Expression of Macrophage–Lymphocyte Fc Receptors in Madin–Darby Canine Kidney Cells: Polarity and Transcytosis Differ for Isoforms with or without Coated Pit Localization Domains

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Abstract. Many cells of the immune system and certain epithelia express receptors for the Fc domain of IgG (FcR). On mouse macrophages and lymphocytes, two distinct receptor isoforms have been identified, designated FcRII-B1 and FcRII-B2. The isoforms are identical except for an in-frame insertion of 47 amino acids in the cytoplasmic tail of FcRII-B1 that blocks its ability to be internalized by clathrin-coated pits. We have recently found that at least one IgG-transporting epithelium, namely placental syncytial trophoblasts, expresses transcripts encoding a receptor similar or identical to macrophage–lymphocyte FcRII. To determine whether FcRII of hematopoietic cells might also function as a transcytotic receptor if expressed in epithelial cells, FcRII-B1 and -B2 were transfected into Madin–Darby canine kidney (MDCK) cells and grown on permeable filter units. The two FcRII isoforms exhibited different patterns of polarized expression: FcRII-B1 was localized mainly to the apical plasma membrane domain, whereas FcRII-B2 was found predominantly on the basolateral surface. As expected for FcR in placenta, FcRII-B2 and to a lesser extent FcRII-B1 mediated transcellular transport of IgG-complexes from the apical to the basolateral plasma membrane. Neither receptor mediated transcytosis in the opposite direction, although FcRII-B2 also delivered ligand to lysosomes when internalized from either the basolateral or apical domains. Furthermore, FcRII-B2 was capable of transporting monovalent antireceptor antibody Fab fragments across the cell, suggesting that transcytosis was not dependent on receptor cross-linking. These findings suggest the possibility that FcRII can mediate transepithelial IgG transport when expressed in placental syncytial trophoblasts in addition to its "classical" endocytic and signaling activities when expressed in macrophages. Because FcRII-B1 and -B2 are expressed with distinct polarities, the results also suggest that interactions with clathrin-coated pits may play a role in generating the polarized distribution of at least some plasma membrane proteins in MDCK cells.

MACROPHAGES, granulocytes, and many lymphocytes express receptors for the Fc domains of IgG (Mellman et al., 1989; Mellman, 1988). In macrophages, Fc receptors (FcR)[1] can be associated with different activities such as clearance of antibody–antigen complexes via receptor-mediated endocytosis (Mellman and Plutner, 1984; Ukkonen et al., 1986; Mellman et al., 1984), phagocytosis of IgG-coated particles (Mellman et al., 1983), and triggering the synthesis and release of potent inflammatory mediators and cytotoxic agents (Unkeless et al., 1981; Nathan and Cohn, 1980).

Some epithelial cells, such as placental syncytial trophoblasts and rat neonatal intestinal epithelium, also express IgG Fc receptors. In these examples, FcR mediate the apical to basolateral transcytosis of maternal IgG and are thus responsible for conferring passive immunity to the fetus or newborn (Abrahamson and Rodewald, 1981; Rodewald et al., 1983; Simister and Rees, 1985; Balfour and Jones, 1978; Matre et al., 1984; Niezgodka et al., 1981).

Macrophage–lymphocyte FcRs have been extensively characterized on both human and murine cells, the best understood being the mouse receptor for murine IgG1/IgG2b, designated FcRII (Unkeless, 1979; Mellman and Unkeless, 1980). cDNAs corresponding to three distinct FcRII isoforms (FcRII-A, FcRII-B1, and FcRII-B2) have been cloned (Mellman et al., 1989), each encoding single-span transmembrane glycoproteins that are members of the immunoglobulin gene family (Hibbs et al., 1986; Lewis et al., 1986; Ravetch et al., 1986). Although the isoforms have identical or nearly identical ectodomains, their cytosolic tails and/or

1. *Abbreviations used in this paper*: FcR, Fc receptor.

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membrane-spanning segments are heterogeneous. In the case of FcRII-B1 and -B2, this heterogeneity probably arises by differential splicing at the 3′ end of mRNA transcribed from a single gene because the two isoforms differ only by a 46-amino acid inframe insertion in the cytosolic tail of FcR-B1 (Lewis et al., 1986; Ravetch et al., 1986). We have recently found that one important functional difference between the two isoforms is their ability to interact with coated pits: when expressed in Chinese hamster ovary (CHO) cells, only FcRII-B2 could accumulate at coated pits and mediate the efficient endocytosis of bound IgG-complexes (Miettinen et al., 1989). FcRII-B2 is usually expressed by macrophages, a cell type highly adept at FcR-mediated endocytosis, whereas FcRII-B1 is found mostly in B lymphocytes, cells that are far less efficient at the internalization of immune complexes (Miettinen, H., unpublished results).

Although there is comparatively little information concerning the structure and function of IgG FcR expressed by epithelial cells, there is reason to believe that they will bear some similarity to the FcR on macrophages and lymphocytes. Firstly, the one extensively characterized transcytotic FcR, the receptor for polymeric IgA/IgM (pIg-R) of hepatocytes and mammary epithelium, is also known to be a member of the immunoglobulin gene family (Mostov et al., 1984). In contrast to epithelial receptors for IgG, however, the pIg-R mediates transcytosis from the basolateral to the apical plasma membrane (reviewed by Mostov and Simister, 1985). Second, the molecular masses of putative transcytotic IgG Fc receptors from placenta and intestinal epithelia are ~40–50 kD (Balfour and Jones, 1978), similar to FcRII of immune cells.

While a neonatal rat intestinal IgG FcR has recently been cloned and found to be related to MHC class I antigens (Simister and Mostov, 1989), little is known about the structure of the placental receptors. We recently cloned the human homologue of FcRII-B2 from a human placental library, sharing 70% amino acid identity throughout its length with FcRII-B2. It was shown by Northern blot analysis to be expressed in different cells of the immune system and in cultures of placental syncytial trophoblasts. Furthermore, in situ hybridization experiments and immunohistochemistry also suggested that syncytial trophoblasts express high levels of macrophage–lymphocyte FcRII (Stuart et al., 1989).

In this paper, we evaluate the potential functional significance of the presence of a macrophage-lymphocyte FcR in placental epithelium by expressing FcRII isoforms in Madin–Darby canine kidney (MDCK) cells, a cell line well characterized for the study of polarized membrane protein transport and receptor-mediated transcytosis (Mostov and Deitcher, 1986; Maratos-Flier et al., 1987). We found striking differences in the polarized expression and pathways of the two FcRII isoforms in MDCK cells, presumably reflecting their different abilities to localize in coated pits. Furthermore, these receptors were found to mediate the unidirectional transcellular transport of IgG-complexes from the apical to the basolateral membrane, the same directionality as would be expected for a transcytotic FcR of placental and intestinal epithelia. Therefore, it is possible that FcRII may represent a second class of epithelial receptors for IgG found in placenta, structurally distinct from, but functionally related to, the MHC class I antigen–like intestinal receptor.

Materials and Methods

Construction of pWE-FcRII-B1 and -B2

DNA manipulations involved standard techniques (Maniatis et al., 1982). A unique Pst I site located 50 bp upstream of the initiation codon and a Bgl I recognition sequence 31 bp downstream of the stop codon were used to isolate the complete coding regions of both FcRII-B1 and -B2 cDNAs (Lewis et al., 1986; Ravetch et al., 1986). The restriction fragments were blunt-ended with T4 DNA polymerase and cloned into the previously blunted Bam HI cloning site in the retroviral vector pWE (Choudray et al., 1986; obtained from R. Mulligan via T. Claudio). The correct orientation of the inserts in the resulting plasmids pWE-FcRII-B1 and pWE-FcRII-B2 was verified by restriction mapping. DNA restriction and modification enzymes were purchased from International Biotechnologies, Inc. (New Haven, CT), Promega Biotec (Madison, WI), or Boehringer Mannheim Biochemicals (Indianapolis, IN).

