# Review
# Transcellular transport of immunoglobulins

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***Abstract*** :

Recent studies on perinatal transmission of immunoglobulins are reviewed with special reference to the cellular mechanism of IgG transcytosis. Histochemical and cytochemical studies have establised the localization of IgG-specific Fc receptors on the cell surface of the intestinal epithelium or on the endosomal membrane of the syncytiotrophoblast or yolk sac epithelium. A widely accepted scenario of IgG transcytosis is this : Fc receptor specifically binds to IgG molecule to form a complex at pH6 on the cell surface or in the endosome, and the complex moves towards the basolateral cell surface to release the intact IgG molecule into the fetal circulation. Recent in vitro studies revealed a family of GTP-binding proteins and vesicle-membrane fusion proteins involved in the regulation of sorting and transport of IgG, or in the fusion event of IgG-carrying endosomes.

***Key words*** : IgG, Fc receptor, rab protein, endosome, transcytosis

It is well known that babies raised up with mothers breast milk are protected from infectious diseases. The breast milk contains leukocytes, immunoglobulins, interferons, growth factors and fatty acids. They all work in immune defense of the new born baby. Among them, antibodies transmitted from the mother provide the baby with a potential of immune response specifically against the antigens to which the mother was recently exposed. IgA, the most abundant antibody in the milk, stays within the baby's intestine and works in the immune defense as the secretory IgA does. IgG, the less abundant antibody in the milk, are absorbed from the baby's intestine. IgG is also transmitted from the mother via the placental barrier to the baby's circulation. These antibodies provide the baby with the passive immunity, and they protect him from various infectious pathogens through the neonatal period until his own immune system developes. Thus, the perinatal transmission of immunoglobulins is one of the attractive and challenging issue for Cell Biologists. However, the cellular and molecular mechanism of transcellular transport of immunoglobulins are little understood. This short report reviews recent studies associated with this issue.

## Perinatal absorption of immunoglobulins

In the mammaly gland, dimeric IgA (dIgA) bound to polymeric immunoliobulin receptor (pIgR) on the basal cell surface is internalized via the coated vesicle. The vesicle fuse with the basolateral endosomes, from which another vesicle is formed to transfer dIgA to the apical cell surface via the apical endosome. In the intestine, human placenta, or in the rodents yolk sac, IgG is taken up into the coated vesicle from the luminal surface of the intestinal epithelium, syncytiotrophoblast, or the yolk sac epithelium, respectively. Afterwards, IgG is basolaterally transported and released into the circulation of the fetus or the new born baby (Fig. 1).

(a) In the small intestine

IgG in the breast milk is not digested in the stomach of the new born baby, and reaches to the small intestine intact. IgG molecules bind to the IgG-Fc receptor (FcR) expressed on the epithelial cell surface[1,2,3]. The lumen of the small intestine of the new born baby is weakly acidic around

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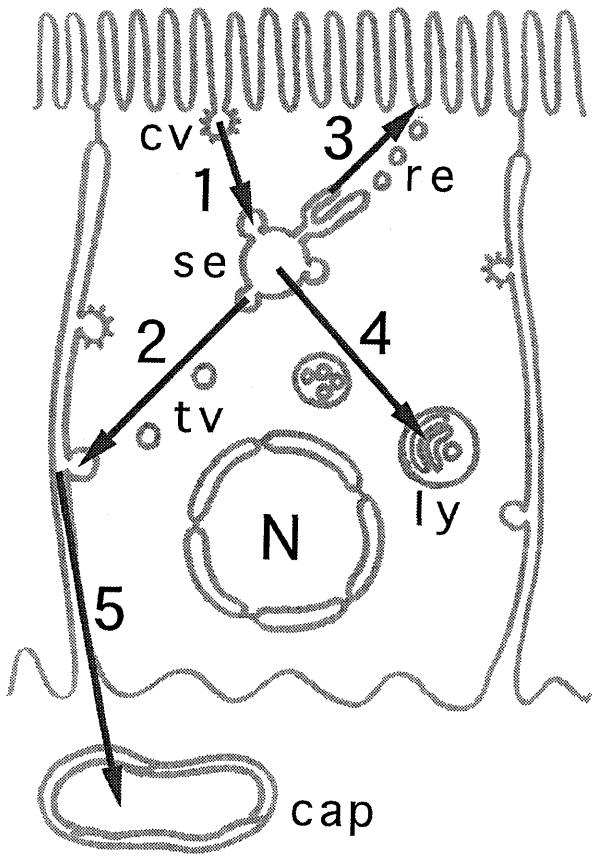


