Receptor-mediated Transport of IgG

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ABSTRACT The intestinal epithelium of the neonatal rat is a model system for the study of receptor-mediated endocytosis in which large amounts of IgG are transferred intact across polarized cells. This review summarizes the ultrastructural pathway followed by IgG during cellular transit and several important properties of the membrane receptor that recognizes the IgG.

Selective transfer of IgG across the intestinal epithelium of the neonatal rat is a well-documented and intriguing model system for the study of receptor-mediated endocytosis by cells. In most examples of this widespread phenomenon (3, 13), a ligand is delivered to an intracellular compartment, where it may be stored or degraded as well as trigger important intracellular metabolic processes. In contrast, receptor-mediated endocytosis of IgG serves in the bulk transfer of extremely large quantities of intact maternal IgG across an epithelial barrier to provide passive systemic immunity for the newborn. As a highly polarized structure, the intestinal epithelial cell also shows a very clearly defined direction of transport that has greatly aided our morphological observations. This brief review summarizes these observations as well as our recent biochemical studies on the receptor responsible for the remarkable specificity for IgG displayed in the process.

Transport Pathway

The small intestine of newborn rats is highly adapted for the efficient uptake of maternal IgG from the lumen. Luminal proteolysis is depressed at this age to give the absorptive epithelium in the duodenum and proximal jejunum a chance to remove intact IgG from the other milk proteins that will pass further down the intestine and be degraded within absorptive cells of the ileum. In the duodenum and jejunum, the epithelial cells responsible for transport differ markedly from their adult counterparts in having a highly developed system of small endocytic vesicles that arise from the luminal surface and participate in the selective transfer of the IgG. In past work my colleagues and I have used ultrastructural tracers for electron microscopy to examine the details of this uptake process and, in particular, the intracellular route IgG follows across the cell (10).

The early steps in transport are quite clear. IgG binds to the luminal plasma membrane, presumably to specific membrane receptors. The receptors, which recognize the Fc portion of the IgG molecule, are most numerous at the base of the microvilli, where frequent membrane invaginations are apparent (Fig. 1). These invaginations frequently exhibit a clathrin-like coating on their cytoplasmic membrane face and hence are truly coated pits. As time-course studies with both ferritin and peroxidase conjugates of IgG have revealed (1, 7, 8), the pits are precursors of endocytic tubules, which rapidly form, lose their clathrin coats, and carry receptor-bound IgG through the terminal web into the apical regions of the cell (Fig. 2).

What happens next is not quite as clear. Apparently, the endocytic tubules, rather than migrating directly to the basolateral surface, transfer their ligand to a second class of distinctly smaller coated vesicles that are responsible for final exocytosis (7). This pathway completely bypasses the Golgi cisternae. Nevertheless, the internalized IgG may enter other membrane compartments, notably apical lysosomes and numerous small vacuoles characteristically found within these cells. I have inferred that IgG transfer may not be fully efficient and that a portion of the internalized IgG may be degraded by the lysosome system.

In the course of these morphological experiments, I was able to demonstrate two very important properties of the membrane receptors critical for transport (9). First, I showed that the receptors on the luminal surface can bind IgG at the normal pH (6.0–6.5) of the luminal contents, as one would expect, but not at pH 7.4! Second, I demonstrated receptors on the basolateral plasma membrane of cells that showed the same pH-dependent binding of IgG. These observations led me to propose a model for transport (9), also suggested by others (14), in which IgG is transferred across the cell as stable receptor-ligand complexes that dissociate only upon exposure to a pH 7.4 environment, presumably upon exocytosis at the basolateral surface. This scheme not only emphasized the reversibility of binding that would be essential for efficient
receptor-mediated transport but also raised the intriguing possibility that receptors could shuttle back and forth between the apical and basolateral membranes. Net directional transfer of IgG could be driven simply by the pH difference across the epithelium. Although I have not yet shown that such receptor shuttling actually occurs, I have been able to demonstrate that there is, in fact, inward movement of vesicles from the basolateral membrane that could provide at least a partial pathway for receptor recycling (9).

Could IgG dissociate from the receptor before reaching the basolateral surface? Although this is possible, I have good evidence that the IgG must remain bound to its receptor at
least beyond a critical stage during transit through the cell. The first observation that suggested this arose from experiments that utilized free peroxidase as a control tracer for fluid-phase uptake by cells. Although most non-IgG proteins do not enter the cell by luminal endocytosis, small amounts of peroxidase could in fact be shown to enter (1, 8). However, this internalized tracer did not cross the cell but instead was rapidly and efficiently segregated within the apical vacuoles and lysosomes. To understand this observation better, I and D. R. Abrahamson performed double-tracer experiments in which cells were exposed simultaneously to free peroxidase and to ferritin-conjugated IgG as a tracer for the selective transport (1). We found that both tracers entered the cell within the same endocytic vesicles but that only the IgG tracer was later released at the basolateral surface in the normal manner. As before, the peroxidase was segregated within the apical vacuoles and lysosomes. We inferred that, in addition to the apical membrane, there was a second site for selection of IgG from fluid-phase tracers inside the cell, and assumed that IgG must remain bound to its receptor during the time this internal sorting takes place.

