Basolateral secretion of kappa light chain in the polarised epithelial cell line, Caco-2

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Summary

The immunoglobulin kappa light chain is constitutively secreted in non-polarised cells. It is therefore unlikely to display any of the signals thought to be required for the selective delivery of proteins to the apical or basolateral borders of polarised epithelial cells. We have transfected the gene for the kappa light chain into a polarised epithelial cell line (Caco-2) and shown that it is secreted predominantly from the basolateral surface. Metabolically labelled endogenous secretory products show the same polarity and we conclude, therefore, that in Caco-2 cells there is a major intracellular trafficking route to the basolateral border that requires no sorting signal.

Key words: epithelial cells, Caco-2 cells, protein secretion.

Introduction

Simple epithelia form boundaries between two different environments. The structure of the cells is generally polarised, reflecting their function. The apical (luminal) and basolateral (serosal) membranes differ in their protein and lipid composition (Simons and Fuller, 1985) and are separated by the tight junctions, which seal the interface between the two environments. Protein secretion from epithelial cells is frequently polarised; for example, the milk protein casein is secreted from the apical surface of mammary gland cells (Parry et al. 1987), whereas the basement membrane components laminin and heparan sulphate proteoglycan are secreted basolaterally in the Madin-Darby Canine Kidney (MDCK) cell line (Caplan et al. 1987). The secretion of these proteins is thought to be constitutive; that is, continuous and requiring no external stimuli. Regulated secretion, in which the secretory product is stored in secretory granules and is only secreted from the cell in the presence of an appropriate stimulus, may also be polarised, as in the pancreatic acinar cell (Jamieson and Palade, 1971).

Various models for sorting of secretory proteins in epithelial cells have been put forward (see Matlin, 1986; Bartles and Hubbard, 1988). One model proposes that every secretory protein probably carries a sorting signal that determines whether it is delivered to either the basolateral or the apical surface. Another postulates that only some secretory proteins have sorting signals, the remainder follow an unselective 'default' route. The latter model is not supported by experiments in the MDCK cell line. When the appropriate DNAs for a variety of secretory proteins (including kappa light chain) were transfected into these cells, which are capable of polarised secretion from both surfaces (Kondor-Koch et al. 1985; Caplan et al. 1987), the foreign proteins were exported to the apical and basolateral surfaces in approximately equal amounts (Kondor-Koch et al. 1985; Gottlieb et al. 1986) and thus did not appear to be sorted. In the COMMA-1-D mammary epithelial cell line, although the secretion of casein is apical, transferrin is secreted both apically and basolaterally (Parry et al. 1987). At the present time therefore there is no compelling evidence for the view that lining epithelial cells contain a polarised default pathway.

We have explored the possibility that a polarised default pathway exists in the enterocyte-like Caco-2 cell line (Pinto et al. 1983). This cell line is derived from a human colon carcinoma and forms a highly polarised epithelium when grown on a permeable substratum. Although these cells are derived from a colon carcinoma, they possess some characteristics of the small intestine. Caco-2 cells express brush border enzymes at the apical surface (Haurie et al. 1985; Stiegerei et al. 1988) and secrete apolipoproteins (Traber et al. 1987) and cholesteryl ester transfer protein (Faust and Albers, 1988) at the basolateral surface. We introduced into this cell line the gene for immunoglobulin kappa light chain, a protein with no known apical, basolateral or other signals for sorting out of the constitutive pathway to the cell surface, and have found that more than 90% of this tracer of the unsorted constitutive pathway is secreted from the basolateral surface.
Materials and methods

Cell culture
Caco-2 cells between passages 94 and 115 were routinely maintained in 75cm² flasks (Costar, NBL, Northumberland) in Dulbecco's modified Eagle's medium (DMEM) with 2mM-glutamine, antibiotics (all from Gibco, Middlesex) and 20% foetal calf serum (FCS) (Flow Laboratories, Rickmansworth, Herts.). Cells were split 1:3 once a week. For experiments, 1×10⁵ to 1.5×10⁶ cells were seeded onto each Transwell filter (Costar), 24.5 mm in diameter, with 2ml of medium in the upper chamber and 5ml in the lower chamber. At this density, cells achieved confluency in about 2 days. The medium was replaced every 2–3 days. Cells were used for experiments between 7 and 14 days after plating. The transepithelial resistance of each filter was tested before use, to ensure integrity of the monolayer, using a device similar to that described by Fuller et al. (1984). Only filters with a resistance greater than 200 ohms cm² were used for experiments.