***Fig. 1.*** Schematic diagram of intracellular transport pathway in the absorptive epithelial cell. IgG molecules are internalized into the cell via the coated vesicle (cv), and moved to the preexisting sorting endosome (se) (pathway 1). IgG-Fc receptor comlex is formed in the sorting endosome, sorted into transfer vesicles (tv), and is forwarded to the basolateral membrane to release IgG extracellularly (pathway 2). Transferrin is recycled back to the apical cell surface through the sorting endosome and the recycling endosome (re) (pathway 3). Low density lipoprotein is forwarded to lysosome (ly) after sorting (pathway 4). The extracellularly released IgG molecules pass through the intercellular space, and enter into the fetal circulation (pathway 5). cap: fetal blood capillary, N: nucleus.

pH6.0, which is optimal for the binding of IgG to FcR[4]. The IgG-FcR complex formed on the cell surface is then internalized into the endosome by receptor-mediated endocytosis via the coated vesicle. The complex is sorted into a new vesicle, and basolaterally transported to fuse with the basolateral membrane. Following the membrane fusion, the IgG-FcR complex is exposed to the basolateral interstitial fluid. This weakly alkaline environment forces FcR to release IgG. FcR is thought to be recycled back to the apical membrane for reuse.

(b) In the yolk sac

In rodents around 15 emblyonic day, yolk sac epithelium comes to be exposed to the uteline lumen, and the receptor-mediated endocytosis of maternal IgG takes place thereafter. Specific Fc receptor has not been identified on the luminal surface of the yolk sac epithelial cell. Cell surface binding and the following endocytosis of IgG is mediated by a non-specific surface receptor. Roberts et al. showed the presence of immunoreactivity of FcR from neonates intestine (FcRn) in the endosomal vesicles and coated vesicles associated with apical and basolateral membrane[4]. FcRn is expressed also on the preexisting endosome in the apical cytoplasm. Thus, IgG-FcR complex is formed in the endosome, sorted into transfer vesicles and forwarded to the basolateral membrane. The vesicles are then fused with the basolateral membrane and release IgG extracellularly.

(c) In the human placenta

Human placental syncytiotrophoblast forms a cellular barrier between maternal and fetal circulation. This cellular barrier allows the passage of maternal IgG except other classes of immunoglobulins[5,6,7]. Bright et al. showed that the FcγRIII subtype of leukocyte Fc receptor FcγR localized to the apical cytoplasm of the syncytiotrophoblast of the term placenta[8,9,10]. This suggests an involvement of FcγRIII in the endocytic uptake of IgG. FcγRII-B2 found on the macrophage has a sequence of 47 amino acid residues in the cytoplasmic domain. This type of

FcγRII, when expressed in the Madine Darby canine kidney (MDCK) cell, is transported from the apical to basolateral membrane[11]. FcγRII-B1 found on the lymphocyte lack the 47 amino acid residues and is not transcytosed when expressed in the MDCK cell. This sequence of amino acid residues may involve the signal of sorting FcγRII-B2-containing endosomes.

Major histocompatibility complex (MHC)-I-related human Fc receptor (hFcRn) is expressed in the human placental syncytiotrophoblast[12]. This receptor binds to IgG at pH6.0 and releases it at pH7.5. Thus, IgG-hFcRn complex is formed in the weakly acidic environment of endosomes of the syncytiotrophoblast. Localization of hFcRn in the cytoplasmic granules was also confirmed by other authors[13]. FcRn is composed of two subunits; a small subunit identical to β2-microglobulin and a large subunit sharing 50% homology with MHC-I[14,15]. Hence, the large subunit is involved in quite different functions of the receptor and antigen.

