

Isolation and Characterization of the Fc Receptor from the Fetal Yolk Sac of the Rat

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Abstract. The yolk sac of the fetal rat and the proximal small intestine of the neonatal rat selectively transport maternal IgG. IgG-Fc receptors are thought to mediate transport across the epithelium of both tissues. We used a mouse mAb (MC-39) against the 45–54-kD component of the Fc receptor of the neonatal intestine to find an antigenically related protein that might function as an Fc receptor in fetal yolk sac. In immunoblots of yolk sac, MC-39 recognized a protein band with apparent molecular mass of 54–58 kD. MC-39 bound to the endoderm of yolk sac in immunofluorescence studies. In immunogold-labeling experiments MC-39 was associated mainly with small vesicles in the apical cytoplasm and in the region near the basolateral membrane of endodermal cells. The

MC-39 cross-reactive protein and β_2 -microglobulin, a component of the intestinal Fc receptor, were copurified from detergent-solubilized yolk sac by an affinity purification that selected for proteins which, like the intestinal receptor, bound to IgG at pH 6.0 and eluted at pH 8.0. In summary, the data suggest that we have isolated the Fc receptor of the yolk sac and that this receptor is structurally and functionally related to the Fc receptor of the neonatal intestine. An unexpected finding is that, unlike the intestinal receptor which binds maternal IgG on the apical cell surface, the yolk sac receptor appears to bind IgG only within apical compartments which we suggest represent the endosomal complex.

MATERNAL IgG is transmitted from mother to young in mammals to protect the young until its immune system is fully developed (for review see Brambell, 1970; Rodewald 1980). The site of transfer varies depending on the species. In primates, transmission occurs before birth across the chorioallantoic placenta. In other species that also transfer IgG before birth, the IgG crosses the yolk sac splanchnopleure and not the chorioallantoic placenta as a result of the dramatically different organization of these tissues (Brambell, 1970; Steven, 1975; Steven and Morris, 1975). In rats and mice, in addition to transfer across the yolk sac before birth, the small intestine of the neonate transmits maternal IgG present in the colostrum and milk after birth. Although in these species the plasma IgG concentration achieved by fetal transport does not reach the level that later results from neonatal intestinal transfer, nevertheless, fetal transport confers significant immunity which can protect the young against infection up through at least the first day after birth (Culbertson, 1938, 1940; Brambell, 1970).

Regardless of the tissue site, transmission is highly selective and is thought to involve specific receptors that bind to the Fc region of the IgG molecule (IgG-Fc receptors) (Brambell, 1970; Rodewald, 1980). In the case of the yolk sac, Brambell and co-workers (Brambell et al., 1958; Brambell, 1970) proposed that the receptors reside within the endoderm of the yolk sac. The apical surface of endodermal cells is bathed by the fluid of the uterine lumen which is similar in composition to the maternal plasma and contains maternally derived IgG (Brambell, 1970). Maternal plasma proteins enter the uterine lumen by diffusion across a thick hyaline layer, Reichert's membrane. Whereas the endoderm takes up many luminal proteins which are digested for the nutrition of the fetus, IgG destined for the fetal circulation is transported by the endoderm intact. After transfer across the endoderm, IgG enters the circulation of the fetus by way of the blood vessels in the yolk sac. Although Brambell and co-workers (Brambell, 1970) postulated that an Fc receptor is responsible for selective transport across the endoderm, this receptor has not been well characterized or isolated. Furthermore, it is not clear where the receptor resides in the endoderm or by what mechanism it transports maternal IgG.

In contrast, more is understood about the receptor-mediated transmission of IgG across the neonatal small intestine. This receptor has been isolated and found to consist of two subunits: a heavy chain (45–54 kD) (Rodewald and Kraehenbuhl, 1984; Simister and Mostov, 1989) and β_2 -

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microglobulin (Simister and Mostov, 1989). Morphological studies have shown that the intestinal receptor binds IgG on the luminal membrane of absorptive cells in a pH-dependent manner and releases IgG at the basolateral cell surface (Rodewald and Abrahamson, 1982; Rodewald and Kraehenbuhl, 1984). It has been postulated that IgG remains bound to the Fc receptor within the acidic, endosomal compartments of the cell thus allowing this ligand to escape transport to and digestion within lysosomes (Rodewald and Abrahamson, 1982).

Our purpose in this study was to isolate and characterize the IgG-Fc receptor of the yolk sac endoderm. We reasoned that since the Fc receptors of neonatal intestine and fetal yolk sac were involved in transmission of maternal IgG, the receptors might be structurally similar. Therefore, we probed the yolk sac with an mAb that recognizes the heavy chain of the intestinal receptor in order to identify any antigenically related proteins that could be candidates for the Fc receptor in this tissue. We detected a cross-reactive protein of the yolk sac which is localized to the absorptive endoderm and copurifies with β_2 -microglobulin in an isolation procedure that selects for proteins which, like the intestinal receptor, bind to IgG at an acidic pH and release IgG at a basic pH. The evidence indicates that we have isolated the Fc receptor of the yolk sac and that this receptor has structural and functional similarities with the intestinal Fc receptor. An unexpected result in light of the similarities between the two receptors, is that, although both receptors are found in apical compartments in their respective epithelial cells, unlike the intestinal receptor (Berryman and Rodewald, 1990), the yolk sac receptor is not present on the luminal surface of endodermal cells. We suggest a model for selective IgG transport, consistent with the differences in cellular function between the neonatal intestine and yolk sac, in which the Fc receptor of the yolk sac binds maternal IgG within the endosomal complex and not on the luminal surface of endodermal cells.

Materials and Methods

Animals

Pregnant Sprague-Dawley female rats or female rats with litters were purchased from Dominion Labs (Dublin, VA) or from Hilltop Lab Animals, Inc. (Scottsdale, PA).

mAb Production

mAbs against the IgG-Fc receptor of rat neonatal intestine were made by isolating brush borders from epithelial cells of neonatal duodenum and jejunum at 10–12 d after birth. Cells were isolated according to Evans et al. (1971). Brush borders were isolated from these cells by the procedure of Forstner et al. (1968), and nuclei were separated from brush borders according to Mooseker et al. (1978). Brush border membranes were then solubilized in 60 mM n-octyl β -D-glucopyranoside (Sigma Chemical Co., St. Louis, MO) and 10 mM D-gluconic acid lactone (Sigma Chemical Co.) to inhibit endogenous glucosidase activity (Gatt, 1966) in PBS, pH 7.4 (0.1 M phosphate buffer, 0.25 M sodium chloride) (5×10^7 brush borders/ml). Solubilized membranes were diluted in 0.1% SDS in saline and were injected in complete Freund's adjuvant (1:1) into the peritoneal cavity of BALB/c mice. Mice were boosted intraperitoneally after 2 wk with ligand affinity-purified intestinal Fc receptor (Rodewald and Kraehenbuhl, 1984) (also see below) in 0.1% SDS in saline mixed 1:1 with incomplete adjuvant. After mice were killed, spleen cells were fused with a Sp2/O-Ag14 myeloma cell line (Shulman et al., 1978) according to Chapman et al. (1984). Cells were cultured in 96-well culture dishes. Media from individual wells were

tested for the presence of anti-Fc receptor antibodies by ELISA (Engvall and Perlman, 1971) and Western blot (see below) employing affinity-purified Fc receptor for detection. Cells whose media tested positive were cloned twice by limiting cell dilution. Media from wells that contained colonies were retested by ELISA and Western blotting. The mAb used for the experiments in this paper is an IgG₁ subclass and is designated MC-39. MOPC 21 (Litton Bionetics, Kensington, MD), a mouse myeloma protein of subclass IgG₁, and mouse IgG (Sigma Chemical Co.) were used as primary antibodies in controls.

