunoglobulin G, is present in avian egg yolks and plays a crucial role in the protection of newly hatched chicks against infectious pathogens (Kowalczyk, et al., 1985). The process of avian maternal IgY transfer comprises 2 steps, the first being the transfer from circulating maternal blood to the yolks of maturing oocytes in ovarian follicles, whereas the second involves IgY transfer from the egg yolks to the embryonic circulation through the yolk sac membrane (Linden and Roth, 1978; Tressler and Roth, 1987). Whereas the second step relies on the IgY Fc receptor, FcRY (West, et al., 2004), the relevant receptor involved in IgY transport is unknown. In our previous study, we observed that an Fc domain of IgY was essential for effective IgY transport into egg yolks (Kitaguchi, et al., 2008). A study using quail IgY Fc and chicken IgY Fc mutants showed that a single substitution, namely that of the Tyr^{363} residue located on the Fc domain to an Ala^{363}, greatly impairs IgY transport into egg yolks. In addition, the absence of an *N*-glycosylated carbohydrate chain at Asn^{407} on the Fc domain also reduces IgY transport into egg yolks (Murai, et al., 2013; Takimoto, et al., 2013). These results support the existence of a specific IgY receptor mediating blood IgY uptake into egg yolks.

The absence of endogenous IgY is a suitable model to gain insight into how IgY is incorporated into egg yolks. Although in mammals the bone marrow is the source of B-dependent lymphocytes for immunoglobulin

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production, immunoglobulin gene rearrangement for B lymphocyte production in birds takes place in the bursa of Fabricius, located between the cloaca and sacrum (Glick, et al., 1956; Scott, 2004). Surgical removal of the bursa of Fabricius before and after hatching, called bursectomy, depletes B cells in peripheral lymphoid organs and practically abolishes IgY production (Cooper, et al., 1969). Yasuda et al. (1998) succeeded in obtaining sexually mature, IgY-depleted chickens after performing bursectomy on day 18 of incubation, and these IgY-depleted chickens produced eggs with very low IgY levels. The IgY concentration in the egg yolks of IgY-depleted chickens was 2 μg/mL of yolk, which is 1,000-fold lower than that in IgY-producing chickens. Because IgY-depleted chickens are viable, evaluation of blood IgY transport ability into egg yolks can provide a clue as to how the IgY receptor contributes to maternal IgY transfer. However, the characteristics of egg production and ovarian IgY transport ability in sexually mature, IgY-depleted chickens are unknown.

In the present study, we generated IgY-depleted chickens by surgical bursectomy at days 17 to 18 of incubation and characterized egg production performance and exogenously injected IgY uptake into egg yolks to gain insight into the mechanisms of maternal IgY transfer. We further assessed which genes were upregulated in oocyte nuclei of IgY-depleted chickens by DNA microarray to determine the candidate IgY receptor that contributes to maternal IgY transfer.

MATERIALS AND METHODS

Chickens

Fertilized White Leghorn-type commercial chicken eggs were purchased from a local supplier (Julia Light; Japan Layer, Gifu, Japan). Animal care complied with the applicable guidelines of the Nagoya University Policy on Animal Care and Use (approval nos: 2015022605, 2016022605, and 2017030220).

Surgical Bursectomy

The fertilized eggs were incubated at 37°C with a relative humidity of 60 to 70%, with turning once per h, until day 17 of incubation. On day 17 to 18 of incubation, surgical bursectomy was performed according to the method of Yasuda et al. (1998). Briefly, eggs were sterilized with tincture of iodine before drilling. A section of the eggshell (1 × 2 cm^2) was cut out with a dental drill without damaging the shell membrane and underlying chorioallantoic membrane. The eggshell was removed, and 3 sides of the shell membrane square were cut off to close the egg after treatment. The chorioallantoic and amniotic membranes were cut to allow gripping the tail of the embryo with forceps. Bursectomy was carried out basically according to the method described in Aitken and Penhale (1986). After treatment, the removed shell was placed in the same position and sealed with surgical tape, following which the eggs were returned to the incubator until hatching. The control eggs were continuously incubated without any operation.

