Maternal Transfer of Immunoglobulins into Egg Yolks of Birds

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In avian species, maternal IgY transferred across egg yolks from mother to offspring plays a key role to protect the hatching chicks against pathogenic attacks. The process of avian maternal IgY transfer is divided into two steps: the first step is the transfer from the maternal circulation to the egg yolks of developing oocytes in the maternal body, and the second step is the transfer from the egg yolks to the embryonic circulation in the developing eggs. The second step relies on IgY-Fc receptor, FcRY, expressing on the yolk sac membrane. However, in the first step, the molecular basis of IgY transfer is still unknown. This review focused on the structural requirements of IgY and heterologous immunoglobulin such as human IgG for transferring into egg yolks of birds, and a plausible molecular mechanism for immunoglobulin uptake in ovarian follicles were discussed. Recent research revealed existence of a selective IgY transfer system that recognizes Cυ3/Cυ4 interface of IgY in ovarian follicles of birds.

Keywords: egg yolk, Fc, IgY, immunoglobulin, maternal immunity, ovarian follicle


Introduction

Maternal immune protective factors transferred across the placenta, colostrum or eggs from mother to offspring play a key role to protect the newborns against pathogenic attacks. This protective action is called as “maternal immunity”, which was first described in mammals and birds more than 100 years ago (Ehrlich, 1892; Klemperer, 1893). It is quite reasonable for newborns being received maternal immune factors, since their acquired immune system is still immature. The maternal immunity has been reported in a broad range of vertebrates such as mammals, birds, reptile and fishes as well as invertebrates such as insects, shrimp and amphioxus (Zhang et al., 2013).

Major component of maternally-derived immune protective factors are immunoglobulins (Igs). In mammals, maternal Igs are transferred to the fetus and neonate through the placenta and breast milk, respectively. In birds, maternal Igs in blood are incorporated into egg yolks of maturing oocytes, and then they are transferred to the embryonic circulation through yolk sac membrane. Of the three Ig classes (IgA, IgM and IgY) in birds, only IgY is actively transferred into the egg yolks, which suggests existence of a selective IgY transport system in maternal ovary. On the other hands, IgY can be utilized as an antigen-specific detection antibody or as a neutralizing antibody for specific pathogen. Many papers have been published on the production of IgY against various bacterial, viral and protein antigens, and the practical application of passive immunization techniques targeting to gastrointestinal infection are in progress. Nevertheless, molecular basis of maternal IgY transfer into egg yolks are still unclear.

This review provides an overview of mode of maternal Ig transfer into egg yolks of birds, especially focusing on the structural requirements of IgY and heterologous human IgG for transferring into egg yolks of birds. The review also proposes a plausible molecular mechanism for Ig uptake into ovarian follicles.

Structure of Ovarian Follicles and Yolk Formation

In ovarian follicles, developing oocyte with yolk is concentrically surrounded by several layers of supporting tissue, from the periphery inwards, (i) the theca layer, which consists of the theca externa (a broad layers of the stratified cells) and the narrower theca interna separated by interstitial cells (ii) an acellular layer commonly designated as basement membrane, (iii) an epithelial layer of granulosa cells and (iv) the perivitelline membrane (Fig. 1). Most of the yolk components are derived from blood plasma yolk precursors that are mainly synthesized in the liver. The thecal layers of ovarian follicles are very well vascularized with permeable capillaries that allow the blood to leak out into the surrounding tissues. Next, the yolk precursors have to pass the basement membrane which plays as a filter preventing passage of
larger plasma components. The yolk precursors then pass through gaps between the granulosa cells and the perivitel- 
line membrane. Finally, they reach to the oocyte plasma
membrane, and then endocytosed into the developing oocyte.