Cell Culture

J774 macrophages were cultured in α-modified minimal essential medium (α-MEM, Gibco Laboratories, Grand Island, NY) containing 3.5% heat-inactivated FCS, 100 U/ml penicillin, and 100 μg/ml streptomycin. MDCK strain II cells (obtained from K. Matlin via M. Caplan) and Ψam cells (obtained from T. Claudio) were grown in DME (Gibco Laboratories) supplemented with 10% FCS, 100 U/ml penicillin, 100 μg/ml streptomycin, and 20 mM Hepes pH 7 at 37°C in 5% CO_2. MDCK cells were passaged 1:5 every week and a fresh stock was used after 15 passages. Polycarbonate filter units (Transwell units) were from Costar Corp. (Cambridge, MA) and experiments were performed on tissue culture-treated filters of 6.5 or 24 mm diameter and 3.0 μm pore size. Similar results were obtained using filters with 0.4 μm pore size. Cells from a confluent flask were trypsinized, washed, and taken up in medium. 1.5×10^5 (6.5-mm filter) or 1.5×10^6 (24-mm filter) cells were plated per well. Growth medium was changed daily and cells were used for experiments on the third day in culture.

Ligands

Fab-fragments of the rat mAb 2.4G2 (2.4G2 Fab; Unkeless, 1979) were radioiodinated to specific activities of $2–5 \times 10^6$ cpm/μg using Iodogen (Pierce Chemical Co., Rockford, IL). Dog holotransferrin (a gift form B. Podbilewicz) was radioiodinated to a specific activity of $\sim 5 \times 10^6$ cpm/μg. Unincorporated ^{125}I (Amersham Corp., Arlington Heights, IL) was removed by ion exchange chromatography on Dowex-1 (Sigma Chemical Co., St. Louis, MO) as described (Mellman et al., 1983). IgG-complexes (RαDNP:DNP-BSA) of rabbit antidinitrophenol (RαDNP) and DNP-BSA were formed as described (Ukkonen et al., 1986), and RαDNP or DNP-BSA was radioiodinated as outlined above.

Generation of MDCK Cells Stably Expressing FcR

10 μg of pWE-FcRII-B1, pWE-FcRII-B2, or pWE without insert were added to 0.5 ml Hepes-buffered saline (Parker and Stark, 1979) and precipitated by the addition of 31 μl of 2M $CaCl_2$ for 45 min at 22°C. This suspension was added to a 10-cm dish of Ψam cells which had been split 1:5 from a confluent dish the previous day. After 20 min, 10 ml of medium was added and the dish incubated for 4 h at 37°C. The medium was then replaced by 2.5 ml of a 15% glycerol solution in Hepes-buffered saline. After 3.5 min at 37°C, the glycerol was removed and the cells were washed once with medium and cultured in 10 ml of fresh medium for 20 h. This medium (designated transient viral supernatant) was filtered through a 0.45-μm filter (Millipore Corp., Bedford, MA) and 2 ml of the transient viral supernatant containing 8 μg/ml polybrene (Sigma Chemical Co., St. Louis, MO) was added to a 10-cm dish of 20% confluent MDCK strain II cells (split the previous day) and incubated for 2.5 h at 37°C. 8 ml of fresh medium was added and the cells were continued to grow for 48 h. Cells were then split 1:100 into media supplemented with 0.5 mg/ml G418 (Gibco Laboratories). The selection media was changed every 4 d and after 10–13 d, well-isolated colonies were picked using cloning cylinders and expanded. After three to four passages, G418 was omitted from the medium.

Labeling of Cells

Confluent cell monolayers were washed three times with PBS and then

starved in MEM lacking cysteine and methionine (Select-Amine Kit; Gibco Laboratories) and supplemented with 10% dialyzed FCS (labeling medium) for 20 min at 37°C. Fresh labeling medium containing 0.25 mCi/ml of each, [^{35}S]cysteine (Amersham Corp.) and [^{35}S]methionine (Translabel; ICN Biomedicals, Costa Mesa, CA) was added and cells were labeled for 1 h. The labeling medium was removed, cells were chased for 15 min in complete DME, and the monolayers were washed three times with PBS. Lysis of cells and immunoprecipitation of FcR with a rabbit polyclonal anti-FcRII serum was essentially as described (Green et al., 1985), except that FcR were stripped from the immunoadsorbant in 0.5% SDS (500 μl; 100°C, 5 min), and after quenching the SDS with excess Triton X-100 (50 μl of a solution containing 0.5 M Tris-HCl pH 8; 1.5 M NaCl; 20 mM EDTA; 20% Triton X-100), FcRs were reprecipitated. SDS-PAGE (10% polyacrylamide, nonreducing conditions) and fluorography were essentially as described (Green et al., 1985).

Immunofluorescence

Cells grown on polycarbonate filters were incubated with 2 μg/ml of the anti-FcR mAb 2.4G2 added apically or basolaterally for 2 h at 4°C, washed five times for 5 min with PBS containing Ca^{2+} and Mg^{2+} (PBS$^+$) and then fixed in 3% paraformaldehyde (in PBS$^+$) for 2 h at 4°C. Filters were subsequently washed three times with PBS, quenched in 50 mM NH_4 Cl for 30 min and washed (all washes were for 5 min at room temperature with PBS). Nonspecific binding sites were blocked by incubation in 10% goat serum for 60 min. A 1:50 dilution of FITC-conjugated F(ab')$_2$ fragments of goat anti-rat IgG (Tago Immunologicals, Burlingame, CA) in 10% goat serum was then added for 60 min. The cells were again washed four times in PBS, once in water, mounted in Moviol and viewed with a Zeiss Axiophot microscope (Carl Zeiss, Inc., Thornwood, NY) using a 40× oil immersion lens, epifluorescence, and fluorescein filters. Pictures were taken with Kodak Tri-X Pan film, exposed and developed at 400 ASA.

Binding of 2.4G2 Fab and IgG-Complexes (Ligand)

Binding assays were essentially carried out as previously described (Mellman et al., 1983; Mellman and Plutner, 1984): saturating concentrations (1–2 μg/ml) of radioactive 2.4G2 Fab in complete DME were added either to the apical or basal compartment of the Transwell unit. Aliquots containing 2 μg/ml cold 2.4G2 IgG were present on the opposite side of the filter to quench binding of any radioactivity diffusing to this compartment. Binding was carried out on ice for 2 h. Intactness of each cell monolayer was determined by counting the media from both compartments in a gamma counter. Generally, <1% of the initially added label diffused to the opposite side. Both sides of the filter were then washed with five changes of ice cold PBS$^+$ over 25 min, filters were cut out of the Transwell unit and counted. In prebinding experiments, prewarmed medium was added to the Transwell unit and the plate was incubated at 37°C for variable periods of time. Specific binding values were obtained by correcting for nonspecific binding in the presence of excess (100 μg/ml) 2.4G2 IgG. Specific binding was typically >90% of total bound radioactivity.

Ligand binding was essentially as described for 2.4G2 Fab. IgG-complexes were used at a concentration of 20 μg RαDNP/ml medium and ~10^6 cpm were added per 6.5-mm filter. Binding was carried out on ice or, in some experiments, at 17°C for 2 h. Cold 2.4G2 IgG (2 μg/ml) was present on the opposite side of the filter to quench binding of any radioactivity diffusing to this compartment. Intactness of cell monolayers was monitored as outlined above and nonspecific binding (<15% of total bound radioactivity) was determined in the presence of excess (100 μg/ml) 2.4G2 IgG.

Binding of Transferrin

Filter-grown cells were washed twice with PBS$^+$ and starved for 30 min at 37°C in DME supplemented with 0.5% BSA. The cells were then placed on ice and 2 μg/ml dog holotransferrin (1–2 × 10^6 cpm) in DME with 0.5% BSA was added either to the apical or the basal compartment. In parallel experiments, a 100-fold excess of cold transferrin was included to estimate nonspecific binding (10–20%). After 1 h on ice, filters were washed, cut out, and counted in a gamma counter. Less than 1% of the initially added radioactivity diffused to the opposite compartment.

Uptake of Methionine

Uptake of [^{35}S]methionine (Amersham Corp.) was carried out essentially as described by Balcarova-Stander et al. (1984), except that after labeling, cells were washed five times with cold complete medium on ice. Filters were then cut out and transferred into 1 ml of 1% SDS, 0.5 M EDTA, boiled for 5 min, vortexed, and cooled on ice. The filters were removed and the lysate briefly sonicated to shear DNA. 250-μl aliquots were then TCA-precipitated (20% TCA, 60 min on ice) and the pellets were washed with cold acetone, dissolved in 4 ml OptiFluor (Packard Instruments, Downers Grove, IL), and counted in a scintillation counter.