Experimental Design

Experiment 1 After allowing 24 h for hatching, female chicks were moved to battery cages and housed there until 3 wk of age. The birds were provided with free access to water and a commercial starter diet. The photoperiod was continuous lighting. At 3 wk of age, the birds were housed in cages placed in a room under controlled temperature (25 ± 2°C) and with a 16 h:8 h light-dark photoperiod with lights on at 08:00. The diets were changed to grower pullet ration, finisher pullet ration, and layer ration at 3, 10, and 15 wk of age, respectively.

Body weight measurement and blood sample collection were performed periodically (2, 4, 6, 8, 12, 16, and 20 wk of age; $n = 5$). The blood samples were collected *via* the wing vein and centrifuged at 16,000 × g for 4 min at 4°C. The supernatant was collected and stored as the serum sample for measurement of IgY and IgA concentrations.

Experiment 2 Seventeen bursectomized chickens were raised, and their blood IgY and IgA concentrations were measured at 8 wk of age for selection of IgY-depleted chickens (<10 μg/mL for IgY and <1 μg/mL for IgA). After 20 wk of age, hen-day egg production (%) of both the control and IgY-depleted groups ($n = 5$) were measured for 25 D. At 21 wk of age, blood, and eggs were collected and their IgY concentrations were measured as described below. For the injection study, chicken IgY (Rockland, Limerick, PA) was labeled with digoxigenin (**DIG**) (Roche Diagnostics, Indianapolis, IN) according to the manufacturer's recommendations. At 25 wk of age and within several h of oviposition, each bird was injected intravenously with 100 μg of DIG-labeled IgY. Laid eggs were collected for 7 D after injection and stored at 4°C until analysis. In general, concentration of IgY in the egg yolks is essentially constant throughout the entire maturation of the oocyte, from the small (0.05 g) to the largest (20 g) oocyte (Kowalczyk et al., 1985). Importantly, IgY transfer into egg yolks maximize at F3 ovarian follicle (3-4 D before laying) because of its maximum growth at hierarchical stage. Therefore, we decided to collect eggs for 2 to 7 D after the injection (equivalent to F1-F6 ovarian follicles at the timing of injection). Yolk extract of IgY was prepared as described in Takimoto et al. (2013), which is a modified version of the water dilution method in Akita and Nakai (1993).

Experiment 3 Control and IgY-depleted chickens ($n = 3$) were prepared as described in Experiment 2. At 25 wk of age, the germinal disc region, including the oocyte nucleus, was collected from F5–F7 yellow ovarian follicles. The collected samples (9 germinal discs of 3 birds) were preserved in RNAlater (Thermo Fisher Scientific, Waltham, MA) for DNA microarray analysis.

Quantitation of Chicken IgY, IgA, and DIG-Labeled IgY by ELISA

Serum and yolk IgY and IgA concentrations were determined using chicken IgG and IgA ELISA Quantitation kits (Bethyl Laboratories, Montgomery, TX). The concentrations of DIG-labeled IgY in the egg yolk extracts were quantified using an original ELISA (Bae, et al., 2009).

DNA Microarray Analysis of Oocyte Germinal Discs (Experiment 3)

Total RNA was isolated from pooled F5–F7 germinal discs using an RNeasy Micro kit (Qiagen, Hilden, Germany). Total RNA from 3 birds per treatment was pooled for each chip. Whole transcripts from germinal discs were measured using a GeneChip Chicken Genome Array (Affymetrix). Raw data were normalized using the MAS5 algorithm in Affymetrix GeneChip Operating Software ver. 1.4. The microarray data have been deposited in the NCBI Gene Expression Omnibus (GSE134668).

Data Analysis

Mean values for body weight, egg production, and DIG-labeled chicken IgY uptake were compared by the Student's *t*-test. All error bars represent the standard error of the mean, and differences between means were considered significant at $P < 0.05$. Statistical analyses were performed in the Excel Statistics package.