The major yolk precursors are the lipoproteins such as
very low density lipoprotein (VLDL) and protein rich-lipo-
protein including vitellogenin (VTG), and they are incorpo-
rated into the yolks by receptor-mediated endocytosis. The
receptor-mediated mechanisms involved in yolk formation
have been described in detail by Schneider (2009). In the
final 7-day period of rapid growth of the ovarian follicles,
oocyte internalizes about 5 g of lipid and protein, reaching a
diameter of nearly 35 mm until it is expelled from the ovarian
follicle by ovulation. The receptor that interacts with LDL
and VTG is called as “LR8”. The LR8 is a 95 kDa molecule
and expresses in the granulosa cells (Bujo et al., 1994). It is
hypothesized that the synthesized LR8 are embedded in the
perivitelline and/or oocyte plasma membranes which inter-
acts with a specific region of apoprotein molecule and VTG.
LR8 belongs to the LDL receptor gene family and is known to
be a multi-binding receptor. It also interacts with ribo-
flavin-binding protein (Mac Lachlan et al., 1994) and com-
plement component C3 (Recheis et al., 2005). Uptake of
yolk precursors into egg yolk is a dramatic example of the
receptor-mediated endocytosis pathway. This process plays
a crucial role in egg formation.

Structure of Avian IgY

There are three classes of Igs in birds, IgY, IgM and IgA. Functionally, IgY is generated mainly in secondary antibody
responses and behaves like mammalian IgG despite some
differences between IgY and mammalian IgG in biochemical
properties (Hatta et al., 1993; Davalos-Pantoja et al., 2000).
IgY exists as a monomeric form in serum, and its concent-
 ration is the highest among three classes of Igs. Similar to
mammalian IgG, avian IgY is made up of light and heavy
chains bridged by disulfide bonds (Fig. 2). Structurally,
there are some differences between IgY and mammalian IgG.
The heavy chain of IgY has an additional constant domain
instead of the hinge region of mammalian IgG. Thus, the
molecular mass of IgY (180 kDa) is rather heavier than that
of mammalian IgG (150 kDa), and IgY resembles to mam-
malian IgE composed of four constant domains. Molecular
analysis indicates that IgY is the evolutionary progenitor of
both mammalian IgG and IgE (Warr et al., 1995). Compari-
on of the constant region sequences of avian IgY and mam-
malian IgG shows that Cυ3 and Cυ4 domains of avian IgY
are most closely related to Cγ2 and Cγ3 domains of mam-
malian IgG (Magor et al., 1994; Parvari et al., 1988). An-
other structural difference between avian IgY and mammali-
an IgG is N-glycosylation pattern of IgY and IgG. Avian
IgY contains two potential N-glycosylation sites located on
the Cυ2 and Cυ3 domains, and one of them located on the
Cυ2 domain (N308) is absent in mammalian IgG. The other
is located in the Cγ3 domain (N407), which corresponds to
the Cγ2 domain of mammalian IgG (N297). Mass spectro-
metry of chicken IgY glycopeptides revealed that chicken
Co3 domain contained only high-mannose-type oligosac-
charides, whereas chicken Co2 domain contained only com-
plex-type N-glycans (Suzuki and Lee, 2004). It has been
indicated that the N-glycosylation pattern of avian IgY is
more analogous to that in mammalian IgE than IgG.

General Mechanism of Maternal Ig Transfer
in Mammals and Birds

In mammals, IgG has long been known to be the only class
of antibody that is actively transferred from mother to off-
spring to confer passive immunity. This specific transport of
IgG is carried out by the neonatal Fc receptor, FcRn, com-
prised of a heterodimer of β2-microglobulin and a 45- to 53-
kDa protein, suggesting that the heavy chain might be a
MHC class I homolog (Simister and Rees, 1985; Simister
and Mostov, 1989). In human, FcRn expresses on the sur-
f ace of the syncytiotrophoblast of placenta, and it transfers
IgG from the mother to the fetus across the placenta. FcRn
also expresses on the cell-surface brush border of enterocytes
in the proximal small intestine of neonates, and it transfers
IgG of the mother’s milk to the neonates. This receptor also
has a unique ability that modulates the half-life of IgG and
albumin. More recently, several publications have shown that
FcRn plays major roles in antigen-IgG immune-com-
p lex phagocytosis by neutrophils, and also in antigen pre-
sentation of IgG immune complexes by professional antigen
presenting cells (Mi et al., 2008; Qiao et al., 2008; Baker
et al., 2011; Liu et al., 2011).

