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A survey of membrane peptidases in two human colonic cell lines, Caco-2 and HT-29

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The expression of cell-surface peptidases was examined in two human colon carcinoma cell lines, Caco-2 and HT-29. Enzymic assays revealed the presence of eight cell-surface peptidases on a Caco-2 cell line (passage number 82–88), namely aminopeptidase N, dipeptidyl peptidase IV, peptidyl dipeptidase A (angiotension-converting enzyme), aminopeptidase P, aminopeptidase W, endopeptidase-24.11, γ-glutamyl transpeptidase and membrane dipeptidase. The presence of dipeptidyl peptidase IV and endopeptidase-24.11 was also confirmed immunochemically. After 15 days culture, the activities of aminopeptidase P, peptidyl dipeptidase A and alkaline phosphatase activities on Caco-2 cells reached a plateau, and that of membrane dipeptidase began to decline. In contrast, aminopeptidase N, dipeptidyl peptidase IV and endopeptidase-24.11 activities were still rising after 26 days in culture. Caco-2 cells of passage number 181–183 were found to lack endopeptidase-24.11, but maintained dipeptidyl peptidase IV expression. Two populations of HT-29 cells were surveyed. Both the standard, undifferentiated population and a differentiated population expressed only three peptidases: dipeptidyl peptidase IV, aminopeptidase W and carboxypeptidase M. In the differentiated HT-29 cells the activity of dipeptidyl peptidase IV after 14–21 days was beginning to plateau whereas aminopeptidase W activity was still rising and that of carboxypeptidase M had begun to decline. These differences in activity profiles observed among this group of cell-surface peptidases indicate that these cell lines, especially Caco-2, are useful models to study the regulation of their expression.

INTRODUCTION

The human colonic carcinoma cell lines, Caco-2 and HT-29, have been widely studied in culture for their ability to differentiate into enterocyte-like cells, expressing some features characteristic of mature small-intestinal cells [for review see Rousset (1986)]. Caco-2 cells spontaneously differentiate on reaching confluency (Pinto *et al.*, 1983), whereas HT-29 cells can be made to differentiate in the absence of glucose, by the replacement of glucose by galactose in culture media (Zweibaum *et al.*, 1985) or by the addition of certain differentiation inducers. Differentiation in these cells is characterized by the formation of tight junctions and the appearance of an apical brush border on which many hydrolases are expressed. For example, sucrase/isomaltase, lactase and alkaline phosphatase have been studied on the Caco-2 and/or HT-29 cells (Pinto *et al.*, 1982, 1983; Hauri *et al.*, 1985; Wice *et al.*, 1985; Trungnan *et al.*, 1987; Le Bivic *et al.*, 1990; Matter *et al.*, 1990; Matter & Hauri, 1991), but with regard to peptidases, only two, namely aminopeptidase N (EC 3.4.11.2, AP-N) and dipeptidyl peptidase IV (EC 3.4.14.5, DPP-IV), have been reported in these cells (Pinto *et al.*, 1982; Hauri *et al.*, 1985; Chantret *et al.*, 1988). These two enzymes are members of a group of cell-surface peptidases, numbering about twelve, widely distributed on many cell types [for reviews see Kenny *et al.* (1987) and Turner *et al.* (1987)]. They appear to play a variety of roles depending on their location. For example, in the nervous system, membrane peptidases are thought to be responsible for terminating the action of neuropeptides. At other sites, the renal brush border and choroid plexus, the battery of cell-surface peptidases may well act in a protective manner, whereas intestinal microvillar peptidases may be responsible for the degradation of dietary peptides enabling absorption of amino acids and dipeptides from the gut.

In the present study we have made a comprehensive survey of membrane peptidases on both human colonic cell lines, Caco-2 and HT-29. In doing so we report that one of these cell lines, Caco-2, is particularly abundant in many membrane peptidases and should prove a useful model with which to study the regulation of their expression.

