Transport of IGF-I across epithelial cell monolayers

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Abstract

Epithelial cells line the lumens of organs including the gastrointestinal tract, kidney tubules and respiratory airways, where they regulate the transport of electrolytes and the movement of macromolecules. The current study aimed to investigate the transport of IGF-I across epithelial cell barriers. Epithelial cell lines derived from gut (IEC-6), kidney (MDBK) and lung (Mv1Lu) were shown to possess high-affinity, functional receptors for IGF-I and formed tight junctions in monolayer culture. To investigate the transport of IGF-I, the three cell lines were grown on microporous filters in a bi-chamber system. In comparison with filters without cells, IEC-6 and Mv1Lu epithelial cell monolayers restricted the passage of ^{125}I -IGF-I and

$[^3\text{H}]$ inulin, whereas the MDBK cells virtually occluded any passage of these molecules. Transport of ^{125}I -IGF-I across the epithelial cell monolayers was significantly less than that of $[^3\text{H}]$ inulin, suggesting that the binding of ^{125}I -IGF-I to high-affinity IGF receptors or IGF-binding proteins retarded its transport. Moreover, ^{125}I -IGF-I transport was not inhibited by the presence of excess unlabelled IGF-I. Our findings provide evidence for the restricted diffusion of intact, free IGF-I across gut, kidney and lung epithelial cell monolayers via a paracellular or low-affinity transcellular pathway.

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Introduction

Transport of macromolecules across epithelial surfaces occurs via either active transcellular or passive paracellular pathways. Proteins such as horseradish peroxidase, insulin, immunoglobulin-G and beta-lactoglobulin have been shown to be actively transported across the epithelium lining the small intestine in membrane-bound vesicles after binding to cell-surface receptors or binding sites (Walker *et al.* 1972, Wheeler *et al.* 1993, Bendayan *et al.* 1994, Jochims *et al.* 1994, Kiliaan *et al.* 1996). In contrast, a component of the transport of horseradish peroxidase and BSA across epithelial monolayers occurs by passive intercellular diffusion (Rhodes & Karnovsky 1971, Warshaw *et al.* 1974, Kiliaan *et al.* 1996). Several growth factors including insulin, epidermal growth factor, nerve growth factor and insulin-like growth factor-I (IGF-I) are taken up by the gut in an active form (Kelly 1960, Aloe *et al.* 1982, Thornburg *et al.* 1984, Philipps *et al.* 1990).

Significant attention has been focused on the transport and possible role of enteral IGF-I. IGF-I is a potent promoter of growth, metabolism and differentiation of numerous cell types including epithelial cells (Corps & Brown 1987). Administered systemically, IGF-I promotes gut growth and maturation (Read *et al.* 1992) and orally administered IGF-I acts locally to stimulate gut proliferation (Baumrucker *et al.* 1994, Burrin *et al.* 1996). IGFs have been detected in the milk of many

species (Baxter *et al.* 1984, Donovan *et al.* 1991, Vega *et al.* 1991, Olanrewaju *et al.* 1996). Orally administered ^{125}I -IGF-I is absorbed by the gut and appears in the circulation intact (Baumrucker *et al.* 1992, Vacher *et al.* 1995, Odle *et al.* 1996, Kimura *et al.* 1997, Xu & Wang 1996).

To exert its mitogenic actions, IGF-I binds to the type I IGF receptor on target cell membranes (LeRoith *et al.* 1995). Type I IGF receptors have been identified on porcine small intestinal mucosal membrane preparations (Schober *et al.* 1990), on the basolateral membranes of enterocytes in piglets (Morgan *et al.* 1996) and the villus and crypt epithelium of the rat (Heinz Erian *et al.* 1991, Ryan & Costigan 1993, Laburthe *et al.* 1996). Intestinal cell lines derived from rat jejunal crypts (IEC-6), as well as the rat, rabbit and human colon (Caco-2) also express IGF type I receptors (Pillion *et al.* 1989, Park *et al.* 1990, Rouyer Fessard *et al.* 1990, Hoefflich *et al.* 1994). Whether these receptors mediate the transport of IGF across epithelial monolayers is currently unknown.