RESULTS

Experiment 1: Body Weight and Blood IgY and IgA Concentrations

Body weight did not differ significantly between the IgY-depleted and control groups during the 20 wk after birth (Figure 1; $P > 0.05$). Blood IgY and IgA concentrations were both sequentially measured to evaluate the success of bursectomy. There was no difference in blood IgY concentrations between the control and the IgY-depleted group at 2 wk of age (Figure 2A), but differences were observed after 4 wk of age. The blood IgY concentration in the control group increased gradually until sexual maturation, peaking at 16 wk of age, followed by a decrease that was likely because of egg production. In contrast, the blood IgY concentration in the IgY-depleted group declined continuously until sexual maturation. A difference in the blood IgA concentration between the control and the IgY-depleted groups could already be observed at 2 wk of age, and the lowered blood IgA levels in the IgY-depleted group was maintained in all ages (Figure 2B). Newly hatched chicks normally retain maternally derived IgY until their acquired immunity is developed. In contrast, relatively little maternally derived IgA is transferred to the next generation. Measuring the blood IgA concentration to evaluate the success of bursectomy soon after hatching proved to be useful.

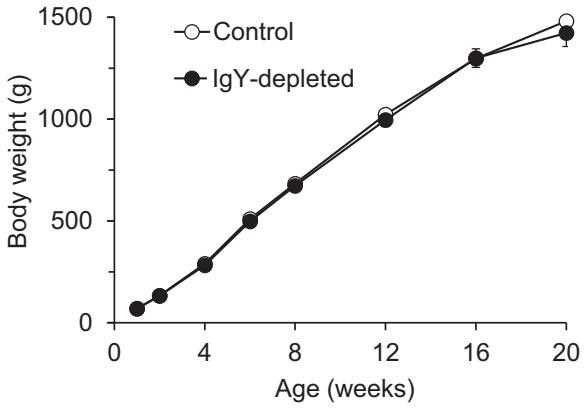

Figure 1. Time course of change in the body weight of control and IgY-depleted chickens. Bars indicate the mean ± SEM of 5 chickens. IgY, immunoglobulin Y.

Experiment 2: Egg Production and Blood and Egg Yolk IgY Concentrations

Egg production at 20 wk of age in both groups was high and did not differ between the 2 groups (Table 1; $P > 0.05$). The blood IgY concentration in the

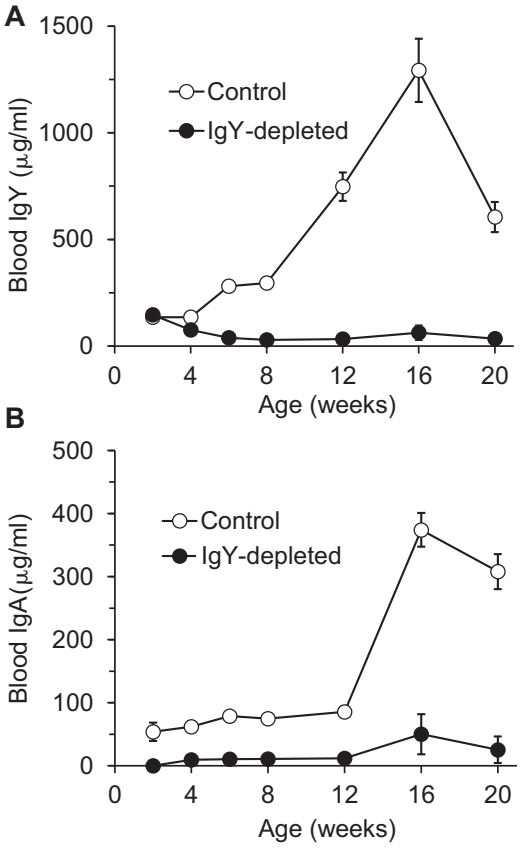

Figure 2. Time course of changes in blood IgY (A) and IgA (B) concentrations of control and IgY-depleted chickens. The IgY and IgA concentrations were measured by ELISA. Bars indicate the mean ± SEM of 5 chickens. IgY, immunoglobulin Y.

Table 1. Hen-day egg production in control and IgY-depleted chickens.