(d) Brambell Hypothesis

The epidermal growth factor (EGF) is internalized by receptor-mediated endocytosis, passes through the sorting endosome, and the ligand-receptor complex is forwarded to the lysosome for degradation. Low density lipoprotein is targeted to the lysosome after uncoupling the ligand-receptor complex in the sorting endosome. The receptor is recycled back to the apical membrane. Tf is also recycled back to the apical membrane as the ligand-receptor complex after liberating ferric ions into the cytosol through the endosomal membrane[16]. Interestingly, pathway of IgG transcytosis was once thought to be almost identical to the endocytic pathway of EGF except for the final step of transporting IgG to the basolateral membrane. Brambell hypothesized that the ligand-receptor complex of IgG is protected from the acid hydrolase in the lysosome until IgG is released extracellularly[17,18]. In the placental syncytiotrophoblast and the rodents epithelial cell of the yolk sac, the Fc-receptors are exclusively localized in the apical endosome of which internal pH is around 6.0 (see below). Within this mildly acidic environment, IgG specifically binds to the Fc receptor and is sorted out to move to the basolateral membrane.

## GTP-binding proteins on the transcytotic pathway

It is now known that a family of GTP-binding rab proteins is involved in the regulation of vesicle transport in endocytosis, transcytosis and recycling[19]. Rab3A is involved in the regulation of transport of synaptic vesicles[20,21,22], rab4 and rab5 in endocytosis[23,24,225,26], and rab8 in vesicle transport in the secretory pathway from the Golgi apparatus. Rab17 and rab18 is expressed exclusively in the epithelial cell[28,29,30], suggesting their involvement in the regulation of epithelial cell-specific transcytosis.

(a) Transport pathway of IgG and Tf

Epithelial cells of the rat yolk sac after 15 days of gestation absorb maternal IgG and Tf from the uterine fluid. This simple epithelial tissue can be maintained in the culture medium for 3 to 6 hours. To see the functional expression of rab proteins in this tissue, we examined the localization of rab4 and rab5A on the endocytic pathway of IgG and Tf[31,32]. To visualize the endocytic pathway of IgG and Tf, the yolk sac epithelial cells were briefly labeled with biotinylated IgG and Tf, and incubated in the culture medium. The tracers were then detected by fluorescent avidin and fluorescent antibody to Tf. Within 5 min of endocytosis, IgG and Tf appeared together in apical endosomes. These apical endosomes are assumed to be the site where the ligand-receptor sorting takes place. Within 15 min after the endocytosis, IgG and Tf are sorted out from the endosomes, and IgG- or Tf-carrying vesicles are formed. IgG-carrying vesicles are forwarded basolaterally, while Tf-carrying vesicles are recycled back to the apical membrane.

(b) Brefeldin-A's effect in IgG transcytosis

Brefeldin-A is known as the antibiotics which interferes the coated vesicle formation by inactivating the adenosine ribosylation factor. Non-coated vesicles and tubules are formed from the

endosomal membrane, and the targeted vesicular transport is interrupted[33,34,35]. Even in the presence of this drug, IgG transport is essentially unaltered, while Tf recycling is dramatically changed. After sorting in the endosome, IgG-carrying vesicles normally move to the basolatereal cytoplasm, but Tf-recycling vesicles are piled up in the apical cytoplasm. This discrepancy means that IgG transport vesicles are formed from the endosomal membrane by an unknown mechanism independent of clathrin or other coat proteins.

(c) Localization of rab4 and rab5A

To visualize the localization of rab4 , rab5A, and rab8 relative to the distribution of IgG and Tf, the rab proteins were immunocytochemically examined after the endocytic labeling of the transport pathway of IgG or Tf in the presence of Brefeldin-A. Biotinylated IgG, Tf, and/or rab proteins were visualized by BODIPY-conjugated avidin, anti-Tf antibody and/or anti-rab antibodies combined with Texas Red-conjugated 2nd antibodies, respectively. The tracers were within the endosomes or vesicles and were detected by avidin or anti-Tf antibody. Since the activated GTP-bound form of rab proteins remain associated with the endosomal or vesicular membranes, the antibodies detect this form of rab proteins. The GDP-bound form of rab proteins are dissociated from the membrane and washed out of the cytoplasm during the tissue preparation. Thus, the presence of IgG, Tf and the GTP-bound form of rab proteins on the same endosomes or the vesicles indicates their coexistence on the same structures in the cell.