In the present study we have examined the transport of ^{125}I -IGF-I across monolayers of a rat small intestinal epithelial cell line and compared this transport with that observed across kidney- and lung-derived epithelial cell lines. We present evidence for the restricted diffusion of intact IGF-I across these epithelial cell monolayers via a paracellular or low-affinity transcellular route.

Materials and Methods

Materials

Recombinant human (rh) IGF-I and IGF-II were purchased from GroPep Pty Ltd (Adelaide, Australia). IGF-I was iodinated with carrier-free Na¹²⁵I from Amersham International (Bucks, UK), to a specific activity of 85 µCi/µg, using a modification of the chloramine T method (Gargosky *et al.* 1990). [³H]inulin (1.18 Ci/mmol) was also obtained from Amersham and [methyl-³H]thymidine (20 Ci/mmol) was purchased from New England Nuclear (Boston, MA, USA). Foetal bovine serum (FBS) was purchased from Cytosystems (Castle Hill, Australia). Hanks' balanced salts solution was purchased from ICN Biomedicals (Costa Mesa, CA, USA) and Dulbecco's modified Eagle's medium (DMEM) was obtained from Flow Laboratories (Irvine, Scotland). Methylene blue, BSA and Tris (tris(hydroxymethyl)amino-methane) were from Sigma (St Louis, MO, USA). Hepes (N-(2-hydroxyethyl) piperazine-N'-(2-ethanesulphonic acid)), trichloroacetic acid (TCA) and di-sodium tetraborate were all from BDH Chemicals (Victoria, Australia). All transmigration experiments were carried out using transwells (6.5 mm diameter, 0.4 µm pore size) from Costar (Cambridge, MA, USA). Disuccinimidyl suberate was obtained from ICN Biochemicals Australasia Pty Ltd (Seven Hills, Australia). Protein molecular mass standards were obtained from Pharmacia (Uppsala, Sweden).

Cell culture

Madin-Darby bovine kidney (MDBK; CRL 6071), normal mink lung (Mv1Lu; CCL 64) and rat small intestinal crypt (IEC-6; CRL 1592) epithelial cell lines were all purchased from American Type Culture Collection (Rockville, MD, USA). All cells were grown in DMEM containing 10% FBS, together with 100 mg streptomycin, 60 mg penicillin and 1 mg fungizone per litre of growth medium. The cells were grown and used for experiments as monolayers at 37 °C in 5% CO₂.

Radioreceptor assays

The procedure for this assay has been described previously (Ross *et al.* 1989). Briefly, epithelial cells were subcultured onto 24-place multiwell plates (Costar) and grown to confluence. The cells were washed twice and incubated for 2 h at 4 °C in Hepes-buffered saline (Hepes (0.1 mol/l), NaCl (0.12 mol/l), KCl (5 mmol/l), MgSO₄ 7H₂O (1.2 mmol/l) and glucose (8 mmol/l), pH 7.6) plus BSA (0.5% w/v). Cells were then incubated for 18 h at 4 °C in 500 µl of the same buffer containing 2 × 10⁴ c.p.m. ¹²⁵I-IGF-I and the indicated amounts of unlabelled IGF-I. Monolayers were harvested by washing twice with

Hanks' balanced salts solution at 4 °C, followed by dissolution in NaOH (0.5 mol/l) containing Triton X-100 (0.1% v/v). Bound radioactivity was determined using a gamma counter. Data were fitted to a four-parameter equation with the aid of a non-linear curve-fitting program (Tablecurve, Jandel Scientific, San Rafael, CA, USA).

Affinity label cross-linking experiments

Cross-linking was performed as described in Kasuga *et al.* (1981). Confluent epithelial cell monolayers in six-place multiwell plates were washed twice and incubated at 4 °C in DMEM plus BSA (0.1% w/v) for 2 h. The medium was removed and the cells incubated for a further 4 h with 0.5 µCi (5 ng) ¹²⁵I-IGF-I in the presence or absence of a 1000-fold excess of IGF-I or IGF-II or a 10 000-fold excess of insulin. The medium was aspirated, the monolayers washed twice on ice with Hanks' balanced salts solution and bound IGFs chemically cross-linked by the addition of disuccinimidyl suberate (0.5 mmol/l) in DMEM for 20 min at 4 °C. The reaction was terminated with the addition of Tris (0.1 mol/l) containing EDTA (1 mmol/l) at pH 7.4. The cells were solubilised in 50 µl SDS-sample buffer containing β-mercaptoethanol, heated at 95 °C for 10 min, and subjected to SDS-PAGE through a 4% stacking and 6% separating acrylamide gel (Laemmli 1970). Gels were stained using Coomassie Blue R-250 and equilibrated in 10% acetic acid and 10% glycerol before being dried and exposed to X-ray film for 4 weeks.