SDS Gel Electrophoresis and Immunoblot Analysis

Tissue from day 19–23 fetuses or from day 12 neonates was Dounce homogenized with 0.1 M phosphate buffer, pH 7.4, at 4°C, containing protease inhibitors (2 mM PMSF, 0.2–0.4 U/ml aprotinin, 100 μ g/ml leupeptin, 1 μ g/ml antipain, and 0.2 μ g/ml pepstatin-A). Samples (0.35 mg/well) (Hartree, 1972) were loaded onto 10% linear SDS polyacrylamide gels which were run in the gel system described by Laemmli (1970). Parallel lanes with high and low molecular weight standards (Sigma Chemical Co.) were used to estimate apparent molecular masses. Proteins were transferred from gels onto nitrocellulose filters (Schleicher and Schull Co., Dassel, FRG) overnight at 140 mA according to Dunn (1986). Transblots were immunostained according to Towbin et al. (1979) with the following changes. Transblots were blocked in 5% powdered milk (Herring et al., 1990) in borate-buffered saline, pH 8.5 (0.1 M borate buffer, 75 mM sodium chloride) (milk-BBS) for 20 min at room temperature (RT) before addition of antibody. Primary antibodies (MC-39 or rabbit antihuman β_2 -microglobulin antiserum [Serotec, Ltd., Bicester, England]) and secondary antibodies (HRP-conjugated goat antimouse IgG [HyClone Laboratories, Inc., Logan, UT]; HRP-conjugated goat antimouse IgG absorbed against rat IgG [HyClone Laboratories, Inc.]; or HRP-conjugated goat antirabbit IgG absorbed against rat IgG [HyClone Laboratories, Inc.]) were diluted 1:1,000 in milk-BBS. Primary antibodies were incubated with transblots overnight at 4°C. After incubation with secondary antibody, the transblots were rinsed in BBS and were reacted at 4°C with DAB (Aldrich Chemical Co., Milwaukee, WI). MOPC 21 or normal rabbit whole serum (Jackson ImmunoResearch Labs, Inc., West Grove, PA) was used as the primary antibody in control immunoblots. Western blots were also stained with 0.1% amido black in 2% acetic acid and 10% methanol for 5 min and destained in 7% acetic acid and 45% methanol until background was clear. Silver staining of 7–15% gradient SDS PAGE gels was performed according to the procedure of Blum et al. (1987).

Ligand-affinity Purification

Whole yolk sacs (day 19–23 gestation) or neonatal jejunum (day 10–12) were Dounce homogenized at 4°C in PBS containing 3 mM EDTA and protease inhibitors (see above). The remaining steps were performed at 4°C. Homogenates were centrifuged at 100,000 g for 60 min. Pellets were solubilized immediately in detergent by resuspension in a binding buffer that consisted of 0.15 M phosphate buffer, pH 6.0, containing 60 mM n-octyl β -D-glucopyranoside, 10 mM D-gluconic acid lactone, 1 mM EDTA, and 0.02% NaN₃ and were stirred for 2 h. Insoluble material was removed by centrifugation at 100,000 g for 30 min to yield a solubilized membrane fraction. Rat IgG immunoabsorbant was prepared by conjugation of rat IgG (Sigma Chemical Co.) to Affigel-10 (Bio-Rad Laboratories, Richmond, CA) according to the manufacturer's instructions. The IgG-Affigel was extensively washed in saline and then was cycled several times through 0.15 M phosphate buffers, pH 6.0 and pH 8.0, and then was washed 6 \times in binding buffer. IgG-Affigel (100–150 μ l packed beads) was then added to detergent-solubilized tissue extracts at pH 6.0 and was rocked overnight. After incubation, beads were microfuged, and the supernatant was saved. Beads were then rapidly washed three times in binding buffer and three more times in diluted binding buffer (0.015 M phosphate buffer, pH 6.0, 30 mM n-octyl β -D-glucopyranoside, concentrations of other components the same). Bound proteins were eluted from pelleted beads at pH 8.0 by adding 100–150 μ l of a releasing buffer (0.15 M phosphate buffer, pH 8.0, concentrations of other components the same). Samples from the purification procedure were run on 7–15% gradient gels (see above). Proteins on the gel were silver stained or transblotted for immunostaining with MC-39 or rabbit antihuman β_2 -microglobulin (see above).

pH Measurements

Uterine pH was measured in two ways. In the first method, capillary tubes (50 μ l; Fisher Scientific Company, Pittsburg, PA) were prepared by inser-

tion of narrow range pH paper (6.8–8.4) (Hydriion Papers, Micro Essential Laboratory, Brooklyn, NY) into the ends of the tubes. Pregnant rats at 19–23 d of gestation were lightly etherized, and an abdominal incision was made to expose the uterus. Small incisions were made in the wall of the uterus from the ovaries to the cervix. Uterine fluid was sampled at each incision by insertion of a capillary tube. The color change of the pH paper was immediately compared with identical reference tubes which had been dipped in 0.1 M phosphate buffer of known pH. In the second method the pH of the uterine fluid was measured in situ by insertion of micro-pH electrodes (models 15874 and 15235; Microelectrodes, Inc., Londonderry, NH) into similar incisions in the uterine wall and measurement with a pH meter (Corning model 125; Corning Scientific Instruments, Medfield, MA). The electrodes were calibrated with phosphate buffers of known pH and were checked periodically throughout the procedure.