Treatment	Hen-day egg production (%)
Control	93.3 ± 3.39
IgY-depleted	95.6 ± 2.16

IgY, immunoglobulin Y.
Daily egg production was measured for 25 D.
Values are expressed as the mean ± SEM of 5 chickens.

IgY-depleted group (1.69 ± 1.7 μg/mL; undetectable in three birds) was 600-fold lower than that in the control group (959 ± 102 μg/mL) (Figure 3A). The egg yolk IgY concentration in the IgY-depleted group (3.78 ± 2.3 μg/mL; undetectable in one bird) was also lower in the IgY-depleted group than in the control group (712 ± 56 μg/mL), similar to that observed for the blood IgY concentration (Figure 3B).

Uptake of Injected IgY Into the Egg Yolk

Following injection, IgY uptake into egg yolks was undetectable 1 D after injection (data not shown) but peaked at 3 and 4 D after injection in both treatments (Figure 4). The uptake of injected IgY into egg yolks was significantly higher in the IgY-depleted group than in the control group at 2 to 7 D (2, 6, and 7 D at $P < 0.01$; 3, 4, and 5 D at $P < 0.05$). The total uptake of injected IgY into the egg yolk for 7 D was 1.8-fold higher in the IgY-depleted group compared with that of the control (425 ng/g of yolk in the control vs. 242 ng/g of yolk in the IgY-depleted group; $P < 0.05$). These results raised a possibility that IgY-depleted chickens might

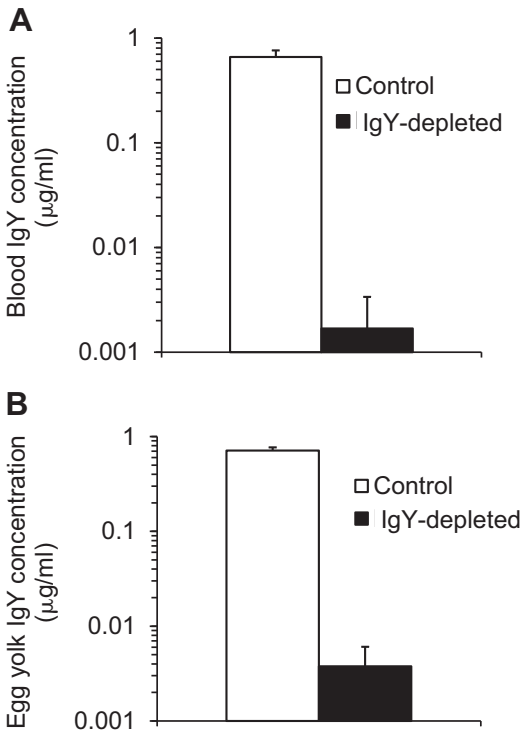

Figure 3. Concentrations of blood IgY (A) and egg yolk IgY (B) in laying control and IgY-depleted chickens at 21 wk of age. Bars indicate the mean ± SEM of 5 chickens. IgY, immunoglobulin Y.

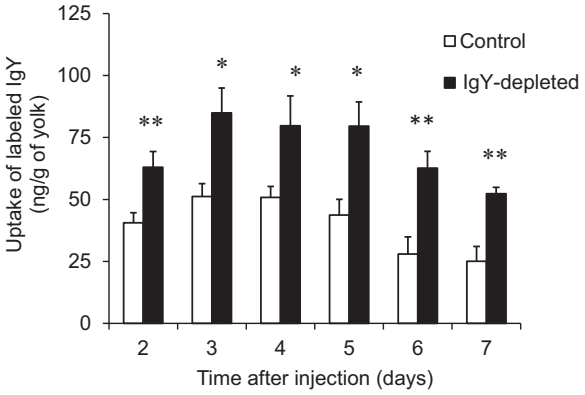

Figure 4. Egg yolk uptake of intravenously injected IgY in control and IgY-depleted chickens at 22 wk of age. DIG-labeled IgY was injected at 100 μg/bird into the wing vein. Laid eggs were collected daily until day 7 after injection, and IgY uptake into the yolks was measured by ELISA. Bars indicate the mean ± SEM of 5 chickens. Significantly different from the control at $^*P < 0.05$, $^{**}P < 0.01$. IgY, immunoglobulin Y.

enhance IgY uptake by upregulation of an as yet unidentified IgY receptor. Therefore, we performed microarray analysis to screen genes upregulated in maturing oocytes of IgY-depleted chickens to identify the putative IgY receptor responsible for maternal blood IgY transfer.