Measurement of DNA synthesis

Confluent monolayers of epithelial cells grown in 24-place multiwell plates were washed twice for 30 min in serum-free DMEM plus BSA (0.1% w/v). Each monolayer was subsequently incubated for 24 h in serum-free medium containing a dilution series of IGF-I. A 10% FBS and serum-free control was also incorporated on each plate. During the last 6 h of this period, 1 µCi (5 nmol) [³H]thymidine was added to each well. Each monolayer was washed with Hanks' balanced salt solution, twice with 5% TCA and once with water. Cells were solubilised by trituration in NaOH (0.5 mol/l) containing Triton X-100 (0.1% v/v), and incorporated [³H]thymidine quantified using a beta counter. Results are expressed as the percentage of DNA synthesis above that observed in serum-free conditions.

Measurement of transepithelial electrical resistance

Measurement of electrical resistance was undertaken to monitor the integrity of each cell monolayer used in the transport experiments. Transwells were seeded with 20 × 10³ epithelial cells per well in DMEM plus 10% FBS, placed in 24-place multiwell plates containing the same

medium and cultured for 16 days with media changes every 2 days. Transepithelial resistance measurements were made every 24 h using a Millicell-Electrical Resistance System (Millipore, Bedford, MA, USA). To calculate the resistance measurement of each cell monolayer, the mean resistance measurements of transwells without cells were subtracted from the monolayer measurements and corrected for the area of the transwell (0.33 cm²).

Transmigration experiments

Epithelial cells were grown for 6 days in transwells as described above for measurement of transepithelial resistance. Before each experiment, control transwells were routinely fixed with methanol, stained with methylene blue (0.1% w/v) in di-sodium tetraborate (0.01 mol/l; pH 8.5) and visualised by light microscopy to monitor confluence. Other control transwells were fixed for 6 h in PBS containing paraformaldehyde (4% w/v), glutaraldehyde (1.25% v/v) plus sucrose (4% w/v), pH 7.2, and processed for transmission electron microscopy (Phillips, CM 100). In addition, the integrity of every monolayer was confirmed prior to each experiment by measuring the transepithelial electrical resistance.

At time zero, the monolayers were washed with serum-free DMEM plus BSA (0.1% w/v) and the transwells transferred to a new lower chamber containing 700 µl fresh medium. The medium from the apical (top) chamber was removed and 150 µl serum-free DMEM plus BSA (0.1% w/v) containing either ¹²⁵I-IGF-I (8 ng) or [³H]inulin (0.1 µg) were added to the apical chamber. After 5 min incubation at 37 °C, the basolateral (bottom) chamber was triturated, a sub-sample (100 µl) taken, and the chamber immediately replenished with the same amount of fresh medium. Further sub-samples were collected at 30, 60 and 120 min. At 120 min, the transepithelial electrical resistance of each transwell was measured again and each transwell was fixed and stained and the confluence of the monolayer confirmed by light microscopy. Transmigrated ¹²⁵I-IGF-I was measured as TCA-insoluble radioactivity. Briefly, portions of the medium from the abluminal chamber at various time points were mixed with ice-cold TCA to a final concentration of 10% TCA, incubated for 30 min at 4 °C and centrifuged at 3500 g. The radioactivity in the TCA-soluble and TCA-insoluble portions was counted in a gamma counter. [³H]inulin samples were counted in a beta counter. Transepithelial migration of each macromolecule was expressed as a percentage of the total of intact macromolecule added to the apical chamber at time zero.