Fluorescence Microscopy of Frozen Sections

Yolk sacs were fixed in situ in paraformaldehyde-lysine-periodate fixative (PLP) (McLean and Nakane, 1974) for 10 min. Tissue pieces were fixed further in PLP overnight at 4°C. Tissue was then incubated with 15% sucrose in cold PBS for 30 min. Frozen sections (5- μ m thick) were cut on a Reichert-Jung 2800 Frigocut-N cryostat (Baltimore Instrument Co., Inc., Baltimore, MD). Sections were treated with methanol for 3 min and were blocked overnight in 1 mg/ml sodium borohydride (Aldrich Chemical Co., Milwaukee, WI) in ethanol at 4°C according to the procedure of Gorbsky and Boris (1986). Sections were washed in 57 mM borate buffer, pH 8.2, (BB) and were blocked in 2% BSA in borate buffer (BSA-BB) for 30 min. Sections were then incubated in a humid chamber in drops of either MC 39 or MOPC 21 at 10 μ g/ml in 1% BSA-BB for 1 h. After a brief wash in BSA-BB, sections were incubated with secondary antibody, rhodamine-conjugated rat anti-mouse IgG (Jackson ImmunoResearch, Avondale, PA), at a dilution of 1:2 in 1% BSA-BB for 1 h. After washing in BB, sections

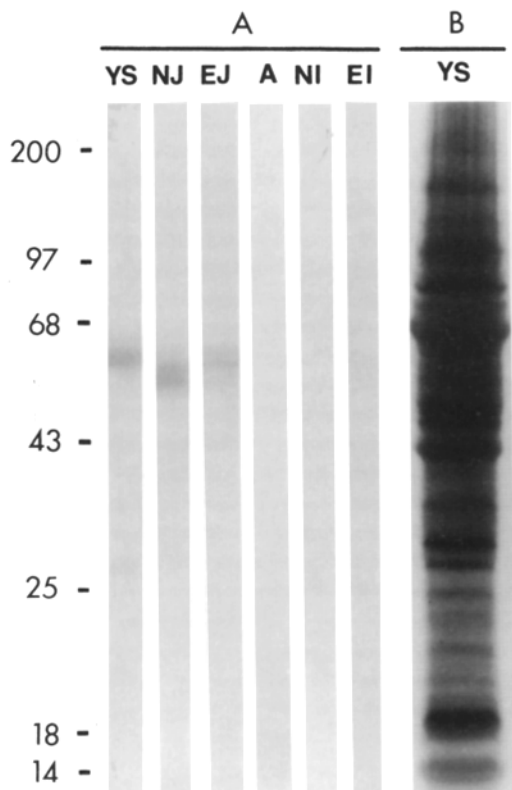


Figure 1. MC-39 staining of immunoblots from SDS gels of whole tissue homogenates. (A) Immunoblot demonstrating that MC-39 binds a protein band from neonatal jejunum (NJ), embryonic jejunum (EJ), and yolk sac (YS). No binding is seen in neonatal ileum (NI), embryonic ileum (EI), or amnion (A). (B) Lane from an identical transblot treated with amido black. All lanes were from gels loaded with equal amounts of protein.

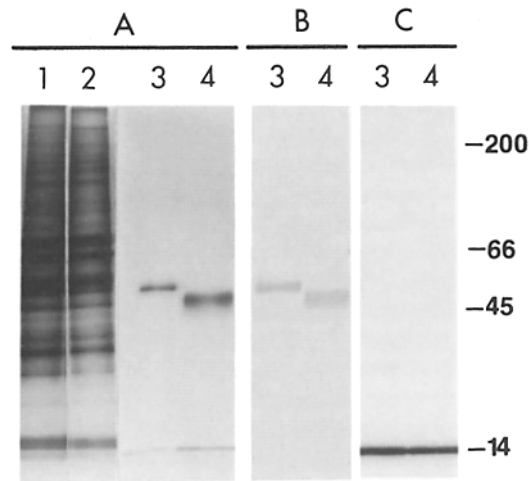


Figure 2. Analysis of samples from the IgG-affinity purification of putative receptors from detergent-solubilized yolk sac and neonatal jejunum. Samples were subjected to SDS-PAGE and were either silver stained to determine purity or transferred to nitrocellulose to determine reactivity with MC-39 and antihuman β_2 -microglobulin. (A) Silver-stained samples. (lane 1) Yolk sac, starting material; (lane 2) yolk sac, pH 6.0 wash; (lane 3) yolk sac, pH 8.0 eluent; (lane 4) neonatal jejunum, pH 8.0 eluent. (B) Immunoblot of pH 8.0 eluates stained with MC-39. (C) Corresponding immunoblot stained with antihuman β_2 -microglobulin.

were mounted in 50% glycerol in BB containing 5% n-propyl gallate (Sigma Chemical Co.) (Giloh and Sedat, 1982). Sections were viewed with epifluorescence, phase and differential interference contrast (DIC) optics on a Zeiss standard photomicroscope (Baltimore Instrument Co., Baltimore, MD).

Fluorescence Microscopy of Plexiglas Sections

The villus region was fixed in PLP as described above and was embedded in Plexiglas (Rohm and Haas Co., Philadelphia, PA) according to the method of Gorbsky and Boris (1986). Sections (1 μ m thick) were cut on a Porter-Blum MT-2 microtome. After removal of Plexiglas and blocking overnight with 1 mg/ml sodium borohydride in ethanol at 4°C, sections were incubated in 1% BSA-PBS for 20 min at 37°C. Sections were then stained with primary and secondary antibodies by the method used for frozen sections except that PBS was used in place of BB. Sections were examined in the same manner as that used for frozen sections.

Immunogold-labeling Experiments

This procedure was adapted from Berryman and Rodewald (1990) with the following changes. Procedures were performed at RT unless stated otherwise. Yolk sacs were fixed in situ for 10 min in either 0.1% glutaraldehyde or 1% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, that contained 0.2% picric acid, 4% formaldehyde, and 0.5 mM calcium. Tissue pieces were fixed for an additional 3 h. Tissue was postfixed in 1% uranyl acetate in either veronal acetate buffer (Kellenberger et al., 1958) or in distilled H₂O for 2 h at 4°C. After embedding was complete, silver sections were cut and were placed on 100 \times 400 mesh nickel grids. Grids were blocked with protein in 1% ovalbumin–5% normal goat serum in TBS, pH 7.4 for 30–60 min. Primary antibody (MC-39, MOPC 21, or mouse IgG) and secondary antibody (goat antimouse IgG conjugated to 10 nm gold) (Janssen, Piscataway, NJ) were diluted in 1% ovalbumin–1% normal goat serum in TBS. Primary antibody was diluted 1:50, airfuge (Beckman Airfuge, Beckman, Fullerton, CA) at 100,000 g for 10 min before use, and incubated with grids for 45 min to 1 h. Grids were incubated with secondary antibody at a dilution of 1:60 for 1 h. All washes were done with 0.1% ovalbumin–0.1% normal goat serum in TBS. After poststaining with 2% aqueous osmium tetroxide followed by lead citrate (Reynolds, 1963), grids were coated with carbon in a vacuum evaporator (Edwards High Vacuum, Inc., Gaithersburg, MD) and viewed at 60 kV in an electron microscope (model 200; Philips Electronic Instruments, Inc., Mahwah, New Jersey).