Experiment 3: Microarray Analysis for Screening of a Candidate IgY Receptor

The GeneChip Chicken Genome Array was used for high-throughput screening of genes upregulated in the oocytes of IgY-depleted chickens. The germinal discs of F5–F7 yellow follicles of 3 birds were pooled, and their total RNA was used for microarray analysis. The sample of germinal discs used here included both germinal disc and adherent granulosa cells, because they were not separated. In advance, we had confirmed that our germinal disc samples expressed oocyte nucleus marker gene (*DAZL* and *WEE2*; Elis et al., 2008) at levels 100-fold to 300-fold higher and expressed granulosa cell marker gene (*ZPC*) at 6-fold lower than granulosa cells alone by real-time PCR (data not shown). These results suggest that germinal disc sample analyzed here were rich in mRNAs originated from oocyte, but included mRNAs originated from granulosa cells.

The array comprises 37,703 probe sets representing 32,773 genes. Among the control and IgY-depleted groups, 278 genes from 331 probe sets were differentially expressed with a significant detection call at $P < 0.002$ (Table 2). A total of 73 genes from 92 probe sets were upregulated > 1.5-fold (data not shown), with 19 genes presenting > 2-fold upregulation in the IgY-depleted group (Table 3). Among the 73 genes, 3 coded for membrane receptors, namely, *PROCR*, *ADRA2C*, and *IL1RL1*. We also focused on already known IgY receptors, PLA2R1 (FcRY), IHSF1 (ggFcR), and very low-density lipoprotein receptor (**VLDLR**) (LR8), a major endocytotic receptor expressed in the oocyte plasma membrane. No change was observed except for the

Table 2. The number of probes and genes with higher expression in the germinal discs of IgY-depleted chickens relative to that of control chickens.

Category	IgY-depleted/control intensity	Number of probes	Number of genes[1]
-	GeneChip Chicken Genome Array	37,703	32,773
1	Significant detection call at $P < 0.05$ in both control and IgY-depleted chickens	18,566	Not counted
2	Significant change call (IgY-depleted > control) at $P < 0.002$ within category 1	331	278 (30)
3	IgY-depleted/control intensity >1.5 within category 2	92	73 (13)
4	IgY-depleted/Control intensity >2.0 within category 3	21	19 (2)

IgY, immunoglobulin Y.
[1]Number in parentheses denotes the number of genes detected by multiple probes.

PLA2R1 gene, which presented a 1.5-fold higher value in the IgY-depleted group compared with the control group.

DISCUSSION

The current study reaffirmed that removal of bursa of Fabricius depletes both IgY and IgA production (Kincade and Cooper, 1973). The changing patterns of blood IgY and IgA concentrations in the control and IgY-depleted groups are in good agreement with characteristics of maternal antibody transfer in newly hatched chicks. Maternal IgA is not transferred into the blood circulation of fetus and hatched chicks (Rose et al., 1974; Rose and Orlans, 1981). In addition, plasma IgA concentration starts to increase within a week after hatching (Hamal et al., 2006). Thus, a difference in the blood IgA concentrations at 2 wk of age between the control and the IgY-depleted groups (Figure 1B) is quite convincing. Concerning about blood IgY, there was no difference in blood IgY concentrations between the 2 groups at 2 wk of age, but differences became wider after 4 wk of age (Figure 1A). These observations are also in agreement with the report of Hamal et al. (2006); the blood IgY concentration of newly hatched chicks decreased up to 2 wk of age because of catabolism of maternal IgY in chicks, and then, it started to increase by 3 wk of age because of appearance of IgY synthesized in chicks.