The birds have a unique IgY transfer system from the ma-
ternal blood circulation to embryonic circulation. The pro-
cess of avian maternal IgY transfer appears to be divided into
two steps: the first step is the transfer from the maternal
circulation to the egg yolks of developing oocytes in the
maternal body, and the second step is the transfer from the
egg yolks to the embryonic circulation through the yolk sac
membrane at the late embryonic stages in the developing
eggs (Kowalczyk et al., 1985). The second step relies on
IgY-Fc receptor called FeRY, a functionally homolog of
mammalian FcRn (West et al., 2004). Unexpectedly, FeRY
is not homology with MHC class I molecules like FcRn, but
is instead a homolog of the mammalian phospholipase A2
receptor, a member of the mannose receptor family (East and
Isaacke, 2002). FeRY plays a critical role in IgY homeostasis
as like mammalian FcRn (Tesar et al., 2008). FeRY is ex-
pressed in multiple tissues including ovary, but FeRY does
not seem to be involved IgY transport into egg yolk (Ward,
2004; West et al., 2004). This speculation is based on the
observation of human IgG uptake into avian egg yolks. The
human IgG can be transported into egg yolk of chickens
(Mohammed et al., 1998; Morrison et al., 2001), although
human IgG does not interact with FeRY. Therefore, it has
been suggested that a distinct receptor is involved in the IgY
transport from the blood into egg yolk.

Concentration of IgY in Egg Yolks

Determination of IgY concentration in yolk and blood pro-
vides us insight into the existence of selective IgY transport
Fig. 1. **Cross-section diagram of an ovarian follicle of birds.** Yolk, designated as oocyte cytoplasm, is concentrically surrounded by follicular tissue layers. Yolk components are incorporated from capillaries embedded in theca layer into oocyte cytoplasm by passing through gaps between the follicular tissues.

Fig. 2. **The domain structure of chicken IgY and IgY-Fc.** Paired inter-heavy chain disulfide bonds are indicated with dotted lines, based on the analysis of Suzuki and Lee (2004) (left panel). The IgY heavy chain contains one variable domain (VH) and four constant domains (Co1, Co2, Co3 and Co4). Variable and constant domains in the IgY light chain are termed VL and CL, respectively. The crystal structure of chicken IgY-Fc (PDB entry 2W59) was drawn using Cn3D 4.3 (right panel). LYT1362-364 and HEAL550-553 motif are located on the Co3/Co4 interface. N407 residue links with N-glycosylated carbohydrate chain.
transported into the egg yolks, probably by a selective IgY results raised the possibility that blood IgY is actively concentrated to some extent in egg yolks of chickens. These plasma regardless of strain, suggesting that blood IgY is plasma fraction were 1.7 fold higher than those of blood concentration. Importantly, the IgY concentrations of yolk varied from 1 to 25 mg/g yolk (Patterson et al., 1962; Cutting and Roth, 1973; Rose et al., 1974; Kowalczyk et al., 1985; Li et al., 1998; Carlander et al., 2001; Hamal et al., 2006). It seems likely that the scattering of the yolk IgY concentration data is caused by multiple reasons including differences in strains of chickens (Gross and Siegel, 1990) and daily fluctuation (Carlander et al., 2001). We determined yolk IgY concentration in three strains of chickens (two commercial layers, Dekalb and Nagoya, and one inbred strain, PNP/DO; Mizutani, 2002), taking into consideration the IgY recovery rate in yolk extract (Kitaguchi et al., 2008a). PNP/DO showed the highest concentration of IgY expressed as mg/g yolk (12.2), and Dekalb and Nagoya had almost the similar concentration of IgY (Dekalb, 6.2; Nagoya, 5.7), and strain difference of yolk IgY concentration has proven to be mainly attributable to a variation of plasma IgY concentration. Importantly, the IgY concentrations of yolk plasma fraction were 1.7 fold higher than those of blood plasma regardless of strain, suggesting that blood IgY is concentrated to some extent in egg yolks of chickens. These results raised the possibility that blood IgY is actively transported into the egg yolks, probably by a selective IgY transport system in avian ovarian follicles.

**Structural Requirements of Ig Transfer into Egg Yolks**

1. Three Classes of Avian Igs and Heterologous Igs

Discrimination of transport ability among homologous and heterologous Igs is important to clarify the molecular basis of selective IgY transport system in ovarian follicles of birds. However, the Ig structure required for selective transport has been confusing.