Degradation of IgG-Complexes

Prebinding of ^{125}I-DNP-BSA complexes was carried out at 4°C as described above. After variable periods of time at 37°C, the Transwell plates were transferred on ice, the apical and basal media were collected, TCA precipitated (20% TCA, 60 min on ice) and the pellet and supernatant counted in a gamma counter. Polycarbonate filters were cut out of the unit and also counted. Specific degradation was calculated by subtracting values obtained in the presence of excess 2.4G2 IgG (100 μg/ml) and expressed in % of initially bound radioactivity.

Transcytosis of IgG-Complexes

The transcytosis assay based on detecting the appearance of IgG-complexes on the surface opposite from the side of addition using binding of ^{125}I-labeled F(ab')$_2$ fragments of goat anti-rabbit IgG (Tago Immunologicals) to the RαDNP component in the ligand. Ligand (20 μg/ml) was added directly in prewarmed medium to the apical (or basal) compartment and after different periods of time at 37°C the Transwell units were cooled to 4°C, washed from both sides five times for 5 min with cold PBS$^+$ and 2 μg/ml of ^{125}I-(Fab')$_2$ fragments of goat anti-rabbit IgG in cold medium was added to the basal (or apical) compartment for 2 h. Binding of radioactive (Fab')$_2$ fragments diffusing to the opposite side was quenched by adding nonradioactive goat anti-rabbit IgG (Tago Immunologicals) at a concentration of 2 μg/ml. The filters were washed, cut out and bound radioactive (Fab')$_2$ fragments quantitated in a gamma counter. Alternatively, ligand was prebound at 4°C or 17°C as outlined above and Transwell units then transferred into prewarmed medium for different periods of time and further processed as above. Nonspecific transcytosis (<15%) was determined in the presence of excess 2.4G2 IgG (100 μg/ml). Diffusion of IgG-complexes and ^{125}I-(Fab')$_2$ fragments to the opposite compartment was <1%. Control experiments confirmed that diffusion did not generate the signal interpreted as transcytosis.

Transcytosis of 2.4G2 Fab Fragments

^{125}I-2.4G2 Fab fragments were prebound apically to filter grown MDCK cells as outlined above. After washing unbound antibody, Transwell units were transferred into prewarmed medium for 90 min. Filters were then cooled on ice. To remove 2.4G2 Fab fragments transcytosed to the basolateral side, filters were washed two times with media adjusted to pH 3.5 with HCl (Miettinen et al., 1989). Subsequently, the apical surface was acid-washed. More than 90% of surface bound 2.4G2 Fab fragments were removed by this method. Nonspecific transcytosis (<15%) was determined in the presence of excess 2.4G2 IgG (100 μg/ml). Diffusion of ^{125}I-2.4G2 Fab fragments to the opposite compartment was <1%.

Preparation of Colloidal Gold Conjugates

Colloidal gold (5–8 nm) was made by tannic acid-citrate reduction of gold chloride according to the method of Slot and Geuze (1985). Gold particles were conjugated to DNP-BSA as described (Ukkonen et al., 1986) and conjugates were purified by linear (10–30%) sucrose gradient centrifugation (Slot and Geuze, 1981). Colloidal gold–tagged IgG-complexes were prepared as described above except that gold-DNP-BSA was used in place of DNP-BSA.

Electron Microscopy

Gold-conjugated IgG-complexes in medium were bound for 2 h on ice from the apical or basolateral compartment to cells grown on polycarbonate filter units. Cells were washed with cold PBS$^+$ and then incubated in prewarmed medium for 2 min. After washing twice with cold PBS$^+$, cells were fixed with 2.5% glutaraldehyde in 50 mM cacodylate buffer (pH 7.4) for 30 min, postfixed in 2% OsO_4 for 2 h at 4°C, and stained with 0.5% uranyl acetate. Samples were then dehydrated with ethanol, infiltrated with propolyene oxide, and embedded in Epon. Sections were examined with a JEM-100 CX electron microscope (JEOL USA, Peabody, MA). Ligand binding was

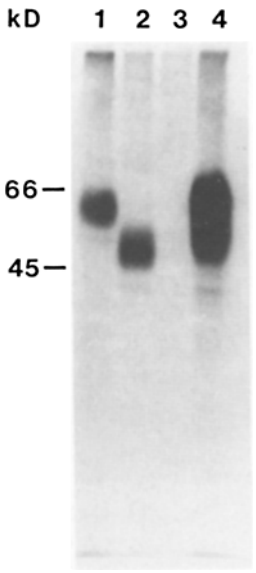


Figure 1. Expression of FcRII-B1 and -B2 in MDCK cells. MDCK-B (lane *1*), -B (lane *2*), -WE (lane *3*), and J774 cells (lane *4*) were metabolically labeled with [^{35}S]cysteine/[^{35}S]methionine for 60 min. Cells were lysed and FcRII immunoprecipitated with a specific anti-FcRII serum. Immunoprecipitates were analyzed by electrophoresis on non-reducing SDS polyacrylamide (10%) gels and fluorography. Positions of molecular weight markers are indicated on the left in kilodaltons.

specific and could be inhibited by the anti-FcRII mAb 2.4G2 (100 μg/ml). Micrographs of cells containing gold particles were taken at 19,000×.

Results

Expression of Macrophage-Lymphocyte Fc Receptors in MDCK Cells

To obtain expression of FcRII-B1 and -B2 in MDCK cells, we used the retroviral expression system previously employed to express the rabbit pIg-R in this cell line (Mostov and Deitcher, 1986). The coding sequences of cDNAs for FcRII-B1 and -B2 (Lewis et al., 1986; Ravetch et al., 1986) were cloned downstream of the chicken β-actin promoter in the retroviral vector pWE (Choudray et al., 1986). This vector provides the signals necessary for the integration of a DNA copy into the host genome, for RNA transcription and processing and carries a neomycin resistance gene as selectable marker. Recombinant amphitrophic virus was produced in Ψam cells (Cone and Mulligan, 1984) and then used to infect MDCK cells (strain II). Stably infected cells, selected by their resistance to the neomycin analogue G418, were efficiently produced, cloned, and expanded for further analysis.

To identify G418-resistant infected clones also expressing FcRII, cells were metabolically labeled with [^{35}S]cysteine/[^{35}S]methionine and FcR detected by immunoprecipitation using a polyclonal rabbit anti-FcRII serum (Green et al., 1985). Two clones designated MDCK-B1 and MDCK-B2 were selected for further study because they contained high levels of labeled FcR. As shown in Fig. 1, MDCK-B1 expressed a polypeptide of ~60 kD corresponding in size to FcRII-B1 (lane *1*); as expected, a slightly smaller protein (47 kD) was immunoprecipitated from MDCK-B2 (lane *2*). No immunoreactive polypeptides were detected in MDCK-WE (lane *3*), a G418-resistant clone obtained by transfection with pWE alone. FcRII-B1 and FcRII-B2 synthesized by the MDCK cells comigrated with the broad band immunoprecipitated from the J774 macrophage cell line (lane *4*) which is known to express both FcRII isoforms (Koch, T., and I. Mellman, unpublished results).

Thus, it was possible to obtain efficient and stable expression of murine macrophage-lymphocyte FcRs in a cell line of epithelial origin.

FcRII-B1 and FcRII-B2 Exhibit Distinct Polarities

To determine if MDCK-B1 and -B2 expressed the transfected FcRs in a polarized fashion, we next grew the cells on permeable supports and analyzed receptor distribution on the apical and basolateral domains by quantitative binding assays and by immunofluorescence. Transfected cells were cultured on polycarbonate filter units (0.4- and 3.0-μm-pore size Transwells) that yield polarized monolayers with intact tight junctions and that allow selective addition of detection reagents to the apical and basolateral cell surfaces (Fuller et al., 1984; Simons and Fuller, 1985).