Results

MC-39 Recognizes a 54–60-kD Yolk Sac Protein

We first used a mAb (MC-39) that recognizes the heavy chain of the IgG-Fc receptor of the neonatal intestine to analyze immunoblots of homogenates from several tissues to determine whether any of these tissues contained antigenically related proteins. MC-39 bound to a protein band in yolk sac homogenates with a range of 54–60 kD apparent molecular mass (Fig. 1). This cross-reacting species migrated with a slightly higher apparent molecular mass than the heavy chain of the intestinal Fc receptor which was detected at 52–56 kD in homogenates of neonatal jejunum (Fig. 1). MC-39 also recognized a protein band in homogenates of embryonic jejunum. Interestingly, this cross-reactive band migrated with an apparent molecular mass similar to that of the yolk sac species. Embryonic and neonatal ileum, tissues which are thought not to have Fc receptors (Rodewald, 1973) as well as fetal amnion did not bind MC-39.

Affinity Purification Isolates the Yolk Sac Protein Recognized by MC-39

We isolated the MC-39 cross-reactive protein from detergent-solubilized yolk sac using a pH-dependent ligand-affinity purification technique that has been used to isolate the intestinal Fc receptor from neonatal jejunum (Rodewald and Kraehenbuhl, 1984). We looked for a yolk sac protein(s) that, like the intestinal Fc receptor, would bind to IgG-Affigel at pH 6.0 and release at pH 8.0. Fig. 2 shows data from one such experiment in which IgG binding proteins were isolated from both yolk sac and neonatal intestine. As revealed in the silver-stained SDS gels, similar protein profiles were seen in both starting material (pH 6.0 detergent-solubilized yolk sac) and the pH 6.0 wash from yolk sac (Fig. 2, lanes *A 1* and *A 2*). pH 8.0 eluates from both detergent-solubilized yolk sac (Fig. 2, lane *A 3*) and neonatal jejunum (Fig. 2, lane *A 4*) contained two proteins: a high and a low molecular weight component. The heavy component in the pH 8.0 eluate from yolk sac migrated with a slightly higher apparent molecular mass (54–58 kD) than the heavy chain (45–54 kD) from the pH 8.0 eluate of neonatal jejunum. The low molecular mass component from both tissues migrated at 14 kD. We will refer to the heavy chain from yolk sac as p57 and to the 14-kD component as p14. MC-39 bound in immunoblots to both the isolated p57 from the yolk sac (Fig. 2, lane *B 3*) and the isolated 45–54-kD component of the intestinal Fc receptor (Fig. 2, lane *B 4*).

We examined the possibility that p14 of the ligand-purified yolk sac membranes might be β_2 -microglobulin since Simister and Mostov (1989) demonstrated that the 14-kD component of the intestinal Fc receptor is β_2 -microglobulin. We stained immunoblots of pH 8.0 eluates from both yolk sac and neonatal intestine with antihuman β_2 -microglobulin antibodies (Fig. 2, lanes *C 3* and *C 4*). These antibodies specifically recognized the 14-kD component in both eluates. These data indicate that p14 from yolk sac is also β_2 -microglobulin.

MC-39 Binds to the Endoderm

We next determined the distribution of the MC-39 cross-reactive protein in yolk sac tissue by immunocytochemistry for light and electron microscopy. The vascularized rat yolk

sac near term has two morphologically distinct areas: villous and nonvillous regions (Ramsey, 1975; Brambell, 1970). Both regions of the yolk sac contain the same three distinct cellular layers: an endoderm that faces into the uterine lumen; a mesenchyme that is located just underneath the endoderm and contains connective tissue and blood vessels; and a mesothelium, a thin mesoderm-derived epithelium that faces the amnion (Wislocki and Padykula, 1953; Padykula et al., 1966). The endoderm is responsible for the absorptive functions of the yolk sac (Everett, 1935; Lambson, 1966; Brambell, 1970).

MC-39, as revealed by the fluorescence of the rhodamine-labeled secondary antibody, stained the endoderm of both villous (Fig. 3, *a* and *b*) and nonvillous (Fig. 3, *c* and *d*) regions in 5- μ m thick cryosections of fixed yolk sac. MC-39 bound to the cytoplasm of endodermal cells and was not found in the nucleus. Bright fluorescence was seen in both the apical and basolateral regions of the cells, although in some cells fluorescence was more intense in one region than the other. Controls in which MOPC 21 was used as a nonspecific primary antibody (Fig. 3, *e* and *f*) exhibited some faint fluorescence in the endoderm, mesothelium, and endothelium. Autofluorescent red blood cells were also observed in all preparations. Thinner Plexiglas sections (1- μ m thick) of the villous region provided better resolution of MC-39 immunostaining within the endoderm (Fig. 3, *g* and *h*). MC-39 bound throughout the cytoplasm of cells in a punctate staining pattern which suggested that binding was to vesicles. The intensity of MC-39 binding within the cytoplasm varied from cell to cell. However, MC-39 did not appear to bind to the brush border region of any of the endodermal cells. In Plexiglas sections the mesothelium and endothelium treated with MC-39 showed no fluorescence.

We determined the ultrastructural distribution of MC-39 staining in the endodermal cell using an indirect immunogold-labeling procedure for EM. Although gold was associated mainly with small vesicles in the apical cytoplasm (Fig. 4 *a*) and in the regions near the basolateral membrane (Fig. 5), very little or no gold was located on the microvilli or invaginations of the apical membrane of the endodermal cells (Fig. 4, *a* and *b*). This observation is consistent with our results of MC-39 binding at the light level. This distribution, however, is unexpected since the intestinal receptor is clearly located on the luminal membrane of epithelial cells (Rodewald, 1976; Berryman and Rodewald, 1990), and since the yolk sac and intestinal receptor have many other similarities in both structure and function. In the apical cytoplasm of endodermal cells, the small vesicles that contained gold were a heterogeneous population in both size and shape exhibiting both circular and tubular profiles (Fig. 4 *a*). Although coated membranes were difficult to visualize, these vesicles did not appear to have a cytoplasmic coat and contained varying amounts of electron-dense material. Large tubules and vacuoles with electron-lucent interiors that are located near the apical surface and may be profiles of deep apical invaginations (Kugler and Miki, 1985) also bound very little or no gold (Fig. 4 *b*). In the basolateral cytoplasm gold was associated primarily with small, uncoated vesicles as well as with less frequent coated vesicles and coated pits on the basolateral membrane (Fig. 5). Virtually no gold was located in any part of the endodermal cell when control primary antibodies were used.