Additional insight into this internal sorting has come from experiments with cationic ferritin (10). This highly charged derivative of ferritin (pI = 8.5–9.0) binds avidly and presumably nonspecifically to a variety of anionic sites on cell membranes. When the epithelial cells are exposed to this tracer, not only does it bind strongly to the apical plasma membrane but it also enters the cell and is found in large amounts in the same vesicles that normally transport IgG (Fig. 3). Surprisingly, cationic ferritin fully traverses the cell, apparently following the same pathway as IgG and accumulating on the basolateral surface after exocytosis (5, 10). If double-tracer experiments are performed, and cells are exposed to both cationic ferritin and peroxidase, again the membrane-bound ligand is sorted from the fluid-phase tracer within the apical cytoplasm in a manner closely analogous to that evident from previous double-tracer experiments with ferritin-conjugated IgG. These results, as a whole, lead to the important conclusion that one essential condition for transport to the basolateral surface is that the ligand must remain bound to a membrane component during a critical period within the cell. The binding may be specific, as is the case with IgG binding to its receptor, or nonspecific, as is the case with cationic ferritin associated with anionic sites on the membrane.

The double-tracer experiments vividly demonstrate the existence of intracellular selection and sorting of ligands from fluid-phase tracers that possibly enter cells accidently. Although I have not yet identified the precise site at which this internal sorting occurs, I strongly suspect that it resides within the small vacuoles in that region of the apical cytoplasm where most fluid-phase peroxidase is confined. It is interesting that these vacuoles bear a striking similarity to structures, variously termed endosomes, intermediate vacuoles, prelysosomes, and receptosomes, found in several other diverse cell systems engaged in receptor-mediated endocytosis (4, 6). As with these systems, the vacuoles in the intestinal cells frequently are found connected with endocytic vesicles and accumulate fluid-phase tracers shortly after their uptake but lack demonstrable lysosomal hydrolases (1). Indeed, IgG tracers and cationic ferritin also entered this compartment to a limited degree in our experiments, but remained preferentially bound to the vacular membrane. If conditions within the vacuoles do not cause rapid degradation or dissociation of IgG from the tubular or vacuolar membranes, I theorize

![Figure 3](https://example.com/figure3.png)

**Figure 3** Endocytosis of cationic ferritin. The tracer is found within the endocytic vesicles, small vacuoles, and lysosomalike structures. Fusion of endocytic tubules with apical vacuoles is illustrated (arrows). × 45,000.
that momentary fusion of the tubules with the vacuoles could allow removal of the fluid contents from the tubules before their continued transport of receptor-bound IgG through the cell. I suspect that, as a sorting site, the vacuoles represent the first step in a divergent pathway for fluid-phase transport to the lysosomal compartment.

Properties of the Receptor

The results of the morphological studies that my colleagues and I have conducted clearly emphasize the importance of both the specific binding properties and the movements of the receptor among membrane compartments during transport. To define the binding properties of the receptor more accurately, we have undertaken a quantitative biochemical study of IgG binding to the apical plasma membrane of brush borders isolated from the neonatal epithelial cells (11; manuscript in preparation). We are able to harvest the brush borders in high yield from intestinal tissue by methods described previously (12). The isolated brush borders are large, open vesicles consisting of virtually the entire microvillar surface and a variable portion of the underlying terminal web of individual cells (Fig. 4). To assay IgG binding, we have used 125I-labeled IgG and simply measured the total radioactivity that binds to brush borders under various conditions.

Equilibrium binding of 125I-IgG is reached by approximately 3 h at 0°C. As anticipated, binding is exquisitely pH sensitive, occurring at pH 4.0-6.0 but not at pH 7.4 or higher. Ligand bound at pH 6.0 can be rapidly and completely dissociated if brush borders are washed at pH 7.4. Binding is competitively inhibited by excess unlabeled IgG or its Fc fragments but not by Fab fragments, indicating that the observed binding is specific for the Fc portion of the IgG molecule. Scatchard analysis of binding at different IgG concentrations indicates that the binding is complex, as has also been shown by others for this system (15). The nonlinear Scatchard plot for binding at pH 6.0 in low-ionic-strength buffer (Fig. 5) suggests either that binding exhibits “negative cooperativity” (bound IgG inhibits binding of additional IgG) or that there are two distinct classes of binding sites that differ markedly in apparent affinities (Kd1 = 2 x 10^7 M^-1 and Kd2 = 4 x 10^5 M^-1). Two observations strongly favor the second possibility. First, if the buffer concentration is increased, the apparent number of low-affinity sites is sharply reduced without any effect on either the number or affinity of the high-affinity sites. Second, and more telling, if we analyze binding to brush borders from 21-d-old rats, an age when selective IgG transport no longer occurs in intact cells, we still observe the low-affinity binding but not the high-affinity binding. These results argue convincingly that the high-affinity sites, present at an average density of ~3 x 10^6 per brush border, are the true receptors involved in the endocytic transport in the intact cell. Conversely, the low-affinity sites are probably an artifact of our assay or at least are not involved in the selective transport of IgG.