Statistical analyses

Transmigration data were analysed by one-way ANOVA with all pair-wise multiple comparisons using Student–

Figure 1 IGF-I radioreceptor assays. Inhibition of ¹²⁵I-IGF-I binding to IEC-6 (○), Mv1Lu (△) and MDBK (□) cells by unlabelled IGF-I. Each point is the mean ± S.E.M. of triplicate determinations. Non-specific binding (amounting to 9.4, 7.5 and 2.0% of total counts bound for the IEC-6, Mv1Lu and MDBK cells respectively) has been subtracted.

Newman–Keuls method. DNA synthesis data and data arising from the effects of excess unlabelled IGF-I on ¹²⁵I-IGF-I transport were analysed by Student's *t*-test.

Results

Competitive binding and ligand–receptor cross-linking experiments

To confirm the presence of IGF-binding sites on epithelial cell lines, competitive-binding studies were performed on confluent cell monolayers. IEC-6 and Mv1Lu epithelial cell lines bound 3.7 and 4.1% of the total ¹²⁵I-IGF-I added to each well respectively, while the MDBK cells bound 23% of total ¹²⁵I-IGF-I. The IGF-I receptor displacement curve is shown in Fig. 1. Fifty per cent of bound ¹²⁵I-IGF-I were displaced by 3.6, 3.8 and 6.0 ng/ml unlabelled IGF-I from Mv1Lu, IEC6 and MDBK cells respectively. To further characterise IGF-binding sites on each of the epithelial cell lines, bound ¹²⁵I-IGF-I was cross-linked to cell-surface receptors in the absence or presence of saturating concentrations of unlabelled IGF-I, IGF-II or insulin. Receptor–ligand complexes identified by SDS-PAGE and autoradiography were visualised at approximately 140 and 270 kDa for both the IEC-6 and Mv1Lu cell lines (Fig. 2a and b). Both bands were sensitive to displacement when cross-linking was performed in the presence of unlabelled IGF-I, IGF-II or insulin. The

Figure 2 Autoradiograph of ^{125}I -IGF-I cross-linked to monolayers of (a) IEC-6 and (b) Mv1Lu cells. Cross-linking was carried out in the presence (+) or absence (-) of IGF-I (8 μg), IGF-II (8 μg) or insulin (80 μg). Cross-linked samples were subjected to SDS-PAGE on 6% acrylamide gels under reducing conditions and bands were visualised by exposure to X-ray film for 28 days. Positions of ^{14}C -labelled molecular mass markers are indicated ($\times 10^{-3}$).

molecular masses of these two bands are consistent with the cross-linked type I IGF receptor α -subunit and dimers of the cross-linked α -subunit respectively. No cross-linking of ^{125}I -IGF-I to MDBK cell membranes was observed. However, a band at 21 kDa was apparent when lysates of MDBK cells incubated with ^{125}I -IGF-I were run on a 12% polyacrylamide gel (not shown).

DNA synthesis assays

The presence of functional type I IGF receptors was further assessed using [^3H]thymidine incorporation as a measure of DNA synthesis. All concentrations of IGF-I tested significantly increased [^3H]thymidine incorporation into the DNA of Mv1Lu cells (Fig. 3). At the highest concentration tested (100 ng/ml), the Mv1Lu cells were stimulated to increase [^3H]thymidine incorporation

Figure 3 [^3H]thymidine incorporation into IEC-6 (open bars), Mv1Lu (solid bars) and MDBK (hatched bars) cells treated with the indicated concentrations of IGF-I. Results are expressed as a percentage above incorporation observed under serum-free conditions (means $\pm$ S.E.M., $n=3$, * $P<0.05$, † $P<0.01$).

by $74 \pm 2\%$ (means $\pm$ S.E.M.; $n=3$). The incorporation of [^3H]thymidine into IEC-6 and MDBK cells was increased only after addition of 100 ng/ml IGF-I, resulting in significant increases of 20 ± 4 and $80 \pm 3\%$ over serum-free conditions respectively (means $\pm$ S.E.M.; $n=3$).