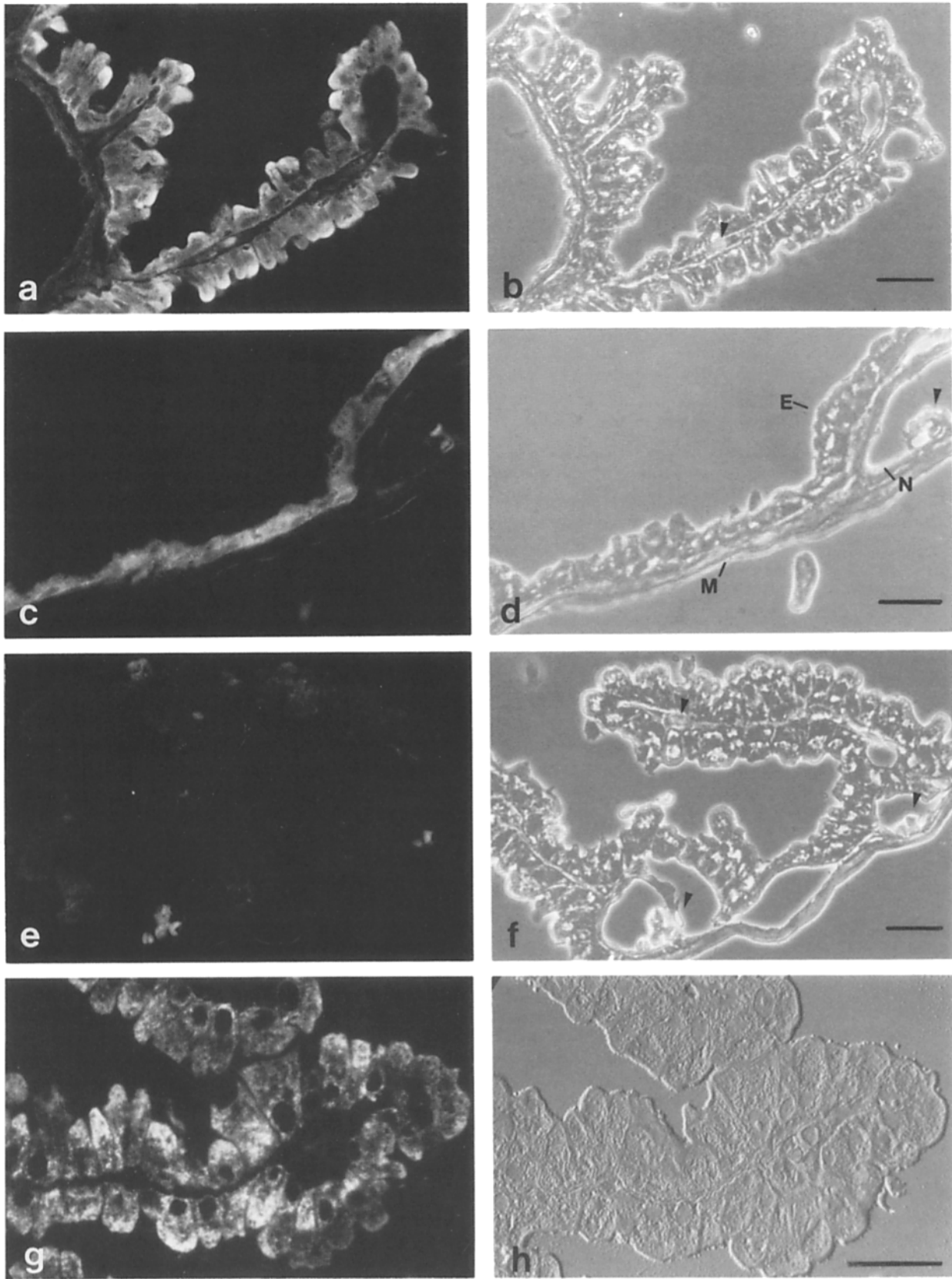
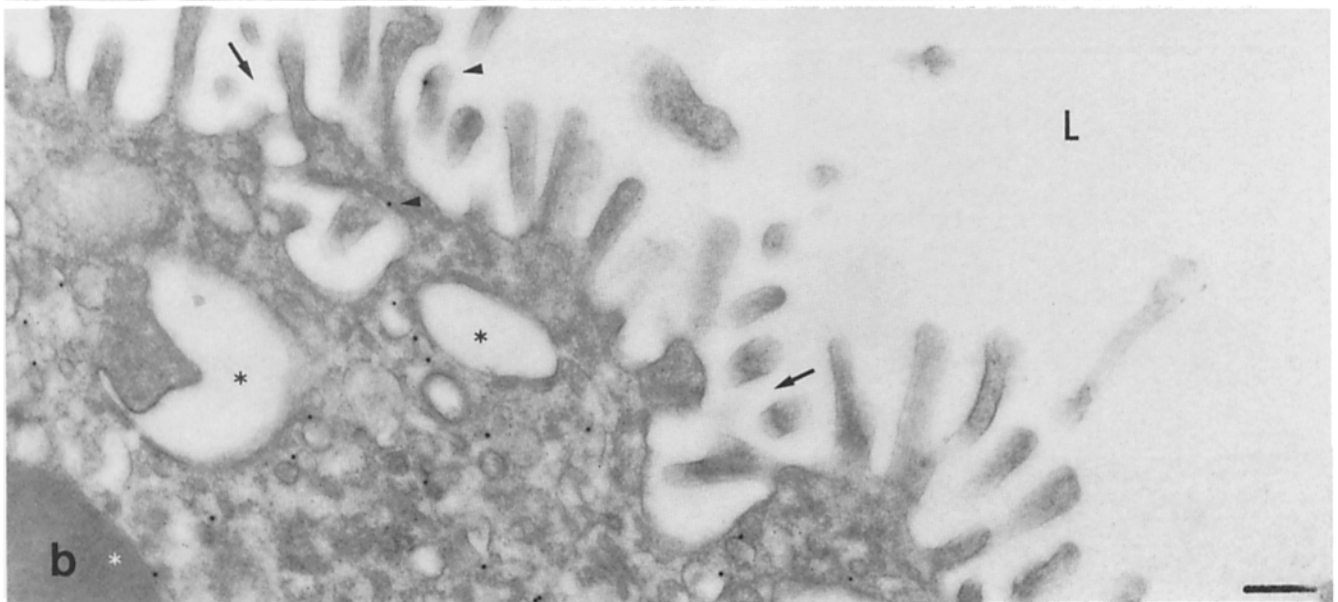
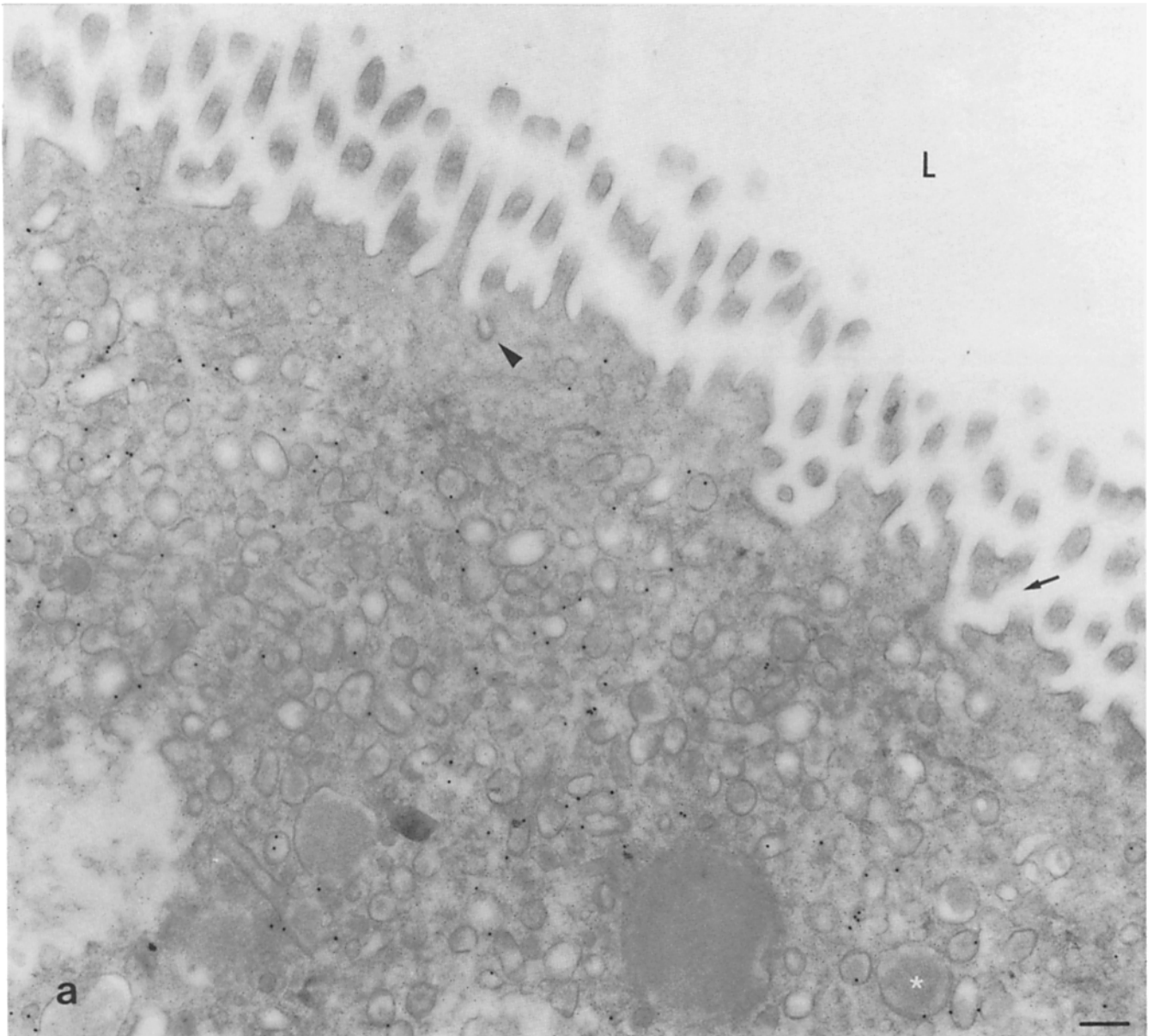