Receptor expression was quantified using iodinated Fab fragments of the rat anti-mouse FcRII mAb 2.4G2 (Unkeless, 1979). By adding Fab fragments to both the apical and basolateral compartments, we first estimated that MDCK-B1 expressed a total of ~2.2 × 10^5 receptors per cell, while

Table I. Polarized Expression of Fc Receptors, Transferrin Receptor, and Methionine Uptake in Transfected MDCK Cells

Cell line	Domain	Fc receptor		Transferrin	Met uptake
		2.4G2 Fab	IgG-complex		
		%	%	%	%
MDCK-B1	Apical	65	71	1	6
	Basal	35	29	99	94
MDCK-B2	Apical	20	14	2	10
	Basal	80	86	98	90
MDCK-tail minus	Apical	80	89	3	–
	Basal	20	11	97	–
MDCK parent	Apical	0	0	1	6
	Basal	0	0	99	94

^{125}I-2.4G2 Fab fragments (1 μg/ml), ^{125}I-IgG-complexes (ligand, 20 μg/ml), and ^{125}I-dog transferrin (2 μg/ml) were bound from the apical or basal side for 2 h on ice. Incorporation of [^{35}S]methionine (25 μCi/ml) was for 5 min at 37°C. Specific values (see Materials and Methods) were expressed as percent of total (apical and basolateral) binding or incorporation. 100% values in counts per minute were: 2.4G2 Fab: 11,700 (MDCK-B1), 5,850 (MDCK-B2), 6,366 (MDCK-tail minus); IgG-complex: 11,333 (MDCK-B1), 4,886 (MDCK-B2); transferrin: ~9,000 (all cell lines); [^{35}S]methionine: ~30,000 (all cell lines). Points were performed in duplicate and standard errors of the mean were <15%. Numbers for binding are the mean of two independent experiments. Total (apical and basolateral) binding of 2.4G2 Fab and IgG-complexes to nontransfected MDCK cell monolayers was 200–300 cpm.

MDCK-B2 expressed somewhat fewer surface receptors (~1.1 × 10^5 per cell). To measure receptor distribution, binding assays were next performed by adding Fab from either the apical or basolateral side. As summarized in Table I, distribution of the two FcR isoforms was found to be both polarized and distinct from each other. In MDCK-B1, ~65% of the receptors were found to be on the apical plasma membrane with only ~35% basolaterally. The polarity of expression in MDCK-B2 was reversed, with only ~20% of the receptors apically and ~80% basolaterally. Analysis of additional clones showed 45–65% of FcRII-B1 and 8–20% of FcRII-B2 on the apical plasma membrane at steady state. Furthermore, uncloned populations of infected cells expressed 58% of FcRII-B1 and 15% of FcRII-B2 apically, indicating that the distributions observed in the cloned cell lines selected were representative. An MDCK clone infected with a mutant FcRII devoid of a cytoplasmic tail (MDCK-tail minus) was similar to MDCK-B1, and expressed most (~80%) of its receptors apically (Table I). The same distributions were obtained using binding of radioiodinated IgG-complexes (i.e., ligand) to detect FcRII (Table I). No specific binding of 2.4G2 Fab or immune complexes was observed on the parental MDCK strain (Table I).

To ensure that the introduction of the FcRII into MDCK cells did not alter the polarized distribution of endogenous markers, we next analyzed the polarity of [^{35}S]methionine uptake and the cell surface distribution of the transferrin receptor. More than 90% of the methionine uptake (Balcarova-Stander et al., 1984) occurred from the basolateral surface of infected and control cells (Table I), similar to previous results for the MDCK II strain (Mostov et al., 1987). For a second basolateral marker, we measured the distribution of transferrin receptors (Fuller and Simons, 1986). Using ^{125}I-transferrin binding at 4°C (see Materials and Methods), >98% of the specific receptor binding activity was restricted to the basolateral plasma membrane (Table I). Because no significant differences were found for methionine uptake or transferrin binding between parental MDCK II cells and any of the infected cell lines, it is apparent that the different distributions of FcRII-B1, -B2, and the tail-minus mutant on the apical vs. basolateral domains were not due to endogenous differences in polarization of the MDCK-B1 and -B2 cell lines.

To ensure that the polarized distribution of the different FcRs did not reflect only a small subpopulation of cells even within cloned cell lines, receptor expression was next examined by indirect immunofluorescence. 2.4G2, an anti-FcR mAb, was added to either the apical or basolateral sides of the cell monolayers, allowed to bind at 4°C and the cells were then fixed with paraformaldehyde in the presence of Mg^{2+} and Ca^{2+} to keep tight junctions intact (Fuller et al., 1984). Bound complexes were visualized with fluorescein-conjugated $(Fab')_2$ second antibody. As shown in Fig. 2, MDCK-B1 and MDCK-B2 showed basolateral staining which was uniform from cell to cell and exhibited a characteristic pattern outlining the cell borders. Intensity of basolateral staining was similar for both MDCK-B1 and -B2, as expected (although a greater proportion of receptors was basolateral in MDCK-B2, the absolute number of basolateral receptors was similar to MDCK-B1; see Table I). MDCK-B1 showed bright, punctate staining on the apical surface of almost every cell, whereas the apical staining on MDCK-B2 was very faint due to the relatively low number of receptors on the apical domain of this cell line (see Table I). In contrast, cells expressing the tail-minus mutant receptor showed predominant apical staining. Using nontransfected MDCK cells, no specific staining was observed following apical or basolateral addition of 2.4G2 IgG and fluorescent second antibody.

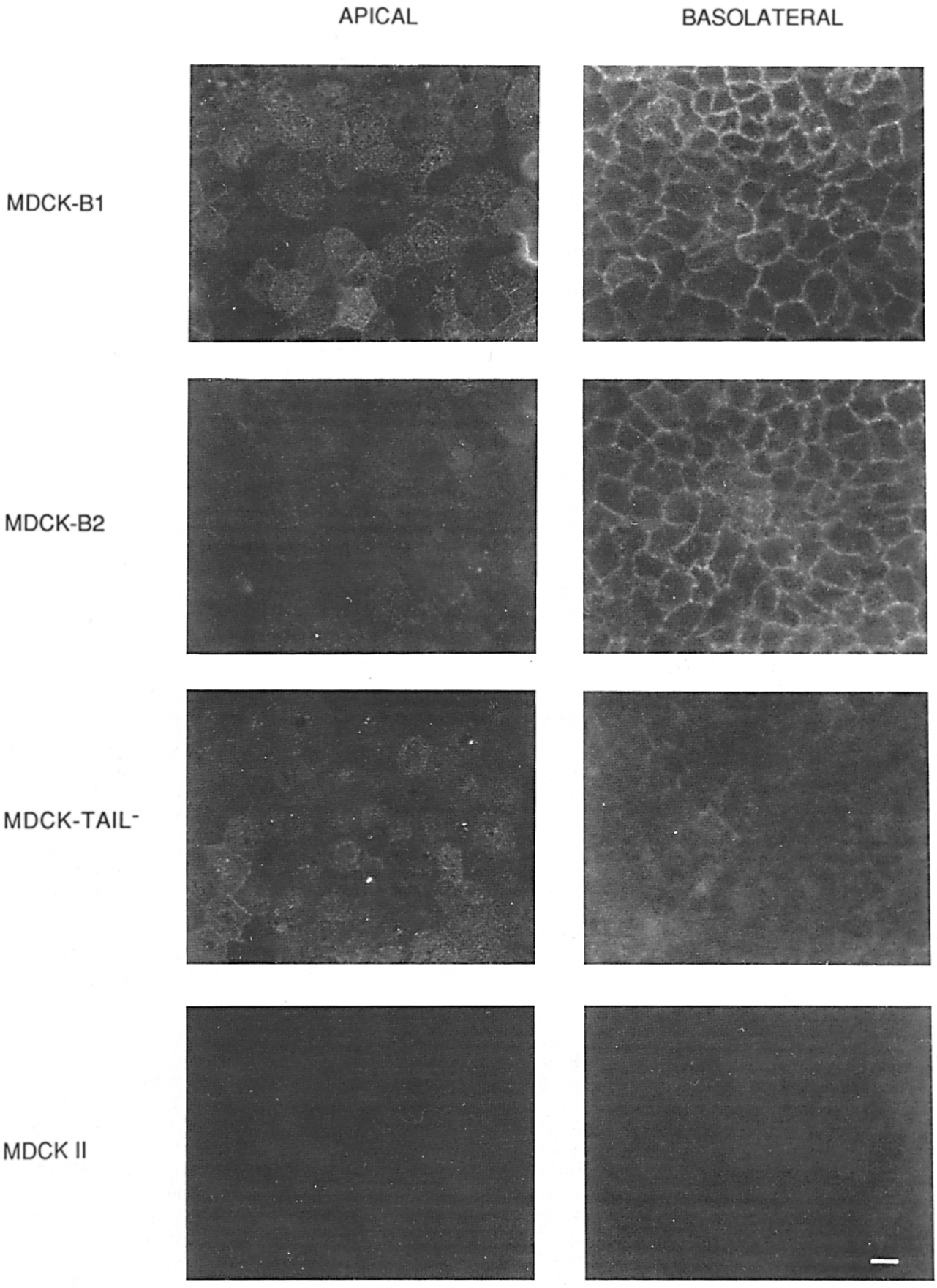
Together, these results demonstrate that FcRII isoforms normally expressed in nonpolarized macrophages and lymphocytes are capable of polarized distribution when expressed in an epithelial cell line. FcRII-B1 and FcRII-B2 also exhibited different patterns of polarized expression, suggesting that their polarity was at least partly determined by the fact that only FcRII-B2 could efficiently localize at coated pits.