We have confirmed this conclusion with morphological tracer studies undertaken to visualize directly the locations of the low- and high-affinity sites (11; manuscript in preparation). The results of these experiments using a variety of IgG tracers can be summarized as follows. At low ionic strength, IgG tracers bind to the plasma membrane of the isolated brush borders as well as binding diffusely to filament-rich regions within the terminal web. At high ionic strength, binding is limited almost exclusively to the plasma membrane (Fig. 6), as would be expected for the high-affinity receptors for transport. We have suggested that terminal web binding, presumably of the lower affinity, may be to actin, which others (2) have shown can bind IgG under conditions similar to ours. Although interaction with actin or another cytoskeletal component of the terminal web is an intriguing possibility, we tend to view this binding merely as a curious artifact.
that, nevertheless, might have led us seriously astray in our subsequent efforts to isolate the true transport receptor.

Receptor Isolation

A primary aim of our brush border-binding studies has been to define relevant conditions for IgG-receptor binding that we could exploit for efficient affinity purification of the receptors. The basic strategy of these studies, currently in progress, is to allow receptors, solubilized with detergents from brush border membranes, to bind to immobilized IgG at pH 6.0 and then to release the receptors from washed complexes by raising the pH to 7.4. Our approach assumes that the detergent-solubilized receptor has the same binding proper-

**Figure 5** Computer-generated Scatchard plot of $^{125}$I-IgG binding to brush borders in 0.05 M phosphate buffer, pH 6.0, at 0°C. The biphasic plot results from the presence of two classes of binding sites with high and low affinity for IgG. $B$, bound; $F$, free.

**Figure 6** Brush borders incubated with a ferritin-IgG tracer in 0.15 M phosphate buffer, pH 6.0. At high ionic strength most of the IgG tracer binds to the apical plasma membrane. Binding within the terminal web is sharply lower than that found in 0.05 M buffer. $\times$ 53,000.
ties, including pH sensitivity, as the receptor in situ. We have chosen the nonionic detergent beta-octylglucoside for membrane solubilization on the basis of preliminary evidence that this detergent preserves binding activity, whereas several other detergents, including Triton X-100, do not. Material that was batch extracted at pH 7.4 from IgG covalently attached to agarose beads was radiiodinated and then analyzed by SDS polyacrylamide gel electrophoresis and autoradiography. A typical gel pattern from such an isolation is shown in Fig. 7. Characteristically, two bands are evident: a broad band with an apparent molecular weight of 52–57 kdaltons under nonreducing conditions and a second band of ~18 kdaltons. Neither band appears on gels of material eluted from control agarose beads without attached IgG, and neither band comigrates with either light or heavy chains of IgG that might have detached from the IgG-complex. Although we have yet to demonstrate retention of IgG binding activity in the material eluted from the SDS gels, even after the removal of the SDS, our preliminary results indicate that the iodinated eluate from the affinity beads can rebinding IgG in the presence of pH-activated IgG before electrophoresis in SDS. We are currently analyzing the affinity, pH dependence, and Fc specificity of this binding to determine whether either or both of the bands resolved on gels fulfills the functional definition of the IgG receptor based on binding properties.

Future Goals

The results of our biochemical analysis, although incomplete, are still consistent in every way with our model for the highly selective transport of IgG across cells based on morphological studies. Most importantly, we have demonstrated that the receptor-IgG interaction is of relatively high affinity but can be easily reversed by a change in pH that likely occurs during transport. The stability of binding at low pH is in stark contrast to what is found in other cells for numerous other receptor-ligand systems that rapidly dissociate at low pH in an as yet poorly defined endosomal compartment (4, 13). The anomalous behavior of the IgG receptor system most likely reflects the very different fate of the IgG that requires release intact at the basolateral surface. In contrast, the ligands in most other examples of receptor-mediated transport are transferred to and degraded within lysosomes. This basic difference is particularly relevant to the concept of the sorting of fluid-phase molecules discussed earlier. We would argue that if IgG-receptor complexes were processed through an acidic endosomal compartment they would be unaffected by the low pH and remain intact, as would be required for continued transit. This could mean not only that there is sorting and removal of fluid-phase molecules from the IgG pathway within an endosomal compartment but also that if this compartment were acidic there could be dissociation of other, non-IgG ligands from receptors that might share part of the IgG transport pathway. Such additional receptor-ligand systems have not been shown for these intestinal cells but remain an intriguing possibility that we wish to examine.

Clearly, we are still severely limited in our knowledge of the movements and possible recycling of the receptors within the cell. Our previous transport experiments, although suggestive of what may happen to the receptors within the cell, provide mainly information on the movements of the ligand that we clearly have shown may easily and rapidly dissociate from the receptors. To overcome this major shortcoming, we plan to use purified receptors to develop specific antireceptor antibodies that can be used as direct immunocytochemical probes to map receptor movements.

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