Figure 3. Indirect immunofluorescence micrographs and corresponding phase or DIC images of yolk sac immunostained with MC-39. Cryosections from villous region (*a*) and nonvillous region (*c*) bind MC-39 in the endoderm (*E*). Control (*e*) was stained with MOPC 21. Thinner Plexiglas sections offered greater resolution of staining within endodermal cells of the villous region (*g*). MC-39 binds throughout the cytoplasm in a punctate staining pattern. MC-39 does not bind to the brush border region. (*arrowheads*) Autofluorescent red blood cells; (*N*) endothelium; (*M*) mesothelium. (*b*, *d*, and *f*) phase; (*h*) DIC. Bars, 33 μm .



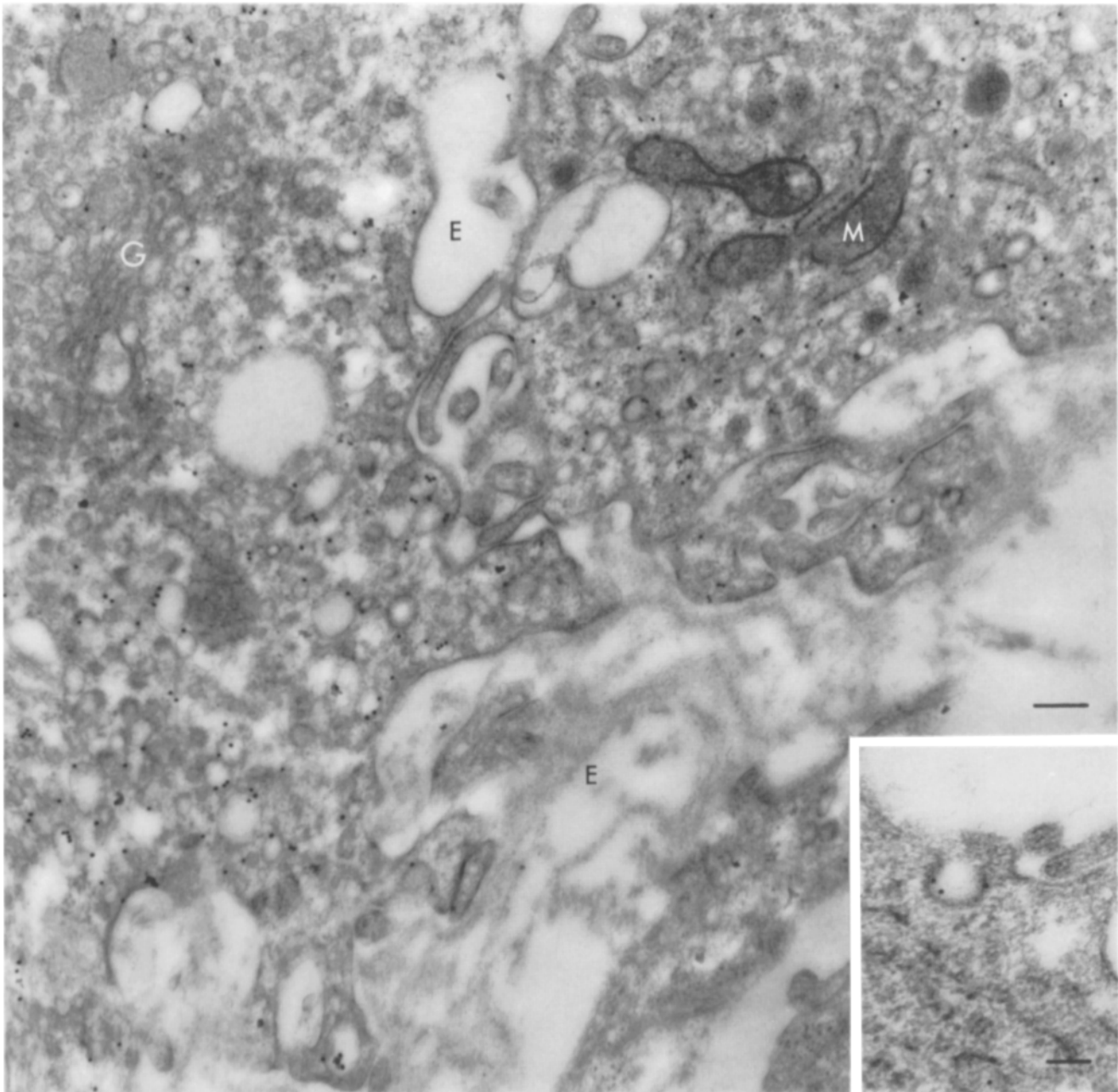


Figure 5. MC-39 binding in the basolateral region of endodermal cells. MC-39 stains numerous small vesicles and a coated pit on the basolateral membrane (inset). (G) Golgi; (M) mitochondria; (E) extracellular space. Bars: 200 nm; (inset) 100 nm.

The Uterine Lumen Has a Slightly Basic pH

The evidence indicates that both the yolk sac and intestinal Fc receptors bind IgG at an acidic pH (pH 6.0) and release IgG at a basic pH (pH 8.0), but have different distributions in their respective epithelial cells. Therefore, we measured

the pH of the uterine fluid to determine if differences in the environments present at the apical surface of endodermal and intestinal epithelial cells might explain the difference in receptor distribution. We used two separate methods to measure uterine pH. Using a colorimetric method with narrow range pH paper, we measured the pH at multiple sites along

Figure 4. Electron microscopic localization of MC-39 binding in the apical region of endodermal cells. (a) MC-39 binds to small tubules and vesicles in the cytoplasm. Apical invaginations and microvilli rarely label with gold. An apical invagination resembling a coated pit is seen (*large arrowhead*) as well as a deep apical invagination (*arrow*). (b) An example is seen of the rare association of gold with microvilli and a deep apical invagination (*small arrowheads*). No gold is associated with electron-lucent vacuoles (*black asterisks*). Occasionally the membrane of large vesicles with electron-dense contents label with gold (*white asterisks*). (L) Uterine lumen. Bars, 200 nm.

Table I. Measurement of Uterine Lumen pH

	n	Mean (\pm SD)
pH paper	28 (6)	7.1 \pm 0.2
pH meter	10 (2)	7.4 \pm 0.3

n = number of measurements (number of pregnant females given in parentheses).

the uterine horn in six pregnant females. The mean of 28 measurements showed a neutral to slightly basic pH (pH 7.1) (Table I). In the second method, we used a pH meter and microelectrodes and measured a mean pH from 10 measurements that was only slightly more alkaline (pH 7.4) (Table I). Thus, the results from both methods agreed closely and showed the uterine fluid to have a consistently neutral to slightly basic pH with the most acidic reading in any case being pH 6.9. By way of comparison, using the pH paper method, Rodewald (1976) determined that the pH of the lumen of the proximal intestine is 6.0–6.5. Importantly, in this acidic pH range, IgG can be bound by the intestinal receptor (Rodewald, 1976; Rodewald and Abrahamson, 1982). These data suggest that the yolk sac Fc receptor would not be able to bind IgG efficiently if it was located on the luminal membrane of endodermal cells.

Discussion