IgY is efficiently incorporated into ovarian follicles, but other classes, IgA and IgM, are much less abundant in the egg yolks. Kitaguchi et al. (2008b) described that why IgA and IgM cannot be efficiently transported into the egg yolk. Three chicken Igs (IgY, mixture of monomeric and polymeric IgAs, pentameric IgM) and two human IgAs (monomeric IgA and polymeric IgA) were intravenously injected into laying quail, and their uptakes into egg yolks were determined. Interestingly, all monomeric Igs (chicken IgY, monomeric chicken IgA, monomeric human IgA) were transported into egg yolks. On the other hand, polymeric Igs (polymeric chicken IgA, polymeric human IgA, pentameric chicken IgM) were less- or untransportable into egg yolks. These results suggest that the retention of the monomeric form contributes to the efficient transport of Igs into ovarian follicles. Possible explanations of lower uptake of polymeric Igs are that their greater physical size caused by polymerization may result in low infiltration across ovarian follicular layers. The rank order of transport ability among intravenously-injected Igs is summarized in Table 1. Although monomeric Igs are preferentially transported into egg yolks, it is difficult to compare their transport ability when experimental conditions are not unified. Our study has shown that 22% of intravenously-injected chicken IgY was incorporated into the egg yolks of quail (Kitaguchi et al., 2008b). It has been reported that human IgG and human IgA were well transported into the egg yolks of chickens, though there was no transport of mouse IgG2b (Mohammed et al., 1998; Morrison et al., 2001). We questioned whether heterologous human IgG and human IgA can be transported into egg yolks with a similar potential of IgY, and then we compared transport efficiency of them under the same experimental condition using quail. The results showed that chicken IgY uptake was approximately 3 to 5-fold higher than those of the human IgG and human IgA (Kitaguchi et al., 2008b; unpublished data). Since amino acid sequence homology on constant domain was only 30% among them (chicken IgY vs human IgG vs human IgA), the gap of transport efficiency is quite reasonable. These results imply the presence of a system facilitating the IgY transport into ovarian follicles; in other words, presence of a system capable of discriminating monomeric Igs. We have also compared the uptake of quail IgY and chicken IgY into egg yolks of quail. Unexpectedly, the uptake of quail IgY was only one-third of the uptake of chicken IgY, implying that heterologous chicken IgY is far preferable as a ligand for transport into egg yolks of quail compared to homologous quail IgY (Bae et al., 2009). The examination of tissue distribution and blood clearances showed that higher deposition of quail IgY in various body tissues might lead to lower uptake of quail IgY into ovarian follicles.

To further identify IgY domains important for the enhanced transport into ovarian follicles, the uptakes of enzyme-digested chicken IgY fragments were measured. The injection of chicken IgY fragments Fc, Fab and F(ab')2 resulted in the largest uptake of Fc fragment, with the same level as that of intact IgY (Kitaguchi et al., 2008b). This result is consistent with the observation in ducks that IgY ($Delta$Fc), truncated forms equivalent to F(ab')2, is less effectively incorporated than full-length form of IgY (Liu and Higgins, 1990). These results suggest the presence of a selective IgY transport system recognizing Fc region in avian ovarian follicles.