In this study, we successfully raised bursectomized IgY-depleted chickens producing markedly lowered IgY levels in egg yolks. We observed that blood and egg yolk IgY concentrations in the IgY-depleted chickens were 600-fold less compared with those of the control chickens. Yasuda et al. (1998) successfully developed IgY-depleted hens producing eggs free of IgY, but the efficiency of egg production was not reported. The present

Table 3. Nineteen genes upregulated >2-fold in IgY-depleted chickens relative to control chickens.

Gene symbol	Gene name	Fold change	Entrez gene
Upregulated			
ELN	Elastin (supravalvular aortic stenosis, Williams-Beuren syndrome)	3.03	396,441
PROCR	Protein C receptor, endothelial	3.03	424,867
CEP63	Centrosomal protein 63	2.83	424,873
LOC423138	Inner centromere protein-like	2.64	423,138
KLHL7	Kelch like family member 7 (Drosophila)	2.46	420,612
BROX	BRO1 domain and CAAX motif containing	2.46	421,334
ALDH1A3	Aldehyde dehydrogenase 1 family member A3	2.46	395,389
ADRA2C	Adrenoceptor alpha 2C	2.30	428,799
FAM129 A (*NIBAN1*)	Family with sequence similarity 129, member A	2.14	424,451
BMP6	Bone morphogenetic protein 6	2.14	420,868
LARP4B	La ribonucleoprotein domain family member 4B	2.14	420,457
LOC771537	Uncharacterized LOC771537	2.14	771,537
FGF13	Fibroblast growth factor 13	2.14	414,831
PAK6	p21 (RAC1) activated kinase 6	2.14	428,837
NR2C1	Nuclear receptor subfamily 2 group C member 1	2.14	373,913
C6ORF57 (*SDHAF4*)	Chromosome 3 open reading frame, human C6orf57	2	421,870
RAD51	RAD51 recombinase (*Saccharomyces cerevisiae*)	2	396,086
UGCG	UDP-glucose ceramide glucosyltransferase	2	427,335
ZYX	Zyxin	2	418,300
Already-known endocytotic receptor and IgY receptor			
VLDLR (*LR8*)	Very low-density lipoprotein receptor	1	396,154
PLA2R1 (*FcRY*)	Phospholipase A2 receptor 1, 180 kDa	1.52	404,304
IGSF1 (*ggFcR*)	Immunoglobulin superfamily member 1	1.07	419,114

IgY, immunoglobulin Y.
Upregulated (>2-fold) genes between control and IgY-depleted chickens identified by DNA microarray analysis were listed. The already-known endocytotic receptor gene expressing in oocyte and 2 IgY receptor genes were also listed.
Fold change was calculated by the gene expression level in IgY-depleted chickens relative to that in control chickens.

study showed that under conventional conditions, IgY-depleted chickens grow normally and exhibit an egg-producing capacity equivalent to that of the control group, suggesting that IgY depletion does not directly impair the reproductive ability of laying birds.

To characterize the IgY transport capacity of IgY-depleted chickens, we intravenously injected DIG-labeled IgY into the chickens and determined its uptake into the egg yolk. The quantity of DIG-labeled IgY taken up into egg yolks was approximately 2-fold higher in the IgY-depleted chickens than that in the control. Although the precise mechanism of the enhanced uptake of injected IgY into egg yolks of the IgY-depleted chickens is not known, 2 possibilities have been proposed: the reduced levels of endogenous blood IgY may increase expression of IgY receptor in oocytes; or the reduced levels of endogenous blood IgY may be outcompeted by the injected IgY during the transfer process into oocytes. To explore the former possibility, we performed a microarray analysis to determine which genes were upregulated in the oocytes of IgY-depleted chickens.