The potential of FcRII-B1 and -B2 to localize in coated pits has been carefully quantitated for CHO cells expressing the two isoforms (Miettinen et al., 1989). We therefore only qualitatively analyzed the ability of FcRII-B2 and -B1 to enter clathrin-coated structures on the apical surface of MDCK cells (Fig. 3). For electron microscopic identification of coated structures involved in FcR-mediated endocytosis, IgG-complexes formed with gold-DNP-BSA were prebound at 4°C from the apical surface of MDCK-B2 or -B1 monolayers grown on polycarbonate filter units and cells were then warmed to 37°C for 2 min. After fixation of the cells with glutaraldehyde, they were further processed for EM. As expected from the low number of receptors present on the apical membrane of MDCK-B2 (Table I), few gold particles were present apically on MDCK-B2 as compared with those on MDCK-B1. More than 10% of the gold particles on MDCK-B2 were found in coated pits and occasionally in coated vesicles. In MDCK-B1, however, <1% of the total gold particles were detected in coated structures (Fig. 3). These results show that the two receptors expressed in MDCK cells behave similar to those in transfected CHO cells with respect to their ability to localize in apical clathrin-coated structures.

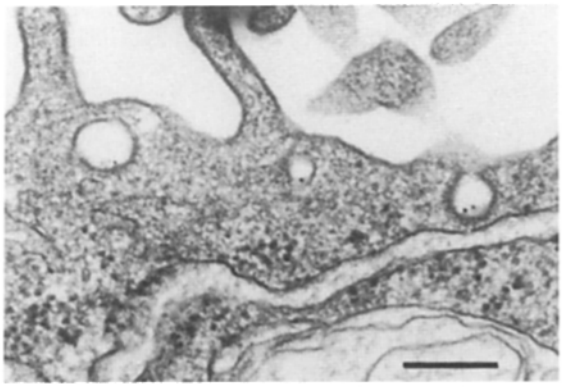
Macrophage-Lymphocyte FcR Mediate Apical to Basolateral Transcytosis

Because authentic epithelial cell FcR mediate the transcellular transport of IgG from the apical to the basolateral domain, we next determined whether either or both receptor isoforms could mediate the transcytosis of bound ligand. For these experiments, we used a transport assay based on detecting the appearance of IgG-complexes on the surface opposite from the side of addition using ^{125}I-labeled $F(ab')_2$ second antibody. This indirect assay was necessary since removal of FcR-bound ^{125}I-IgG-complexes from either the apical or basolateral surfaces required harsh proteolysis (Mellman and Plutner, 1984; Miettinen et al., 1989) that disrupted the integrity of the monolayers.

To measure apical to basolateral or basolateral to apical transcytosis, saturating concentrations of ligand (20 μg/ml rabbit IgG anti-DNP:DNP-BSA complexes) were added to either side of confluent MDCK monolayers grown on polycarbonate filter units. After incubation for various times at 37°C, the filters were transferred to ice and unbound complexes were removed by extensive washing in the cold. Specific binding of ^{125}I-$F(ab')_2$ goat anti-rabbit IgG to the basolateral or apical plasma membrane was then determined at 4°C.


APICAL
BASOLATERAL
MDCK-B1
MDCK-B2
MDCK-TAIL⁻
MDCK II

MDCK-B2



MDCK-B1

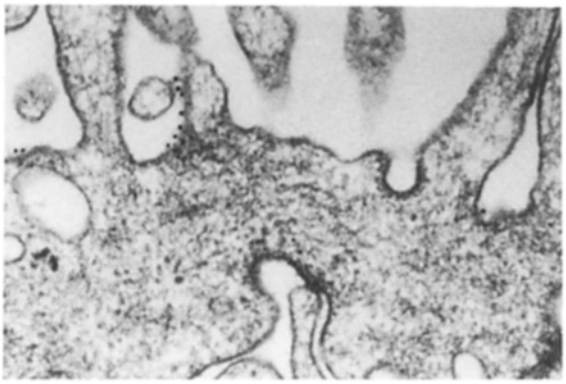


Figure 3. Coated pit localization of FcRII-B2 on the apical membrane of MDCK cells. MDCK-B2 and MDCK-B1 cells grown on polycarbonate filter units were incubated with 20 μg/ml gold-IgG-complexes added apically for 2 h at 4°C. Cells were washed, transferred to 37°C for 2 min, and returned on ice. After fixation with glutaraldehyde cells were processed for EM and embedded in Epon. Bar, 0.25 μm.

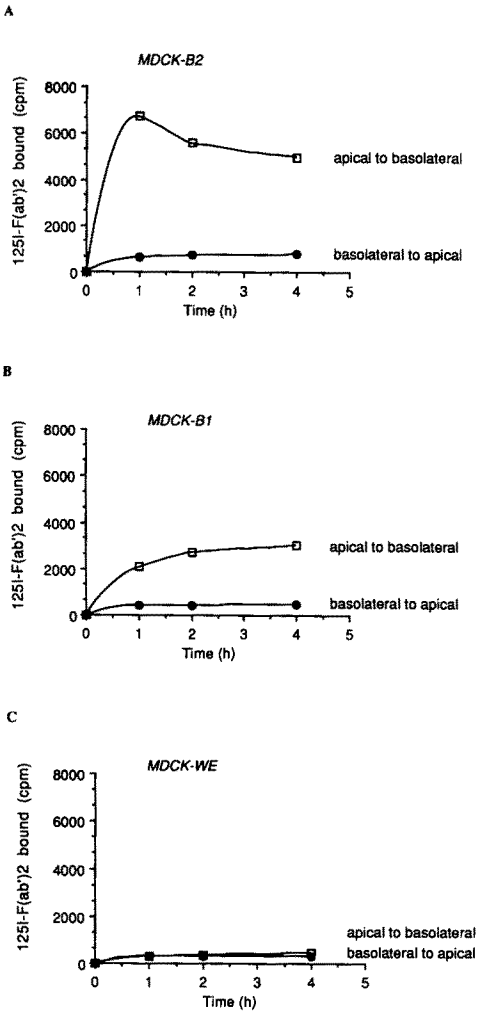


Figure 4. Transcytosis of IgG-complexes. MDCK-B1, -B2, and -WE monolayers grown on polycarbonate filter units were incubated for different periods of time at 37°C with 20 μg/ml IgG-complexes added either from the apical or basal side. Filter units were cooled and washed and IgG-complexes appearing on the surface opposite from the side of addition were detected with ^{125}I-F(ab')$_2$ fragments of goat anti-rabbit IgG. After washing, filters were cut out and counted in a gamma counter. Points were performed in duplicate; standard errors of the mean were <15%. Values for nonspecific transcytosis, determined in the presence of excess 2.4G2 apically during the 37°C incubation, were subtracted and represented <10%. (*A*) MDCK cells transfected with FcRII-B2. (*B*) MDCK cells transfected with FcRII-B1. (*C*) FcR-negative control, MDCK cells transfected with the retroviral vector pWE.

When the IgG-complexes were added to the apical medium of either MDCK-B1 or -B2 cells, significant amounts of the ligand were detected at the basolateral surface after only 1 h at 37°C (Fig. 4). Transport was more rapid in MDCK-B2 cells, basolateral appearance reaching a maximum within 1 h and declining slightly thereafter (Fig. 4 *A*). In contrast, transcytosis in cells expressing FcRII-B1, the isoform less capable of accumulating at coated pits, did not reach a maximum until after 2 h (Fig. 4 *B*).

Transcytosis via FcRII-B2 was also generally more efficient than via FcRII-B1. MDCK-B2 expressed only about half as many receptors as MDCK-B1 and only 20% of the FcRII-B2 molecules (as compared with 65% of the FcRII-B1 molecules) were on the apical surface and thus available to mediate transcytosis (Table I). Nevertheless, after 1–2 h, more than threefold the number of IgG-complexes appeared on the basolateral surface of MDCK-B2 than in MDCK-B1.