Since the Fc receptors of both the neonatal small intestine and the yolk sac endoderm are involved in transmission of maternal IgG, we reasoned that the two receptors could be structurally similar or even identical. Therefore, we initiated these studies by searching for antigenically related proteins with an mAb (MC-39) to the 45–54-kD component of the intestinal receptor. We have found that MC-39 recognizes a 54–58-kD endodermal protein, p57, which, in addition to sharing an antigenic epitope, has other similarities with the 45–54-kD component of the intestinal receptor. Most significantly, β_2 -microglobulin, a subunit of the intestinal receptor (Simister and Mostov, 1989), copurifies with p57. Both the Fc receptors of the yolk sac and the intestine, therefore, appear to have two components: a related heavy chain recognized by MC-39, and β_2 -microglobulin. Since the heavy chain of the intestinal receptor has significant amino acid homology with the heavy chain of class I major histocompatibility antigens (Simister and Mostov, 1989), which also contain β_2 -microglobulin (Bjorkman et al., 1987; Nakamuro et al., 1973), we predict that p57 has structural homology with these antigens. We note that Schlamowitz and co-workers (Cobbs et al., 1980) isolated a high molecular mass complex (>1,500 kD) with Fc receptor activity from the rabbit yolk sac, but the composition of this complex was not further defined.

We have shown that, in addition to the structural similarities, both yolk sac and intestinal Fc receptors (Rodewald and Abrahamson, 1982; Rodewald and Kraehenbuhl, 1984) bind to IgG at an acidic pH (pH 6.0) and release IgG at a more basic pH (pH 8.0), properties that allowed us to affinity purify the receptors. As with the intestinal receptor (Rodewald and Abrahamson, 1982), we suggest that this pH dependence of binding may be important for the transport of IgG across endodermal cells.

An unexpected finding in light of the many similarities between the yolk sac and intestinal receptors is that they have different subcellular distributions. Although both receptors are found in vesicles in the apical and basolateral cytoplasm, only the intestinal and not the yolk sac receptor is clearly located on the luminal surface of cells (Berryman and Rodewald, 1990; Rodewald, 1976). Importantly, the luminal contents of the neonatal proximal intestine has an acidic pH (Rodewald, 1976), whereas the uterine fluid has a neutral to slightly basic pH, suggesting that even if the yolk sac receptor were located on the apical surface, it would not bind IgG. The intriguing question remains as to what establishes the differences in subcellular distribution of the yolk sac and intestinal receptors. Endodermal cells, for example, may have a different pathway for protein trafficking which does not allow the receptor access to the apical surface. Alternatively, the yolk sac receptor may lack a functional signal for apical sorting or contain a signal for an internal target. Possibly relevant to this question is that p57 of the yolk sac receptor has a slightly but significantly greater apparent molecular mass than the heavy chain of the intestinal receptor. The factors contributing to this difference are unknown. We note, however, that Miettinen et al. (1989) have recently shown that two isoforms of a macrophage–lymphocyte Fc receptor which differ by a 47 amino acid insert also differ in their subcellular distributions. In addition, we detect in homogenates of embryonic small intestine an MC-39 cross-reactive protein that has the same molecular mass as p57. This observation suggests that the presumptive Fc receptor of embryonic intestine and the yolk sac receptor could be a fetal form of the neonatal receptor. Our mAb, MC-39, will be a useful tool enabling us to analyze the structure of these receptors to determine if they are the same protein with differences in posttranslational modifications, from different but related genes, or from the same gene but arising by differential splicing from the mRNA.