Transmigration experiments

The ability to grow confluent epithelial cell monolayers on transwell membranes was confirmed by transmission electron microscopy (Fig. 4). Each cell line formed a polarised monolayer of cells with a clearly defined apical membrane. Formation of tight junctions is also evident from the micrographs and they are indicated by arrows. Trans-epithelial electrical resistance values for all cell lines on the first 16 days after seeding at a standard concentration of 20×10^3 cells per transwell are shown in Fig. 5. As all monolayers were confluent at day 6 and produced consistent transepithelial electrical resistance values, all trans-epithelial transport studies were undertaken at this time point. The mean transepithelial electrical resistance values on day 6 post-seeding were 61.5 ± 7.9 , 12.6 ± 1.7 and $7.9 \pm 1.0 \Omega \text{ cm}^2$ for MDBK, IEC-6 and Mv1Lu cells respectively (means $\pm$ S.E.M.; $n=5-7$ cultures). Integrity of the monolayers between experiments was routinely monitored by measuring transepithelial electrical resistance values on all cultures prior to and at the end of every transmigration experiment. These values did not significantly differ. In addition, staining and light microscopy

Figure 4 Transmission electron microscopy of epithelial cell monolayers grown on transwells. (a) IEC-6, (b) Mv1Lu and (c) MDBK cells were seeded on transwells in DMEM plus 10% FBS and the media changed every 2 days. On day 6 post-seeding, the cells were fixed and stained for transmission electron microscopy as described in Materials and Methods. Size bars represent 1 μ m. Tight junctions are indicated by arrows.

of all cultures confirmed the presence of confluent monolayers at the end of each experiment.

The transport of ^{125}I -IGF-I across transwell filters alone and both ^{125}I -IGF-I and $[^3\text{H}]$ inulin across confluent

Figure 5 Transepithelial electrical resistance measurements of cell monolayers. IEC-6 (○), Mv1Lu (△), and MDBK (□) cells were seeded on transwells in DMEM plus 10% FBS (20×10^3 cells per well). Electrical resistance values were measured every 24 h, each value representing the means $\pm$ S.E.M. of three transwell cultures.

monolayers of IEC-6, MDBK and Mv1Lu cells is shown in Fig. 6. Addition of ^{125}I -IGF-I to the apical side of each cell monolayer resulted in a time-dependent increase in the amount of this peptide appearing intact in the basolateral chamber. At 2 h, 7.9 ± 0.8 , 10.8 ± 0.7 and $1.2 \pm 0.2\%$ of total intact ^{125}I -IGF-I added to the apical chamber were transported by IEC-6, Mv1Lu and MDBK cell monolayers respectively (means $\pm$ S.E.M., $n=18$ cultures). The flux of ^{125}I -IGF-I across filters without cell monolayers was significantly greater ($34.1 \pm 0.9\%$, means $\pm$ S.E.M., $n=18$) than that across the epithelial cells, indicating that the IEC-6 and Mv1Lu cells had greatly restricted and the MDBK cells virtually excluded the passage of ^{125}I -IGF-I. In addition, the flux of ^{125}I -IGF-I across the epithelial cell monolayers was significantly less than that observed for $[^3\text{H}]$ inulin.

Gel-permeation chromatography of the inulin tracer confirmed that all $[^3\text{H}]$ was present as a high molecular mass species. TCA precipitability of ^{125}I -IGF-I immediately prior to its addition to the apical chamber was $95 \pm 0.6\%$ ($n=72$ cultures). For IEC-6 and Mv1Lu cell monolayers, the majority of radioactivity appearing in the basolateral chamber was TCA precipitable. TCA precipitability gradually increased from $52 \pm 2\%$ at 5 min to $63 \pm 2\%$ by 2 h and from $64 \pm 4\%$ at 5 min up to $76 \pm 3\%$ by 2 h for the IEC-6 and Mv1Lu cells respectively (means $\pm$ S.E.M., $n=18$ cultures), indicating the preferential movement of free ^{125}I at the earlier time points. In contrast, less than 30% of radioactivity appeared as

Figure 6 Transport of ^{125}I -IGF-I (○) and $[^3\text{H}]$ inulin (□) across (a) IEC-6, (b) Mv1Lu and (c) MDBK cell monolayers. Transport of ^{125}I -IGF-I across control transwell filters without cells is shown in each case (△). Transport of ^{125}I -IGF-I and $[^3\text{H}]$ inulin transport is expressed as a percentage of total intact ^{125}I -IGF-I and total $[^3\text{H}]$ inulin added to the apical chamber at time zero respectively. Data are the pooled means $\pm$ S.E.M. of triplicate transwells from six experiments. * $P < 0.05$, † $P < 0.01$ vs ^{125}I -IGF-I across epithelial cell monolayers.