Figure 2. Immunofluorescence localization of FcR. MDCK-B1, -B2, -tail minus, and nontransfected MDCK strain II cells grown on polycarbonate filter units were incubated on ice with 2 μg/ml of the anti-FcR mAb 2.4G2 added apically or basolaterally for 2 h before fixation and staining with fluorescein-conjugated F(ab')$_2$ fragments of goat anti-rat IgG. Pictures were exposed and printed under identical conditions. Bar, 20 μm.

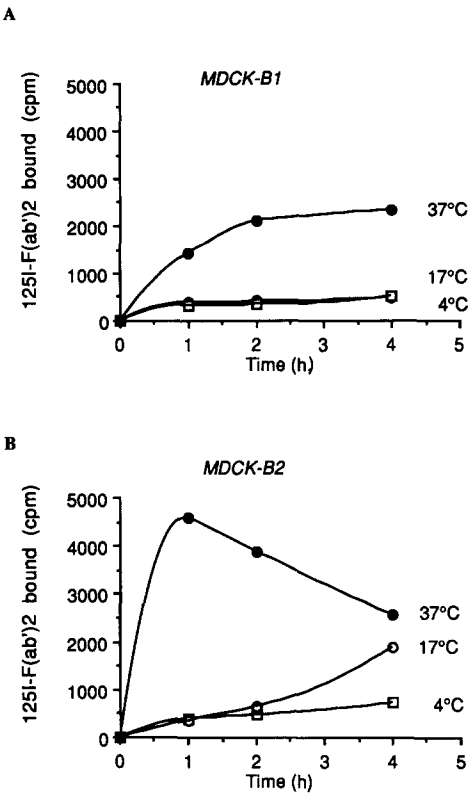


Figure 5. Temperature dependence of transcellular transport. MDCK-B1 and -B2 grown on polycarbonate filter units were incubated with 20 μg/ml IgG-complexes added from the apical surface at 4, 17, or 37°C. After varying periods of time, filter units were transferred on ice, washed, and transported ligand was detected with ^{125}I-F(ab')$_2$ fragments of goat anti-rabbit IgG added to the basal compartment on ice. Values for nonspecific transcytosis in the presence of excess 2.4G2 (<10%) were substracted. Points were carried out in duplicates; SEMs were <15%. (*A*) MDCK cells transfected with FcRII-B1. (*B*) MDCK cells transfected with FcRII-B2.

This amount corresponded to ~20–30% of the IgG-complexes initially bound at 4°C to the MDCK-B2 apical cell surface (as detected using the second-antibody binding assay). In contrast, only 5–10% of apically bound ligand reached the basolateral surface of MDCK-B1 in the same period of time. A significant fraction of transcytosed ligand was not released into the basal medium (not shown).

For both cell lines, transcellular transport was unidirectional, because IgG-complexes added basolaterally were never detected at the apical plasma membrane (Fig. 4, *A* and *B*). Apical to basolateral transport was also judged to be receptor-specific because it was completely inhibited by blocking ligand binding to apical FcR by including 100 μg/ml of the antireceptor antibody 2.4G2 IgG in the apical medium (not shown). However, addition of 2.4G2 to the basolateral medium did not prevent appearence of ligand at the basolateral surface, indicating that IgG-complexes were not simply gaining direct access to basolateral receptors by paracellular diffusion. The lack of nonspecific diffusion or transport of IgG-complexes across the monolayers was further demonstrated by the observation that transcytosis (in either direction) was not observed in the FcR-negative control cell line MDCK-WE (Fig. 4 *C*).

Thus, macrophage-lymphocyte FcRII, like their epithelial cell counterparts, are capable of mediating the apical to basolateral transcytosis of ligand in polarized cells. The efficiency of transcytosis, however, appeared to correlate with the ability of one receptor isoform, i.e., FcRII-B2, to accumulate at clathrin-coated pits.

Temperature Dependence and Kinetics of IgG-Complex Transcytosis

We next determined whether FcRII-mediated transcytosis was temperature dependent, as is the case for the basolateral to apical transport of polymeric IgA (Mostov and Deitcher, 1986) and epidermal growth factor (Maratos-Flier et al., 1987) in MDCK cells. IgG-complexes were added apically to confluent cells on filter units and incubated at 17°C or 37°C, or kept on ice for various periods of time. As shown in Fig. 5, transcellular transport was greatly inhibited at 4°C and 17°C in both MDCK-B1 and -B2 cells. Although not directly demonstrated, it is likely however that incubation at 17°C (but not 4°C) permitted internalization of at least a fraction of apically-bound IgG-complexes and their delivery to endosomes, by analogy to the uptake of IgG-complexes by macrophages at 17°C (Ukkonen et al., 1986).

To study the kinetics of transcytosis in more detail, cells were allowed to bind IgG-complexes apically at 4°C or 17°C

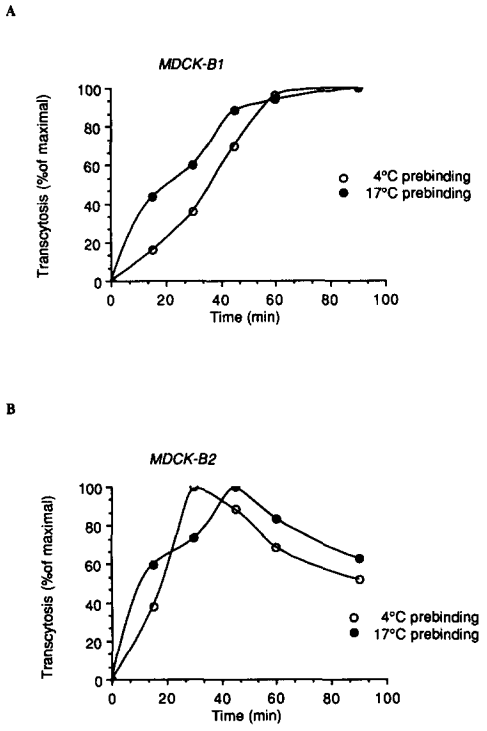


Figure 6. Single round transcytosis of IgG-complexes. IgG-complexes (20 μg/ml) were prebound apically for 2 h at 4°C or 17°C to MDCK-B1 or -B2 grown on polycarbonate filter units. After washing, filter units were transferred to 37°C for different periods of time and then returned on ice. Ligand appearing on the basolateral surface was detected with ^{125}I-F(ab')$_2$ fragments of goat anti-rabbit IgG added basally on ice. Numbers for nonspecific transcytosis in the presence of excess 2.4G2 (<10%) were substracted. Points were performed in duplicate. SEMs were <15%. (*A*) MDCK cells transfected with FcRII-B1. (*B*) MDCK cells transfected with FcRII-B2.

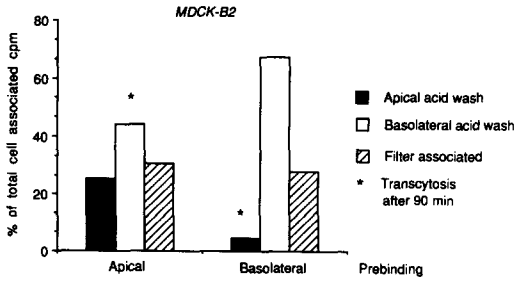


Figure 7. Transcytosis of 2.4G2 Fab fragments. ^{125}I-labeled Fab fragments of the monoclonal anti-FcR antibody 2.4G2 were prebound on ice to MDCK-B2 cells grown on polycarbonate filters from the apical side. Filter units were washed and then transferred to 37°C for 90 min. Cells were then returned on ice and acid-washed twice basolaterally with cold media (pH 3.5) for 10 min each, followed by two apical acid washes. The radioactivity in the acid washes and filters was determined and added to give 100%. Nonspecific values (<10%, obtained in the presence of excess cold 2.4G2) were substracted. 100% values were 1.1×10^4 cpm for apical and 9×10^4 cpm for basolateral prebinding. Points were performed in duplicate; SEMs were <15%.

for 2 h, washed extensively in the cold, and then rapidly warmed to 37°C for 0–90 min. The transcytosis of ligand to the basolateral domain was determined as described above. As shown in Fig. 6, IgG-complexes prebound at 4°C began to reach the basolateral surface of both MDCK-B1 and -B2 cells within 15 min after warming. However, the apparent initial rate for transcytosis via FcRII-B1 was significantly slower than via FcRII-B2. Transport across MDCK-B2 cells reached a maximum after only 30 min as compared with 60 min in MDCK-B1 cells.