Transfection
To make a Caco-2 cell line synthesizing kappa light chain, two plasmid expression vectors (from Drs Linda Matsuuchi and Regis Kelly, University of California) were used as described (Matsuuchi et al. 1988). These were pRSV Δ5' MPC-11 k-1, which contained the DNA for mouse immunoglobulin kappa light chain (MPC-11) and pSV2 neo, which confers resistance against G418, the selecting drug. Transfections were performed essentially as described (Schweitzer and Kelly, 1985). Chloroquine (200 μM) was included in the medium while the cells were incubated with the DNA precipitate. Cells were selected using 1 mg ml⁻¹ G418 sulphate drug (Geneticin: Gibco). Resistant colonies could be picked out after 2–3 weeks, then the drug concentration was halved and the colonies were expanded for screening.

Metabolic labelling and immunoprecipitations
To screen the drug-resistant cell lines for kappa light chain synthesis, 6cm dishes of cells were labelled with [³¹S]Translabel (ICN, High Wycombe, Bucks.) for 4–6h in cysteine/methionine-free medium containing 1% foetal calf serum (FCS) and 2mM-glutamine. The cells were then placed on ice, washed in phosphate-buffered saline (PBS) and lysed in 1 ml of 1% Nonidet-P40 (Sigma) plus 10mM-deoxycholate, 66mM-EDTA, 10mM-Tris-HCl, pH7.4, supplemented with protease inhibitors (NDET buffer). The lysate was collected by centrifugation for 5 min in a microfuge. SDS was added to the post-nuclear supernatant to give a final concentration of 0.3% and the supernatant was then pre-absorbed with 100 μl of washed, fixed Staphylococcus aureus (Pansorbin, Calbiochem), spun to clear the sample, and the volume was adjusted to 7 ml with NDET/SDS before incubation with a polyclonal rabbit antibody against mouse kappa light chain (ICN) overnight at 4°C. Pansorbin (60 μl) was added and the incubation continued for 1–3 h, followed by several washes of the Pansorbin pellet in the detergent buffer before resuspension in 50 μl of a reducing sample buffer. The immunoprecipitated protein was analysed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and fluorography. To label filter-grown cells, normal DMEM was replaced with cysteine/methionine-free medium supplemented with FCS; 1 ml apical and 2 ml basolateral. 0.5–1.0 μCi of [³¹S]Translabel was added to the basal part of the chamber and the monolayers were incubated for 6h. Apical and basolateral media were collected separately, proteinase inhibitors were added and cell debris removed by centrifugation. An equal volume of NDET (2×) was added to the media samples. With the omission of the pre-adsorption step with Pansorbin, all of these samples (i.e. 1 ml apical and 2 ml basolateral) and the filter-grown cells were then processed as described above. Quantification of endogenous secretion was performed by precipitation with trichloroacetic acid (TCA) of media samples onto filters, followed by liquid scintillation counting.

Electron microscopy
Filter-grown cells were washed in PBS and then fixed in 1% glutaraldehyde in 0.2m-sodium cacodylate (pH7.4) for 30 min. They were processed for electron microscopy by standard techniques. Silver sections were cut and examined in a Philips CM12 electron microscope.

Immunofluorescence
Monolayers were washed in PBS, fixed in 3% paraformaldehyde in PBS and quenched in 50 mM-ammonium chloride. Saponin (0.05%) was included in all subsequent solutions. Pieces of filter were incubated in two changes of PBS/0.2% gelatin and labelled with a mouse monoclonal antibody against either the insulin receptor or sucrase-isomaltase (kind gifts from Dr Ken Siddle, Cambridge and Dr Hans-Peter Hauri, Basel, respectively) for 30 min. After thorough washing in PBS/gelatin, the cells were labelled with a fluorescein-labelled goat antibody against mouse IgG for 30 min. This was followed by further thorough washing, and the pieces of filter were mounted in PBS/glycerol containing n-propyl gallate (Sigma) to prevent bleaching. Some samples were examined in a Lasersharp MRC laser scanning confocal apparatus in conjunction with a Nikon Optophot microscope.