Among the 73 $>$ 1.5-fold upregulated genes, 3 coded for membrane receptors. PROCR, a cellular receptor for protein C expressed mainly in endothelial cells, is a type 1 transmembrane protein that exhibits sequence and 3-dimensional structural homology with the major histocompatibility class 1/CD1 family (Oganesyan, et al., 2002). PROCR is primarily responsible for protein C activation and plays a crucial role in the protein C anticoagulant pathway (Fukudome and Esmon, 1994). Additionally, PROCR also binds to other ligands such as factor X, factor VIIa, and the γδ T-cell antigen receptor (Mohan Rao, et al., 2014), but no information is available for PROCR binding to immunoglobulins in any animal species. The alpha-2C adrenergic receptor, ADRA2C, is predominantly expressed in the central nervous system and adrenals. As the ADRA2C protein binds to epinephrine (MW 183) and norepinephrine (MW 169). Meanwhile, IL1RL1 (interleukin 1 receptor like 1) is an IL33 receptor belonging to the IL1 cytokine family and is expressed as both a membrane-anchored receptor (called ST2L) activated by IL33 and a soluble variant (called sST2) exhibiting antiinflammatory properties (De la Fuente, et al., 2015). ST2 was considered an orphan receptor for many years, until its association with IL33 was demonstrated (Schmitz, et al., 2005). Together, these findings indicate that PROCR, ADR2AC, or IL1RL1 are unlikely to be the receptor participating in maternal IgY transfer in maturing oocytes.

We also focused on the gene expression of a critical multiligand receptor for yolk precursors, the VLDLR, as well as that of the already known IgY receptors IHSF1 and PLA2R1. Chicken VLDLR (LR8) is a single 95 kDa protein expressed in the oocyte plasma membrane, and mainly binds yolk lipoproteins, very low-density lipoproteins, and vitellogenin (Bujo, et al., 1994). The IHSF1 receptor, called *gg*FcR in the chicken, is an IgY receptor with 4 extracellular Ig domains and transmembrane regions expressed predominantly in blood cells and binds the Fc region of IgY (Schreiner, et al., 2012). However, the expression of these 2 receptors in germinal discs did not differ between the control and IgY-depleted groups (Table 3). Finally, PLA2R1, called FcRY in the chicken, is the chicken counterpart of the mammalian muscle type phospholipase A2 receptor. FcRY is a 180 kDa protein with a single transmembrane region responsible for the transfer of yolk IgY to the embryonic circulation in developing eggs (West et al., 2004). In our previous study, *FcRY* gene expression was detected in the theca layer of the ovarian follicle, although only at low levels in the granulosa cell layer, including the germinal discs (Kitaguchi, et al., 2010). Indeed, the present microarray data showed that FcRY gene expression was relatively low compared with that of the major oocyte receptor, VLDLR, although *FcRY* expression was slightly induced (1.52-fold) by IgY depletion (Table 3). In the present study, therefore, we could not identify a convincing candidate receptor from the microarray analysis. *FcRY* gene expression levels in the theca layer of ovarian follicles in IgY-depleted chickens, as well as the physiological function of FcRY in the thecal layer of the chicken ovary, should be investigated in the future.

As mentioned above, the mechanism responsible for the enhanced uptake of injected IgY into the egg yolks of IgY-depleted chickens is unknown. The latter possibility may be that the reduced levels of endogenous blood IgY are outcompeted by the injected IgY during the transfer process, resulting in enhanced uptake of injected IgY into the egg yolk. In our previous study, we determined the blood IgY concentration and exogenously injected IgY Fc uptake into egg yolks in 6 quail strains. Interestingly, IgY Fc uptake was inversely correlated with endogenous blood IgY levels when all the strain data were pooled (Murai, et al., 2016). The most plausible explanation is that the injected IgY Fc competed with endogenous blood IgY during the transfer process, resulting in reduced IgY Fc uptake into eggs. The enhanced uptake of injected IgY into the egg yolks of IgY-depleted chickens provides indirect evidence of the existence of a specific IgY transfer system, likely receptor-mediated, in avian oocytes.

In conclusion, using IgY-depleted chickens with a normal egg production capacity, we found that IgY depletion increases intravenously injected IgY uptake into the yolks of maturing oocytes. Although we were unable to identify a putative candidate gene for the IgY receptor by microarray analysis, we found that the expression levels of 73 genes were increased $>$ 1.5-fold in response to IgY depletion in the germinal discs of oocytes. Based on the present and recent results, we again propose that an IgY receptor involved in maternal IgY transfer exists in the ovaries of avian species.

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