In contrast, IgG-complexes preinternalized at 17°C before warming reached the basolateral surface much more rapidly than 4°C-bound ligand and at similar apparent initial rates for both MDCK-B1 and MDCK-B2 (Fig. 5, *A* and *B*). In both cell lines, transcytosis reached a maximum within 45 min and proceeded with similar apparent rates. However, it was difficult to establish a precise half-time for FcRII-B2-mediated transport due to the loss of ligand due apparently to degradation either en route or after reinternalization from the basolateral surface (see below).

Because the only major difference in the apparent rates of transcytosis between MDCK-B1 and -B2 cells occurred using ligand prebound at 4°C, it seems likely that the slower rate of apical to basolateral transport via FcRII-B1 reflects its inability to enter coated pits. By preinternalizing ligand at 17°C, presumably allowing it to reach an endosomal compartment from which transport to the basolateral surface is no longer dependent on endocytosis via clathrin-coated structures, FcRII-B1 and -B2 appeared to be almost equally efficient at completing the transcytotic pathway.

Transcytosis Does Not Require Receptor Cross-Linking

To analyze whether FcR cross-linking (as is the case for polyvalent IgG-complexes) was necessary for transcellular transport, we determined if FcR also mediated transcytosis of Fab fragments of the anti-FcR mAb 2.4G2, a well-characterized monovalent tag (Mellman et al., 1984). MDCK-B2 cells grown on permeable supports were allowed to bind ^{125}I-2.4G2 Fab fragments apically at 4°C, extensively washed and rapidly warmed to 37°C for 90 min. Transcytosed Fab fragments were then removed from the basolateral surface by washing with acid. As shown in Fig. 7, ~30% of the cell associated Fab fragments were found in an acid-resistant, presumably intracellular compartment, irrespective of whether ligand was prebound apically or basolaterally. Prebinding ligand basolaterally, ~70% of the cell-associated Fab fragments were still on the basolateral surface and <5% reached the apical side. In contrast, however, if ligand was prebound apically, up to 40% of the cell associated Fab fragments had reached the basolateral surface after 90 min.

Thus, FcRII-B2 also mediated efficient unidirectional transcytosis of a monovalent ligand, suggesting that cross-linking of receptors is not a prerequisite to divert them into the transcytotic pathway. This further suggests that FcR are also capable of mediating transcellular transport of monomeric IgG, although due to the low affinity of FcRII for monomeric IgG (10^6–10^5 M^{-1}; Leslie, 1980; Stengelin et al., 1988) we were not able to experimentally demonstrate apical internalization or transcytosis of murine IgG by MDCK-B2.

Only FcRII-B2 Efficiently Delivers IgG-Complexes to Lysosomes

In macrophages and transfected CHO cells, FcRII normally mediate the delivery of IgG-complexes to lysosomes for

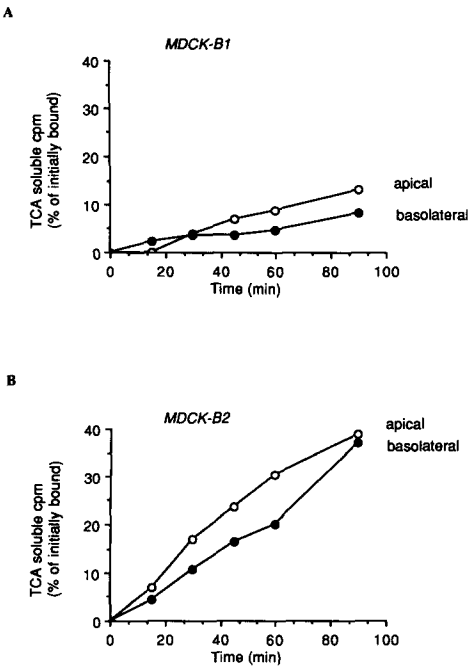


Figure 8. Lysosomal degradation of IgG-complexes. IgG:^{125}I-DNP-BSA complexes (20 μg/ml) were prebound on ice to MDCK-B1 or -B2 cells grown on polycarbonate filter units from the apical or basal side. Filter units were washed and then transferred to 37°C for different periods of time. Apical and basal medium was pooled for TCA precipitation, filters were cut out, and the radioactivity in the TCA pellet, supernatant, and filters was determined. TCA soluble counts were plotted as percent of total initially bound counts per minute. Nonspecific degradation values, determined in the presence of excess 2.4G2 during prebinding, were subtracted and represented <10%. Points were performed in duplicate; SEMs were <15%. (*A*) MDCK cells transfected with FcRII-B1. (*B*) MDCK cells transfected with FcRII-B2.

degradation. Although capable of transcytosis when expressed in MDCK cells, we next determined whether transcellular transport was also accompanied by degradation of internalized ligand. Rabbit anti-DNP IgG:^{125}I-DNP-BSA complexes were bound at 4°C to the apical or basolateral surfaces of MDCK-B1 and MDCK-B2 monolayers grown on filter units. After washing to remove unbound ligand, the cells were warmed to 37°C and the amount of TCA-soluble ^{125}I released into the apical and basolateral media was determined. As shown in Fig. 8, degradation of IgG-complexes by MDCK-B1 cells was severalfold less efficient than degradation by MDCK-B2 cells. After 90 min, up to 40% of the bound IgG-complexes had been degraded by MDCK-B2 (Fig. 8 *B*), as compared with ~10% in the MDCK-B1 cells (Fig. 8 *A*). Similar results were obtained using nonpolarized CHO cells transfected with FcRII-B1 and FcRII-B2 (Miettinen et al., 1989).

Interestingly, the amount of degradation by MDCK-B2 was the same whether the ligand was bound at the apical or the basolateral plasma membrane (Fig. 7 *B*). Thus, in the case of MDCK-B2, it was likely that a fraction of the ligand internalized apically never reached the basolateral surface but was degraded intracellularly, presumably reflecting its diversion to lysosomes. Further work will, however, be needed to absolutely rule out the possibility that all ligand internalized apically appears first on the basolateral surface before being rapidly reendocytosed for degradation. Because much of the degradation occurred after the time of maximum appearance of IgG-complexes at the basolateral surface (30 min, Fig. 5 *B*), a significant proportion of the degradation is likely to reflect lysosomal delivery of ligand reinternalized after reaching the basolateral surface. Therefore, the fraction of IgG-complex transcytosed by FcRII-B2 (20–30%, see above) probably represents a minimal estimate.

Discussion

A variety of cells of the immune system and different epithelia express receptors recognizing the Fc domain of IgG. Although a considerable structural heterogeneity has been identified among FcR in the immune system, relatively little information has been obtained about functional differences among various receptor species. On the other hand, IgG FcR in epithelia are all known to function in the transcellular transport of maternal IgG; until recently, however, no structural information was available for this class of FcR. cDNA cloning of a rat intestinal receptor has recently suggested homology of this epithelial cell FcR to MHC class I antigens (Simister and Mostov, 1989). In the present paper, we report functional evidence for a previously unexpected relationship between a second major class of epithelial FcR and FcR of the immune system. Recent cDNA cloning, in situ hybridization and immunohistochemistry experiments (Stuart et al., 1989) suggest a close structural relationship between the macrophage FcRII-B2 isoform and a putative placental receptor. By expressing two closely related FcRII isoforms in MDCK cells, we found the macrophage–lymphocyte receptors to transport ligand across an epithelial cell in the same directionality as expected for epithelial FcR for IgG, suggesting that FcRII may represent (or be related to) a second, distinct class of transcytotic FcR found in placenta.