TCA-precipitable counts in the basolateral chamber of the MDBK cell cultures by the end of the 2 h experiment.

In order to examine whether the transport of ^{125}I -IGF-I is concentration dependent, a 1000-fold excess of unlabelled IGF-I was added to the apical chamber 30 min prior to the addition of ^{125}I -IGF-I. As can be seen from Fig. 7, excess unlabelled IGF-I did not alter the transport of ^{125}I -IGF-I across epithelial cell monolayers, suggesting the non-saturable flux of intact ^{125}I -IGF-I across epithelial cell barriers.

Discussion

The major function of any epithelium is to form a selective barrier to the movement of solutes including peptides and proteins. Solute flux across an epithelial barrier can occur by either a transcellular route, involving transport systems located in the apical and basolateral membranes, or a paracellular route via intercellular spaces. The absorption of biologically active intact protein species from the intestine is well described. Thus absorption of $[^3\text{H}]$ -labelled BSA has been demonstrated in adult rats (Warshaw *et al.* 1974). IgG is transported from the gut lumen to the circulation by a receptor-mediated pathway in newborn calves (Jochims *et al.* 1994). Intestinal absorption of metabolically active insulin occurs by a transcellular pathway in rats, causing increased circulating levels of insulin followed by significant decreases in blood glucose (Bendayan *et al.* 1994).

Of direct relevance to the current study are several reports demonstrating intestinal absorption of intact IGF-I. It has been shown that plasma IGF-I levels increase in a dose-dependent fashion in calves orally administered IGF-I (Baumrucker *et al.* 1992). In 1 day old calves, supraphysiological amounts of immunoreactive IGF-I or ^{125}I -IGF-I administered into a clamped segment of the jejunum result in the appearance of trace amounts of IGF-I or ^{125}I -IGF-I in the mesenteric vein draining the segment (Vacher *et al.* 1995). Orally administered ^{125}I -IGF-I is absorbed by piglets and calves with some 20% of total plasma radioactivity appearing as intact ^{125}I -IGF-I, the majority of which is associated with plasma IGF-binding proteins (IGFBPs) (Odle *et al.* 1996, Xu & Wang 1996). Donovan *et al.* (1997) have reported that after oral administration of ^{125}I -rhIGF-I to pigs, some 18–20% of total radioactivity in both the portal and arterial blood were intact, with absorbed ^{125}I -rhIGF-I representing 0.205% of the total plasma pool. In rats, rhIGF-I was absorbed into the systemic circulation where it was found to be associated with high molecular mass IGFBPs (Kimura *et al.* 1997). Importantly, Philipps *et al.* (1997) have shown that metabolically active IGF-I is absorbed. Rat pups fed rat milk substitute containing rhIGF-I gained more weight, had increased brain and liver wet weight, demonstrated increased small intestine and liver protein content, and had

Figure 7 Effect of excess IGF-I on transport of ^{125}I -IGF-I across epithelial cell monolayers. Transport is shown as a percentage of total intact ^{125}I -IGF-I added to the apical chamber at time zero appearing in the basolateral chamber of (a) IEC-6, (b) Mv1Lu and (c) MDBK cultures in the presence ($\square$) and absence ($\circ$) of a 1000-fold excess of IGF-I. Data represent the means $\pm$ S.E.M. of triplicate transwells from three experiments.

serum IGF-I levels 2-fold those of animals fed a rat milk substitute devoid of IGF-I.