2. Studies using Recombinant Ig and Site-directed Mutagenesis Technique

By utilizing recombinant human IgG and site-directed mutagenesis technique, Morrison et al. (2001) have identified several regions within the IgG molecule important for its uptake into the egg yolks of chicken. Their data has demonstrated that Fc region of IgG and hinge region but not the Fc-associated carbohydrate chain is required for IgG transport. They also reported that the amino acid residues located on the $C\gamma2/C\gamma3$ interface contribute to IgG transport into the egg yolks. A simultaneous mutation of residues 252–254 (from MIS to GGG) located on the C'y2 domain abolished IgG uptake into the egg yolks. We further expanded target amino acid residues for alanine and glycine-scanning mutagenesis on 16 amino acid residues located along the $C\gamma2/C\gamma3$ interface (Bae et al., 2010b). Wild-type human IgG1-Fc
(WT) and its mutants were synthesized, and their uptakes into the egg yolks of quail were determined. The triple mutation of MIS252-254 to GGG resulted in a 40% decrease in Fc uptake in comparison to that of the WT, which is partly consistent with the Morrison’s result. Furthermore, quartet substitution of HEAL429-432 to GGGG located in an exposed loop at the \( \gamma_3 \) domain completely abolished Fc uptake into the egg yolks. Next, the residues HEAL429-432 were individually substituted with either alanine or glycine. Regardless of the glycine and alanine substitution, single mutations of H (429), E (430) and L (432) significantly reduced Fc uptake compared with WT uptake. These results suggest that the MIS252-254 and HEAL429-432 located on the \( \gamma_2/C_\gamma_3 \) interface are important for human IgG transport into the egg yolks.

Recombinant IgY-Fc is a useful tool to identify the critical amino acid residues for IgY transport and to isolate the specific IgY-Fc receptor. However, a current proposed model for IgY inter-chain disulfide bonds is that C252 pairs with C340 for X-shaped linking, and C347 pairs with another C347 (Suzuki and Lee, 2004; see Fig. 2). The characteristic features of \( \nu \) heavy chains make it difficult to synthesize functional recombinant chicken IgY-Fc. Taylor et al. (2008) first synthesized a small-sized IgY-Fc including a part of the \( \nu_2 \) domain and the full-length \( \nu_3 \) and \( \nu_4 \) domains (designated Fc\( \nu_3-4 \)). The Fc\( \nu_3-4 \) includes two cysteine residues, C340 and C347, but the protein formed the additional disulfide bonds between two cysteine residues. Then, they mutated “C340” to serine, which led to the expression of functional protein (Taylor et al., 2008, 2009, 2010). Pürzel et al. (2009) also synthesized a large-sized IgY-Fc including the full-length \( \nu_2 \), \( \nu_3 \) and \( \nu_4 \) domains (designated IgY-Fc\( \nu_2-4 \)). They mutated “C347” to the serine of IgY-Fc\( \nu_2-4 \), and this recombinant also retained high affinity binding to the chicken leukocyte IgY-Fc receptor, CHIIR-AB1. By introducing this methodology, we have succeeded in producing two recombinant chicken IgY-Fc(s) with different sizes, IgY-Fc\( \nu_2-4 \) (C347S) and IgY-Fc\( \nu_3-4 \) (C340S), retaining a high transport ability into the egg yolks of quail (Bae et al., 2010a). Interestingly, the transport ability of the IgY-Fc\( \nu_3-4 \) into the egg yolks corresponded closely with that of the IgY-Fc\( \nu_2-4 \), suggesting that the presence of \( \nu_3 \) and \( \nu_4 \) domains but not \( \nu_2 \) domain is important to maintain the transport ability of IgY.

By utilizing recombinant chicken IgY-Fc\( \nu_3-4 \) and site-directed mutagenesis technique, we investigated critical amino acid residues of IgY required for egg yolk transport (Murai et al., 2013). Among the 17 amino acid residues