In the presence of Brefeldin-A, IgG-containing vesicles appeared basolaterally, while rab4-associated vesicles piled up apically. Coexisting IgG and rab4 were not abundant. In the presence of Brefeldin-A, rab5 coexisted with IgG in the apical endosomes, but not appeared in the apical zone where rab4-associated vesicles accumulated. This partial distribution of rab4 and rab5 suggested that rab4 and rab5 are involved in the fusion of sorting endosomes and vesicles, while rab4 rather than rab5 plays a similar role in the recycling pathway. Rab8 was always isolated from the IgG-containing compartment. This separation means that rab8 is not involved in the membrane fusion events along the pathway of IgG transcytosis.

## Intraendosomal pH

Brambell hypothesis assumued that IgG is protected from degradative enzymes by forming a stable complex with FcR. The optimal pH for IgG to form a stable complex with FcR in vitro has been shown to be around 6.0[4,36]. Whether the internal pH of the endosome to which IgG-loaded vesicles fuse is set to this optimal pH has not been confirmed yet.

The first quantitative measurement of vesicular pH has been the microscopic fluorometry combined with the ratiometry of fluorescence excited by alternative illumination of FITC at 495 and 450 nm. With using this technuque, Ohkuma et al. obtained the intralysosomal pH around 4.7-4.8[37]. If we use appropriate endocytic probe doubly labeled with FITC and rhodamine, another reliable means of measuring intraendosomal pH becomes feasible. Recently, we have performed a direct measurement of this endosomal pH with using FITC/rhodamin dual-lebeled dextran as the endocytic pH probe and with the confocal laser scanning microscope[38,39]. Dextran is an endocytic tracer destined to lysosome and share the endocytic pathway with IgG before leaving the sorting endosome. Our measurement of pH within this endosome gave pH 6.1 after 5 minutes' endocytosis. Electron microscopy of the endocytic pathway of horseradish peroxidase, a similar lysosome-destined endocytic tracer, showed its arrival to the endosome. Thus, the pH measured 5 min after endocytic labeling is assumed to indicate the intraendosomal pH. This observation has confirmed that the endosome, most likely the sorting endosome, maintains around pH 6.1. This finding strongly suggests that IgG-FcR complex is actually formed within this endosome. Thus, the intact complex can be transported to the basolateral membrane transcellularly.

## Sorting, transport, and fusion of vesicles

Sorting, transport, and fusion of vesicles and their interaction with cytoskeletons are well studied in the IgA transport system rather than IgG transport system. In the IgA transpport system, there are several signal sequences responsible for transcytosis of pIgR[40]. 103 amino acid sequence in the cytoplasmic C-terminal of pIgR has a motif necessary to determine the direction of vesicular transport. Two tyrosine motifs and a serine motif are thought to be possible candidates of endocytosis signal[41,42]. 17 amino acid sequence in the cytoplasmic domain, a known sorting signal in the trans-Golgi network secretory pathway, is thought to be a sorting signal which determines the direction of vesicle transport from basolateral endosome to the apical endosome[43]. This basal-to-apical transport of vesicles is disrupted by Brefeldin-A[44]. pIgR internalized into basolateral endosomes are sorted into transport vesicles, and are forwarded to fuse with apical endosomes before fusing with the apical membrane[45,46]. The vesicle transport from the basolateral endosome to the apical endosome is blocked by nocodazol[47,48,49]. This shows that the basal-to-apical transport of vesicles is dependent on intact microtubules.

Docking and fusion of vesicles and endosomes are hypothetically explained in terms of the soluble NEM-sensitive attachment protein receptor (SNARE)[51]. This model assumes that the docking takes place when v-SNARE on vesicles specifically bind to t-SNARE on the target membrane. The two membranes are then fused in the presence of SNARE-dependent N-ethyl maleimide-sensitive fusion protein NSF[52,53]. Although the SNARE model does not explain all of the vesicle fusion process, evidences suggest their involvement in the fusion event of transport vesicles and the apical membrane[54,55,56]. The cellular mechanism of IgG transport including endocytosis signal, sorting signal, their interactions with coat proteins and cytoskeletons, roles of pathway-specific rab proteins and SNAREs remain to be elucidated.

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