Epidermal growth factor (EGF) internalisation
Human EGF (kindly given by Dr Steven Felder, Philadelphia) was iodinated as described elsewhere (Hughson and Hopkins, unpublished). Filter-grown monolayers were incubated with serum-free medium (DMEM plus 0.1% bovine serum albumin) for 1 h and then [¹²⁵I]-labelled EGF (5×10⁴ cts min⁻¹ ml⁻¹) in serum-free medium was added either basolaterally (1.5 ml) or apically (1 ml) to the filters in the presence or absence of excess cold ligand (100 ng ml⁻¹). The cells were incubated at 37°C for 3 h. The cells were then placed on ice, washed thoroughly with cold PBS, followed by two acid washes (0.15 m-NaCl, 0.5 m-acetic acid, pH2.7) and the filters were cut out of their holders and counted.

Results
The parent cell line
Wild-type Caco-2 cells, when grown on polycarbonate Transwell filters for at least 7 days, routinely show a resistance of greater than 200 ohms cm². Their morphology resembles that of an enterocyte, with a brush border at the apical surface and junctional complexes joining cells on the lateral surfaces (Fig. 1). Plasma membrane proteins are expressed in a polarised manner, with brush border enzymes confined to the apical surface (see below) and other proteins such as the insulin receptor expressed only on the basolateral membrane (Fig. 2).

The transfected cell line
Caco-2 cell lines expressing a kappa light chain cDNA
Fig. 1. Electron micrograph of a wild-type Caco-2 cell. Cells were grown on a Transwell filter for 14 days, fixed and embedded. Sections were stained with lead citrate. The method used to process the cells does not stain glycogen (g). Bar, 2 μm.

Fig. 2. Indirect immunofluorescence of wild-type Caco-2 cells. A filter-grown monolayer was fixed in 3% paraformaldehyde and labelled by a monoclonal antibody for the insulin receptor followed by a fluorescein-labelled goat anti-mouse antibody. The micrograph is taken in the plane of the lateral membrane so that staining from the basal membrane is not seen. No fluorescent label is present on the apical surface. Bar, 25 μm.

were made as described in Materials and methods. When screened by immunoprecipitation, four out of 10 neomycin-resistant colonies were positive for kappa light chain synthesis. The cell line showing the highest level of expression, 1.1 (Fig. 3), was used for further study. Several assays were employed to ensure that this cell line retained the polarised characteristics of wild-type Caco-2 cells. The morphology of the cells resembled that of the parent cells (data not shown) and expression of the brush border enzyme sucrase–isomaltase was exclusively apical (Fig. 4A).

Uptake of radiolabelled EGF was used as a quantitat-

ive measure of cell surface polarity. Duplicate filters were allowed to internalise $^{125}$I-EGF from either the apical or basolateral surface in the absence or presence of excess cold ligand, and then the filters were washed and counted. For both wild-type and 1.1 cell lines, the uptake of EGF was at least 30 times greater from the basolateral surface than from the apical surface (Fig. 4B). This basolateral expression of the EGF receptor in Caco-2 cells has been previously described (Hidalgo et al. 1989).

The transepithelial resistance of the 1.1 cell line was consistently higher than that of the wild-type cells. From two separate experiments, the mean value for the wild-type filters (total 15) was 287 (standard error, 11) ohms cm$^{-2}$, whereas that for the 1.1 filters (total 16) was 484 (standard error, 34) ohms cm$^{-2}$.

To see if the secretion of kappa light chain was polarised in the 1.1 cell line, monolayers of cells were labelled basolaterally with $^{35}$S Translabel for 6h, and then the cells and media were analysed by immunoprecipitation, SDS–PAGE and fluorography. Quantification of the gel showed that most of the kappa light chain was secreted into the basolateral chamber (Fig. 5, experiment a). Although there was some variation in the number of counts immunoprecipitated from each filter (compare lanes 1 and 7, Fig. 5), the ratio between counts

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Light chain secretion in Caco-2 cells
Antibody  | + | + | + | −
Cell line | 2.2 | 2.1 | 1.1 | 1.1

Table: Screening of G418-resistant cell clones after transfection:

| M (kDa) | 92 | 69 | 46 | 30 |

Fig. 3. Screening of G418-resistant cell clones after transfection: 6 cm dishes were labelled with 35S-labelled amino acids, the cells were lysed and the lysates immunoprecipitated with an anti-kappa light chain antibody before SDS-PAGE. Molecular weight standards (M, × 10^2) are indicated on the left. Cell lines 2.1 and 2.2 do not express detectable levels of kappa light chain.