EXPERIMENTAL

Materials

Antibodies. Polyclonal antibodies RP161 and RP143 were generated in rabbits immunized with pig endopeptidase-24.11 (EC 3.4.24.11, E-24.11) and pig DPP-IV respectively. They were affinity-purified and shown to be monospecific (Barnes *et al.*, 1991). Anti-rabbit fluorescein isothiocyanate (FITC)-conjugated IgG was obtained from Sigma Chemical Company, Poole, Dorset, U.K.

Substrates. Ala-7-amino-4-methylcoumarin, Gly-7-amino-4-methylcoumarin and Gly-Pro-7-amino-4-methylcoumarin were obtained from Bachem (UK) Ltd., Saffron Walden, Essex, U.K. [D-Ala^2,Leu^5]Enkephalin was obtained from Cambridge Research Biochemicals Ltd., Cambridge, U.K. All other peptides were obtained from Sigma.

Inhibitors. Phosphoramidon was obtained from Peninsula Laboratories Europe Ltd., St. Helens, Lancs., U.K. 1,10-

Abbreviations used: ACE, peptidyl dipeptidase A ('angiotensin-converting enzyme'); AP, alkaline phosphatase; AP-A, aminopeptidase A; AP-N, aminopeptidase N; AP-P, aminopeptidase P; AP-W, aminopeptidase W; CP-M, carboxypeptidase M; Dip-F, di-isopropyl fluorophosphate; DPP-IV, dipeptidyl peptidase IV; E-24.11, endopeptidase-24.11; FITC, fluorescein isothiocyanate; γ-GT, γ-glutamyl transpeptidase; MDP, membrane dipeptidase; PBS, phosphate-buffered saline; PI-PLC, phosphatidylinositol-specific phospholipase C; DMEM, Dulbecco's minimal essential medium; PBS/GT, 0.04% (w/v) gelatin made up in PBS.

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Phenanthroline was obtained from Aldrich Chemical Co., Gillingham, Dorset, U.K. Enalaprilat {MK422, *N*-[(*S*)-1-carboxy-3-phenylpropyl]-L-alanyl-L-proline} and cilastatin [MK0791, monosodium *Z*-*S*-(6-carboxy-6-{[2,2-dimethyl-(*S*)-cyclopropyl]carbonylamino}-5-hexenyl)-L-cysteine] were gifts from Dr. A. A. Patchett and Dr. H. Kropp respectively, both of Merck Sharp and Dohme Research Laboratories (Rahway, NJ, U.S.A.). Amastatin and di-isopropyl fluorophosphate (Dip-F) were purchased from Sigma.

Cells and culture conditions

Caco-2 cells, kindly donated by Dr. D. Swallow, MRC Human Biochemical Genetics Unit, University College, London, U.K., were routinely grown in 75 cm^2 plastic flasks (Cell-cult, Sterilin Ltd., Hounslow, U.K.) using Dulbecco's minimal essential medium (DMEM) (Imperial Laboratories, Hounslow, Middx., U.K.) supplemented with 20% heat-inactivated fetal calf serum (Imperial Laboratories), 1% non-essential amino acids (Imperial Laboratories), and penicillin and streptomycin (Gibco). They were seeded at 9×10^5 cells per flask at 37 °C in a 5% CO_2/95% air atmosphere, and the medium was changed 48 h after seeding and daily thereafter. Cultures were used between passages 83 and 88 and 181 and 183. HT-29 cells were kindly donated by Dr. A. Zweibaum (INSERM U178, Villejuif, France). Standard HT-29 cells, cells that have been cultured in 25 mM-glucose since the strain was originated, referred to as HT-29 (Std), were grown for 30 passages in a glucose-depleted medium and then repassaged in a standard medium, thus producing the differentiated phenotype, referred to as HT-29 (Glc^-,Glc^+). Both HT-29 (std) and HT-29 (Glc^-,Glc^+) cells were then routinely grown in DMEM supplemented with 5% heat-inactivated fetal calf serum and penicillin and streptomycin. HT-29 (Std) and (Glc^-,Glc^+) cells were seeded at 1.5×10^6 cells per flask and studied between passages 176 and 179 and 30 and 32 respectively.