Polarity and Sorting in MDCK Cells

FcRII-B1, -B2, and a mutant lacking the entire cytoplasmic tail were found to be distributed with distinct polarities on MDCK cells. While FcRII-B1 and the tail-minus mutant, both excluded from coated pits (Miettinen et al., 1989) were predominantly found on the apical surface, ~80% of FcRII-B2 was found basally, suggesting that their different abilities to accumulate in coated pits could account for their different distribution on MDCK cells. Because the mutant lacking the cytoplasmic tail was found mainly on the apical side and, in addition, a soluble anchor minus receptor mutant was predominantly secreted apically (Hunziker, W., and I. Mellman, unpublished results), both receptor isoforms could preferentially be targeted to the apical surface after leaving the Golgi. FcRII-B2, but not -B1 or the mutant lacking the cytosolic tail, might then be efficiently redistributed by transcytosis to the basolateral plasma membrane, resulting at equilibrium in the observed differences in polarity. Because monovalent ligand (i.e., Fab fragments of the anti-FcR mAb 2.4G2) is also transcytosed in the apical-to-basolateral direction by FcRII-B2 in MDCK cells, cross-linking of receptors is not required for diversion into the transcytotic pathway. Experiments are in progress to determine if both isoforms are delivered to the apical membrane domain during biosynthesis. Therefore, the efficiency with which proteins assemble in coated pits could represent a mechanism by which epithelial cells generate and maintain the polar distribution of at least some plasma membrane components.

Alternatively, it is conceivable that FcRII-B2 carries a "signal" for basolateral delivery in the biosynthetic pathway that is partly disrupted by the insertion in FcRII-B1, or deleted in the mutants lacking the membrane anchor and/or cytoplasmic domains. Apically missorted FcRII-B1 and -B2 receptors could then be resorted basolaterally, similar to VSV G-protein artificially implanted in the apical domain of MDCK cells (Matlin et al., 1983; Pesonen and Simons, 1983). Structural homology of FcRII-B2 with a putative placental receptor (see below), however, suggests that transcytosis is of functional relevance and does not merely reflect resorting of receptors.

In the intestine of the suckling rat, transcytosis of maternal IgG is accompanied not only by appearance at the basolateral surface but also by the delivery of a significant fraction of ligand to lysosomes (Morris and Morris, 1974, 1976; Abrahamson and Rodewald, 1981), similar to the pathway taken by FcRII-B2 from the apical surface of MDCK cells. A large fraction (perhaps >50%) of the fluid internalized at the apical surface of MDCK cells is transcytosed as opposed to being delivered to lysosomes; precisely the opposite is true for endocytosis at the basolateral domain (von Bonsdorff et al., 1985). This result suggests that a major transcellular route exists in the apical to basolateral direction in MDCK cells, and it is conceivable that no signal is needed to direct apical proteins to the basolateral surface: simply by entering coated pits, apical proteins may already have a high probability of transcytosis to the basolateral surface. In this case, inefficient accumulation in coated pits could represent a mechanism to generate the distribution of plasma membrane proteins on both surfaces, similar to that of FcRII-B1.

If, however, a constitutive recycling pathway is also present on the apical domain that returns internalized proteins

(i.e., pIg-R) back to the apical surface (Breitfeld et al., 1989), diversion of internalized receptors off of this pathway may be required for transcytosis and/or delivery to lysosomes. Therefore, the FcR could either contain a determinant that specifies its postendocytic transport, or lack a determinant present on the pIg-R responsible for targetting it back to the apical plasma membrane. As previously shown for receptor transport to lysosomes in macrophages (Mellman et al., 1984; Mellman and Plutner, 1984; Ukkonen et al., 1986), a polyvalent ligand increases the efficiency with which ligand-receptor complexes are degraded. Receptor cross-linking is, however, not required for transcytosis because FcRII-B2 transports both monovalent 2.4G2 Fab fragments and polyvalent IgG-complexes across the cell. It remains to be determined, however, if postendocytic sorting of FcR occurs in MDCK cells and if so, what the effect of ligand valency is.

At the basolateral surface, simply removing internalized receptors from the constitutive recycling pathway is clearly not sufficient to cause transcytosis. In the case of FcRII-B2, internalized ligand never reaches the apical surface but is delivered only to lysosomes. In contrast, resident basolateral proteins, such as the transferrin receptor, avoid lysosomes after internalization and recycle back to the basolateral domain (Fuller and Simons, 1986). Other basolateral receptors, such as the pIg-R and the receptor for epidermal growth factor, mediate transcytosis from the basolateral to the apical surface (Mostov and Deitcher, 1986; Maratos-Flier et al., 1987) and appear to avoid lysosomes en route. Together, these observations suggest differences in the mechanisms of sorting of receptors destined for recycling, transport to lysosomes, and transcytosis at the basolateral versus the apical surfaces. Basolateral-to-apical transcytosis may require specific determinants not needed for transcellular transport from the apical surface.

Relationship between FcR of Epithelia and the Immune System

The apical-to-basolateral transepithelial transmission of maternal IgG or immune complexes across fetal and neonatal tissues is essential for the survival of mammalian offspring and has best been described in the proximal small intestine of the suckling rat and in the placental syncytial trophoblast in many species, including humans. In the neonatal rat intestine, FcR recognize both monomeric and complexed IgG and mediate its transport from the intestinal lumen to the blood circulation (Abrahamson et al., 1979; Abrahamson and Rodewald, 1981; Rodewald and Kraehenbuhl, 1984). Net transport results from preferential binding of ligand at the intestinal pH, whereas dissociation of ligand is favored at the higher blood pH (Rodewald, 1976, 1980). FcRII of macrophages and lymphocytes, however, does not exhibit pH dependent binding of IgG or immune complexes. Although a putative intestinal rat Fc receptor for IgG has recently been shown to be clearly distinct from FcRII (Simister and Mostov, 1989), little structural and functional information has been available on the placental receptor.

The ability of macrophage–lymphocyte FcR to mediate apical-to-basolateral transcytosis in MDCK cells suggests that FcR of the immune system may be expressed in epithelia and/or share structural features with IgG receptors found in IgG transporting epithelia. The molecular weight of placental receptors for IgG has been reported to be similar to the intestinal and lymphoid receptors (40–50 kD; Balfour and Jones, 1978). Recently we cloned a human FcR isoform from a placenta cDNA library. This FcRII isoform shares 70% amino acid identity throughout its length with FcRII-B2 and was found to be expressed in various macrophage and lymphoid cells lines and, most surprisingly, in cultured placental syncytial trophoblasts depleted of tissue macrophages. Furthermore, in situ hybridization and immunohistochemistry experiments showed high levels of expression of this isoform in syncytiotrophoblasts, but not in cytotrophoblasts, suggesting that it in fact represents an authentic epithelial receptor (Stuart et al., 1989). Although our results (obtained with the murine homologue) suggest a role of this receptor in placental transcytosis, expression in syncytiotrophoblasts could be unrelated to the transport of maternal IgG and instead reflect a generalized expression of monocytic markers in this epithelium. Furthermore, polar distribution and intracellular traffic of the same receptor could possibly be different in MDCK cells and placental syncytiotrophoblast.

Almost nothing is known of the efficiency of transcellular transport or the oligomeric structure of the ligand transcytosed by placental FcR but like FcRII (Leslie, 1980; Stengelin et al., 1988), they have been reported to bind both monomeric and aggregated IgG (Niezgodka et al., 1981). IgG concentrations in the maternal circulation are certainly several orders of magnitude higher than in the neonatal intestine. Thus it seems possible that the placental transport FcR would exhibit a comparatively low affinity for IgG, because a high-efficiency transport system might result in unfavorably high concentrations of maternal immunoglobulin in the fetal circulation. Due to the high concentration of monomeric IgG in the maternal circulation (in the order of 5–10 mg/ml), it is likely that IgG will bind to FcRII (with an affinity for monomeric IgG of 10^6–10^5 M^{-1}; Leslie, 1980; Stengelin et al., 1988) in vivo. As FcRII-B2 cross-linking is not required for transcytosis, it is well possible that monomeric IgG present at high concentrations apically, could bind to FcRII, be transcytosed and on route, or at the basolateral side, dissociate from the receptor. Furthermore, it is conceivable that FcRII transport maternal IgG present in the form of dimeric or multimeric aggregates. Alternatively, FcRII-B2 could share the determinants needed for transcytosis with the putative placental receptor, or structural differences between the two receptors could account for differences in ligand affinity.

Although our results combined with the fact that a human homologue of FcRII-B2 is expressed in syncytiotrophoblasts suggest that FcRII may represent a class of epithelial receptors distinct from the MHC class I-like intestinal receptors, clearly additional work will be required to prove a functional relationship between leukocyte and placental FcR. We do not know at present if FcRII-B2 or a homologous receptor is expressed in mouse placenta. If however, a FcRII isoform can also be demonstrated in mouse placenta, the murine system will allow to directly test the role of this receptor in transferring immunity to the fetus.

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