We have demonstrated the presence of functional IGF receptors on IEC-6 and Mv1Lu cells by radioreceptor, affinity label cross-linking and [^3H]thymidine incorporation studies. The increased sensitivity of Mv1Lu cells to IGF-I in the latter study may reflect the production or secretion of lower levels of IGFBPs compared with those of the other cell lines. Our results confirm earlier data indicating that IEC-6 cells possess type I IGF receptors (Martin & Baxter 1992, Simmons *et al.* 1995). Interestingly, our finding that MDBK cells possess type I IGF receptors using radioligand binding and DNA synthesis assays could not be extended to cross-linking experiments, possibly reflecting the radioligand cell-binding kinetics over the time course of the study. In this regard, receptors for IGF-I and IGF-II have been identified on cultured canine and opossum proximal tubule cells (Hammerman & Rogers 1987, Fawcett & Rabkin 1995). While the type II IGF receptors were shown to be equally distributed on the basolateral and brush border membranes, the distribution of the type I IGF receptor was asymmetrical, with the basolateral membrane exhibiting a several-fold higher specific-binding capacity (Hammerman & Rogers 1987). As MDBK cells display properties of proximal tubule cells, it may be that the radioligand is unable to access basolateral surface receptors over the time course of the cross-linking experiments. The binding of ^{125}I -IGF-I to the surface of these cells by a competitive mechanism may reflect binding to a cell-surface binding protein. Indeed, a band at 21 kDa was observed when cross-linked ^{125}I -IGF-I was run out on a 12% polyacrylamide gel.

Cells cultured on a supporting microporous membrane that permits separation of the apical and basolateral solutions provide a useful model to investigate the mechanisms of solute transport across cell monolayers. In the present study, epithelial cell monolayers were maintained in serum-free conditions to minimise potential interference of serum-derived IGFs or IGFBPs. The presence of confluent cell monolayers was checked using light and electron microscopy as well as transepithelial electrical resistance measurements. Under these conditions the epithelial cell monolayers produce tight junctions. The electrical resistance measured across MDBK cells in the present studies was much higher than that of Mv1Lu and IEC-6 cell monolayers, but comparable with the value of $83.4 \pm 15.8 \Omega \text{ cm}^2$ cited in Cereijido *et al.* (1978). In addition, the MDBK cells virtually excluded any passage of ^{125}I -IGF-I or [^3H]inulin. MDBK cells are derived from proximal tubule cells, which are not involved in protein transport but are the site of re-absorption of amino acids originating from the small amount of protein present in the glomerular filtrate. Our results thus reflect the role of these cells *in vivo*. The IEC-6 and Mv1Lu cell lines produced low transepithelial electrical resistance values, yet still greatly restricted the passage of ^{125}I -IGF-I or [^3H]inulin

compared with filters without cells. In contrast, previous studies have shown T₈₄ colonic carcinoma cells generate high electrical resistance values, while greatly impeding the movement of electrolytes as well as macromolecules such as insulin, inulin and albumin (Milton & Knutson 1990), and thus display characteristics more comparable with the kidney-derived MDBK line than with the gut-derived line in the current experiments. This observation possibly reflects the role of colonic epithelium as the site of water rather than protein absorption. IEC-6 and Mv1Lu cells exhibit similar characteristics to endothelial cells, which produce tight junctions and low transcellular resistance, although significantly impede the transport of macromolecules (Milton & Knutson 1990, Bastian *et al.* 1997). We have recently shown that the movement of ¹²⁵I-IGF-I across human umbilical vein endothelial cells occurs by restricted paracellular diffusion (Bastian *et al.* 1997).

The majority of ¹²⁵I-IGF-I that bound to each of the three epithelial cell monolayers was almost totally displaced by relative concentrations of unlabelled IGF-I (Fig. 1) which were 10-fold less than the concentration of excess unlabelled IGF-I used in the transmigration experiments (Fig. 7). The transport of ¹²⁵I-IGF-I across epithelial cell monolayers was unaltered in the presence of this excess concentration of IGF-I, suggesting a non-saturable transport mechanism. We interpret these results as demonstrating that ¹²⁵I-IGF-I crosses IEC-6, Mv1Lu and MDBK epithelial cell barriers via a paracellular or low-affinity transcellular route, and not by a receptor-mediated pathway. Indeed, as ¹²⁵I-IGF-I transport across each epithelial cell monolayer was significantly less than inulin, ¹²⁵I-IGF-I transport may have been retarded by binding to cell-surface IGF receptors or IGF-BPs. Transport studies utilising epithelial cell monolayers grown in a bicameral system represent a simple model which generates information regarding IGF-I transport across a well-defined homogeneous cell population and as such are a first step in the investigation of IGF-I transport from the gut lumen. However, the overall IGF effector system at the tissue level is complex and will require future *in vivo* studies where all components of IGF effector systems are present.

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