We propose the following model for IgG transport across the yolk sac. Instead of selective binding on the luminal cell surface, the yolk sac receptors bind IgG only after non-specific uptake with other proteins from the uterine lumen in keeping with the digestive function of these cells. Selective binding occurs after endocytosed contents are delivered to a receptor-bearing compartment where the pH would be sufficiently acidic for specific binding. We suggest that the endosomal complex is this site, consistent with the sorting function and the acidic nature of the endosome (Helenius et al., 1983; Mellman et al., 1986). Further support for this notion is our immunogold localization of MC-39 in small tubules and vesicles in the apical cytoplasm. An apical vesicular–tubular complex is a structure common to endosomes in many cell types (Kugler and Miki, 1985; Helenius et al., 1983; Geuze et al., 1983; Hatae et al., 1986, 1988; Griffiths et al., 1989). Once IgG is bound by receptor, small vesicles containing the receptor–ligand complex bud off the endosome and travel to the basolateral surface where these vesicles then fuse with the membrane and release IgG into the extracellular space. Since our studies demonstrate that the receptor releases IgG at a basic pH, we suggest that the interstitial fluid (\sim pH 7.4) could trigger release, although the transport vesicles from the endosome could develop a basic pH en route to the basolateral surface.

Brambell et al. (1958) were first to postulate that maternal

IgG is taken up with other proteins in a nonspecific manner by the endoderm of the yolk sac. These authors suggested that protein from the uterine lumen was nonspecifically absorbed by the cell and was delivered to phagolysosomes. Here, Fc receptors (Brambell, 1970), by the direct result of binding, would protect IgG from lysosomal hydrolases within the phagolysosomes. Phagolysosomes would fuse with the basolateral surface of the cell and release both functionally intact maternal IgG and products of digestion. Our model agrees with several points of this model, namely, a nonspecific uptake of IgG at the apical surface and binding to Fc receptors within an apical compartment. In our model, however, the receptors protect IgG from lysosomal digestion by the removal of IgG from a prelysosomal compartment consistent with the presence of a sorting compartment in the endocytic pathways of many cell types (Helenius et al., 1983; Mellman et al., 1986; Salzman and Maxfield, 1989).

Considerable biochemical (Williams et al., 1975) and morphological (Lambson, 1966; King and Enders, 1970; Slade, 1970; Seibel, 1974) evidence indicates that a large amount of nonselective uptake occurs at the apical surface of endodermal cells where proteins appear to adsorb nonspecifically to a characteristic dense coat on the luminal surface of apical invaginations. Presuming this coat contains a nonspecific protein receptor (Rodewald, 1980; King 1982), we would expect that this receptor would bind IgG as well as other proteins at the more basic pH of the uterine lumen and would release these proteins in the more acidic environment of the endosomal complex. This raises the interesting possibility that the nonspecific receptor and specific IgG receptor, with opposite pH-dependent binding properties, function sequentially for delivery of IgG to its proper destination. This model is consistent with a conclusion by Douglas and King (1988) that passage through an acidic interior compartment is required for efficient transport of IgG across the endoderm.

Our evidence does not support the model of Moxon et al. (1976) who proposed that the IgG that is transported intact across the yolk sac is taken up by a separate, specific route by way of Fc receptors in coated pits at the apical surface. Coated vesicles would then transport IgG directly to the basolateral surface bypassing any other intracellular compartments. King (1982) proposed a variation of this hypothesis in which the coated vesicles involved in specific transport could also originate from the deep invaginations of the apical membrane. Although we cannot completely rule out the coated pit as a site for specific entry into the cell, we find no evidence of receptors in coated pits or in any other type of invagination at the luminal cell surface.

Fc receptors transfer IgG across the yolk sac endoderm in the rabbit, guinea pig, and chicken (Brambell, 1970). In each case, it is postulated that these receptors are present on the apical surface (Johanson et al., 1985; Tressler and Roth, 1987; Douglas and King, 1988). Although these receptors have not been fully characterized, they all appear to bind IgG in the same pH-dependent manner as the receptor in the rat yolk sac. In the chicken where the pH of the yolk, which bathes the apical surface of endodermal cells, is reported to be pH 6.0 (Tressler and Roth, 1987), surface receptors may selectively bind IgG. In the rabbit, however, where the pH of the uterine lumen is pH 7.3 (Johanson, 1985), we predict that Fc receptors would bind IgG within the acidic endosomal complex after nonspecific entry.

Maternal IgG, in primates, is also transmitted selectively before birth (Brambell, 1970). However, because of the different organization of the fetal membranes, the yolk sac is not used for transport, and instead, IgG crosses the syncytiotrophoblast of the chorio-allantoic placenta. As in the yolk sac endoderm, transfer is presumed to occur via a specific IgG-Fc receptor (Brambell, 1970). Like the endoderm, the syncytiotrophoblast is polarized, contains microvilli on its apical surface, and is capable of endocytosis. As in the endoderm, the pH at the luminal and abluminal surfaces of the syncytiotrophoblast may be near pH 7.4 since the apical surface is bathed by the maternal blood and the basolateral surface contacts the fetal capillaries. Interestingly, a human homologue of the mouse macrophage Fc receptor is expressed in human syncytiotrophoblast (Stuart et al., 1989) and the mouse macrophage Fc receptor can transcytose IgG when experimentally expressed in MDCK cells (Hunziker and Mellman, 1989). This suggests that a macrophage-like receptor, which is not related to the Fc receptor of the neonatal intestine, could function in the syncytiotrophoblast to transfer IgG. We predict that such a receptor in the syncytiotrophoblast would have diverged enough from the macrophage receptor to gain a pH-dependent binding that would allow net transfer of IgG across the syncytium by a mechanism similar to that in the yolk sac.

Our model for IgG transport across the yolk sac differs from the mechanism proposed for transport across the neonatal intestine (Rodewald, 1973; Rodewald and Abrahamson, 1982), mainly in the cellular location for specific binding. Differences in tissue organization suggest reasons for the apparent differences in transport. In the neonatal small intestine, selective transcellular transport of IgG and nonspecific uptake of proteins for digestion are segregated between two different cell types found in the proximal and distal regions of the small intestine, respectively (Rodewald, 1973; Rodewald, 1980). The location of Fc receptors directly on the surface of cells which are specialized for IgG transport may be an adaptation for enhancing IgG uptake that is only possible where digestion and selective transport can be separated. In the yolk sac endoderm, in contrast, one cell type must perform both functions, resulting in transcellular IgG transport that may be less efficient than in the neonatal intestine, but which nevertheless provides important passive immunity to the newborn.

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