<table>
<thead>
<tr>
<th>Class of Ig</th>
<th>Ig species</th>
<th>Molecular form or mutation</th>
<th>Uptake (% of control)</th>
<th>Remarks</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>Native Igs</td>
<td></td>
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<td></td>
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<tr>
<td>IgY</td>
<td>chicken</td>
<td>monomeric</td>
<td>100</td>
<td>Control of native Igs. The 22% of the injected IgY was transported.</td>
<td>Kitaguchi et al. (2008b)</td>
</tr>
<tr>
<td>quail</td>
<td>monomeric</td>
<td>31</td>
<td></td>
<td></td>
<td>Bae et al. (2009)</td>
</tr>
<tr>
<td>IgG</td>
<td>human</td>
<td>monomeric</td>
<td>20-25</td>
<td></td>
<td>unpublished data</td>
</tr>
<tr>
<td>IgA</td>
<td>chicken</td>
<td>monomeric &amp; polymeric</td>
<td>30</td>
<td>No uptake of polymeric IgA.</td>
<td>Kitaguchi et al. (2008b)</td>
</tr>
<tr>
<td>human</td>
<td>monomeric</td>
<td>40</td>
<td></td>
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<td>human</td>
<td>dimeric</td>
<td>5</td>
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<td>IgM</td>
<td>chicken</td>
<td>pentameric</td>
<td>undetectable</td>
<td></td>
<td>Kitaguchi et al. (2008b)</td>
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<td>Recombinant Igs</td>
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<tr>
<td>IgY-Fc(\nu_3-4)</td>
<td>chicken</td>
<td>Y363 to A</td>
<td>undetectable</td>
<td>vs WT IgY-Fc(\nu_3-4)</td>
<td>Murai et al. (2013)</td>
</tr>
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<td></td>
<td></td>
<td>Y363 to F or W</td>
<td>80</td>
<td></td>
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<td></td>
<td></td>
<td>N407 to A (deletion of carbohydrate chain)</td>
<td>12</td>
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<td></td>
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<tr>
<td>IgY-Fc(\nu_2-4)</td>
<td>chicken</td>
<td>WT</td>
<td>100</td>
<td>vs. WT IgY-Fc(\nu_3-4)</td>
<td>Bae et al. (2010a)</td>
</tr>
<tr>
<td>IgG</td>
<td>human</td>
<td>MIS252-254 to GGG</td>
<td>undetectable</td>
<td>vs. native IgG</td>
<td>Morrison et al. (2001)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N297 to A (deletion of carbohydrate chain)</td>
<td>85</td>
<td></td>
<td></td>
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<tr>
<td>IgG1-Fc</td>
<td>human</td>
<td>MIS252-254 to GGG</td>
<td>60</td>
<td>vs. WT IgG1-Fc</td>
<td>Bae et al. (2010b)</td>
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</table>
located on the C\(_\gamma3/C\gamma4\) interface, the LY1362-364 motif at the C\(_\gamma3\) domain is required for efficient IgY-Fc transport from blood to the egg yolks. In particular, our results highlight the importance of the Y363 as a critical amino acid residue exerting potent effects on the transport ability of IgY-Fc, because the substitution of the Y363 to alanine seriously damaged the IgY-Fc transport into egg yolks. In addition, aromatic amino acid residues are indispensable at Y363 residue for maintaining the transport ability of IgY-Fc. The most plausible explanation is that the aromatic side chain at Y363 residue directly or indirectly contributes to an interaction with an unidentified IgY receptor for egg yolk transport. The structure of the IgY-Fc fragment consisting of C\(_\gamma3\) and C\(_\gamma4\) domains reveals a remarkable conservation with the previously determined human IgG-Fc fragment (Taylor et al., 2009), despite the relatively low level of amino acid identity (about 30\%) between chicken IgY-Fc and the human IgG-Fc (Parvari et al., 1988). The LY1 motif at the C\(_\gamma3\) domain is conserved as an LMI motif at the C\(_\gamma2\) domain of human IgG. Several conserved amino acids located at the C\(_\gamma2/C\gamma3\) interface were identified that play a central role in the interaction of FcRn with IgGs (Ghetie and Ward, 2000). Especially, I253 of human IgG, aligned with I364 of chicken IgY, is a key player in the interaction with FcRn (Kim et al., 1994; Raghavan et al., 1995; Medesan et al., 1996; Medesan et al., 1997). These findings suggest that a critical domain required for maternal Ig transfer is evolutionarily conserved between avian species and mammalian species.

The deglycosylation of the N-linked carbohydrate chain by substituting N407 at the C\(_\gamma3\) domain with alanine also caused a marked reduction of IgY-Fc uptake (Murai et al., 2013). Deglycosylation of mammalian IgG by enzymatic cleavage or site-directed mutagenesis also weakened IgG binding with Fc\(_\gamma\R\) (Tao and Morrison, 1989; Lund et al., 2000; Radnitz and Sun, 2001; Shields et al., 2001), which is partly due to conformational changes in the C\(_\gamma2\) domain. Thus, it is plausible that deglycosylation of IgY reduces the interaction with the unidentified IgY receptor responsible for egg yolk transport.