Fig. 4. Cell surface polarity in 1.1 Caco-2 cells. A. A filter-grown monolayer was fixed, permeabilised, labelled for sucrase-isomaltase by indirect immunofluorescence and examined in the confocal scanning microscope. This optical section, taken perpendicular to the monolayer, shows that the label is found only on the apical surface. The band of fluorescence, 3 μm thick, corresponds to the microvilli of the brush border. Arrow indicates the interface between the cells and filters. Bar, 10 μm. B. Duplicate filters were incubated with 125I-EGF in either the apical or basal chamber for 3 h at 37°C. The cells were washed and counted. Specific uptake of 125I-EGF was calculated by subtracting the number of counts internalised in the presence of cold excess ligand from the total. Filled bars, counts internalised with 125I-EGF applied basolaterally; hatched, counts internalised with 125I-EGF applied apically. Error bars indicate the range about the mean. wt, wild-type.

We therefore determined whether the apparent polarity of kappa light chain secretion is due to proteolytic degradation of apically released immunoglobulin by brush border enzymes. Caco-2 cells grown on a filter were radio-labelled and the basolateral medium containing metabolically labelled kappa light chain was split into halves. One portion was stored and the other was incubated overnight in the apical chamber of a Transwell on which an unlabelled confluent monolayer was growing. The medium was collected and processed with its
corresponding untreated sample. Fig. 5 (experiment c) shows that there is no apparent degradation of the kappa chain after incubation in the apical chamber.

If the kappa light chain is acting as a marker for constitutive secretion from Caco-2 cells, then the secretion of endogenous proteins may reflect a similar apical/basolateral distribution. To investigate the polarity of secretion of endogenous products from our Caco-2 cells, filter-grown cells were labelled as before, and apical and basolateral media were collected. A sample of each was analysed by scintillation counting of TCA-precipitable material. A total of 89% of the counts were found in the basolateral medium. SDS-PAGE and fluorography showed (as previously demonstrated by Rindler and Traber, 1988) that none of the detectable proteins were secreted primarily from the apical surface (data not shown). Thus the ratios of apical to basolateral secretion for kappa light chain and total endogenous secretory proteins are similar.

**Discussion**

We have shown that Caco-2 cells transfected with the gene for immunoglobulin kappa light chain secrete this protein predominantly from the basolateral surface. We have ruled out the possibility that this is an artificial effect due to degradation of the protein at the apical surface. We think that it is unlikely that the small amount of kappa light chain that appears in the apical medium is due to leakage across an incompletely sealed monolayer, because less than 0.5% of basolaterally applied radiolabelled tracers (transferrin, horseradish peroxidase) appears in the apical chamber after 3h (Hughson & Hopkins, unpublished). We have also shown that the majority of
kappa light chains are secreted from these cells in a simple pulse–chase experiment.

Recently, Rindler and Traber (1988) have transfected Caco-2 cells with the gene for human growth hormone (HGH) and shown that this exogenous protein is released from the basolateral surface. However, the interpretation of this result is complicated by the fact that, in pituitary cells, HGH is secreted in a regulated fashion. Moreover, it has been previously shown that when the DNA for HGH is transfected into cells possessing a regulated secretory pathway, the protein is still targeted to secretory granules (Moore and Kelly, 1985). Although a classic regulated secretory route has not yet been described in Caco-2 cells, there is evidence that one may exist. Thus in a recent study by Trahair and colleagues (1988) it was found that when a fusion gene containing the DNA for HGH was expressed in transgenic mice, the protein was found to be present in small vesicles in the enterocytes of the intestine. It is reasonable to expect that, if such a regulated secretory route is present in Caco-2 cells, HGH will probably be sorted into it. Thus, the observation of Rindler and Traber that HGH was secreted predominantly basolaterally by Caco-2 cells may not illustrate the polarity of the constitutive secretory pathway in these cells. Our results showing that kappa light chain, which has no such signal for regulated secretion, exhibits the same basolateral preference clearly suggest that both proteins are sorted in these cells. They therefore act as tracers for bulk-flow constitutive secretion and demonstrate that this pathway is polarised in these cells.

Caco-2 cells secrete apolipoproteins and transferrin basolaterally (Rindler and Traber, 1988) but little secretion occurs apically. We found that only 10% of total endogenous secretory products, which are metabolically labelled, are present in the apical chamber of a Caco-2 monolayer. We do not know whether this polarised secretion of endogenous products reflects the active sorting of proteins into a basolateral pathway or simply reflects the relative amount of vesicular traffic to each surface. The behaviour of our tracer protein kappa light chain, which should not interact with endogenous sorting machinery, implies that the latter alternative may be correct.

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