3. Relevance of IgY-Fc Receptor on Ig Uptake into Egg Yolks

The fundamental question of our study is which receptor or carrier protein is involved in IgY transport into avian egg yolks. The microscopic detection of the recombinant IgY-Fc and its Y363A mutant in ovarian follicles showed that the WT IgY-Fc was concentrically accumulated in yolk granules, whereas the Y363A mutant was hardly accumulated in yolk granules. Nevertheless, the Y363A mutant had infiltrated into the granulosa cell layer, suggesting that a major hurdle disturbing the infiltration of the Y363A mutant lies on the inside of the granulosa cell layer such as the perivitelline layer and/or oocyte plasma membrane (Murai et al., 2013). Our previous study using whole-mount sections of quail ovarian follicles also showed that potential IgY binding substances interacting with the Fc domain are present around the perivitelline layer (Kitaguchi et al., 2010). These results strongly support the classical idea that a receptor involved in maternal IgY transfer exists in the perivitelline layer and/or oocyte plasma membrane.

Three receptors that interact with chicken IgY have been described to date. First, Fc\(_\gamma\R\), a receptor referred to as a yolk sac IgY receptor, is present and is functionally equivalent to FcRn. As mentioned above, Fc\(_\gamma\R\) is responsible for the transfer of IgY to chick embryos, and it interacts with the C\(_\gamma3/C\gamma4\) interface (He et al., 2011). However, human IgG does not bind to Fc\(_\gamma\R\) (West et al., 2004), even though human IgG is transported into egg yolks. Taken together, the relevant results suggest that Fc\(_\gamma\R\) is not involved in the transfer of IgY from maternal circulation into chicken egg yolk. A second receptor, ggFcR, highly expresses peripheral blood mononuclear cells and selectively binds to chicken IgY but not to chicken IgM or chicken IgA (Viertlboeck et al., 2009). A recent study showed that ggFcR mainly interacts with the C\(_\gamma2\) domain of IgY (Schreiner et al., 2012). However, the C\(_\gamma2\) domain is not essential to maintain IgY-Fc transport capability into egg yolks (Bae et al., 2010a), suggesting that the likelihood of ggFcR involvement in IgY transport is low. A third receptor, CHIR-AB1, is a member of the leukocyte receptor family (Viertlboeck et al., 2005, 2007). A mutational analysis identified critical IgY residues interacting with CHIR-AB1; a total of four mutations (Y362A, I363A, H550A and R556A) completely abolished IgY binding to CHIR-AB1 (Pürzel et al., 2009). The mutated residues of chicken IgY, Y362, I363, H550 and R556, are located at the C\(_\gamma3/C\gamma4\) interface. Thus, the critical IgY residues interacting with CHIR-AB1 overlap partly with the residues that are critical for IgY transport into the egg yolk. The CHIR family was massively expanded recently, with over 100 CHIR genes, and microchromosome 31 as a genomic location of the CHIR is not included in the chicken genome assembly (Lochner et al., 2010; Viertlboeck et al., 2010). Therefore, the functions of the CHIR family remain largely obscure, with the exception of CHIR-AB1. Although the potential relevance of CHIR family members on IgY transport into egg yolks is unknown, the isolation of IgY receptor from the inner layer of ovarian follicles and a thorough binding study of isolated IgY receptor are necessary to identify a true receptor for maternal IgY transfer in future.

Conclusion

Recent research clearly revealed existence of a selective Ig transfer system in ovarian follicles of birds. This Ig transport system recognizes amino acid residues located on C\(_\gamma3/C\gamma4\) interface of IgY. Although the precise molecular mechanism of IgY transport into ovarian follicles is currently unknown, our data suggest existence of receptor-mediated endocytosis on the most inner layer of ovarian follicles. The identification of a key factor (maybe an IgY-Fc receptor) discriminating amino acid residues located on C\(_\gamma3/C\gamma4\) interface will be essential to clarify “the big picture” of maternal IgY transfer in avian species. In addition, our mutational data raise possibility that IgY transport ability into egg yolks can be modified by substitution of amino acid residues located on
the Co3/Co4 interface. It might be possible to develop transgenic birds producing novel IgY mutants or therapeutic humanized Igs that are highly transportable to avian egg yolks, which could provide a new strategy for antibody production employing eggs.

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