Preparation of cell membranes

The cell culture medium was removed and the cell monolayer rinsed twice with phosphate-buffered saline (140 mM-NaCl, 10 mM-sodium phosphate buffer, pH 7.4, PBS). The cells were then scraped off the flask into 10 ml of ice-cold 5 mM-Tris/HCl, pH 7.4, and stood for 10 min at 4 °C. Homogenization was then performed with a glass/Teflon Potter–Elvehjem homogenizer for 65–70 s with 11 strokes at 1100 rev./min. $CaCl_2$ (18 mM final concentration) was added and after standing for 10 min at 4 °C, the homogenate was centrifuged (10 min, 950 *g*). The supernatant was further centrifuged (90 min, 31000 *g*, 4 °C) to yield a small pellet (P2) which was resuspended in 1 ml of 50 mM-Hepes/NaOH, pH 7.4, and stored at −20 °C until required for assay. The need to determine many enzyme activities on the cell membrane precluded a second Ca^{2+} treatment and the isolation of a more enriched P4 fraction.

Indirect immunofluorescent staining

Unless otherwise stated, all the staining steps were carried out at room temperature. Caco-2 cells were grown on glass coverslips in six-well plates (Linbro, Flow Laboratories, Rickmansworth, U.K.). After various times in culture, coverslips were transferred to clean six-well plates and washed gently three times with 2 ml of PBS warmed to 37 °C. Cells were fixed with 1 ml of paraformaldehyde (4%, w/v, in PBS, 37 °C) and then stood at room temperature for 20 min. Coverslips were washed and incubated with 2 ml of 50 mM-NH_4Cl in PBS for 10 min. After further washing, coverslips were incubated with 2 ml of 0.04% (w/v) gelatin made up in PBS (PBS/GT) for 15 min. Coverslips were then placed on a 100 μl drop of polyclonal antibody RP143 or RP161 diluted 1:500 in PBS/GT. A donkey anti-rabbit FITC-conjugated IgG diluted 1:50 in PBS/GT was centrifuged (10 min, 8000 *g*) and the supernatant collected. After 1 h, coverslips were washed in PBS/GT and placed on a 100 μl drop of the supernatant for 30 min at 4 °C. Coverslips were then washed and mounted in semipermanent mounting medium (Mayer & Walker, 1990) on microscope slides. Slides were kept at 4 °C and viewed by epi-illumination using a Zeiss microscope.

Phosphatidylinositol-specific phospholipase C (PI-PLC) release of membrane proteins from P2 membranes

Caco-2 cells (P87) were cultured for 26 days after which time P2 membranes were prepared as described above. P2 membranes (100 μg) were incubated with 10 mM-Hepes, pH 7.0, and *Bacillus thuringiensis* PI-PLC (kindly donated by Dr. M. G. Low, Department of Physiology and Cellular Biophysics, Columbia University, New York, NY, U.S.A.) for 1 h at 37 °C. Released proteins were separated from membrane by centrifugation (31000 *g*, 90 min at 4 °C) and the supernatants stored at −20 °C until required for assay.

Enzyme assays

E-24.11 and peptidyl dipeptidase A (EC 3.4.15.1, 'angiotensin-converting enzyme'. ACE) were assayed using h.p.l.c. methods of Turner *et al.* (1989) and enzyme specificities controlled by inhibition with phosphoramidon (1 μM) and enalaprilat (10 μM) respectively. Membrane dipeptidase (EC 3.4.13.11, renal dipeptidase, MDP) was assayed by an h.p.l.c. method (Hooper *et al.*, 1987) using Gly-D-Phe (1 mM) as substrate and specificity checked by inhibition with cilastatin (0.1 mM). Aminopeptidase P (EC 3.4.11.9, AP-P) was assayed using the h.p.l.c. method of Hooper & Turner (1988) with Gly-Pro-Hypro (1 mM) as substrate. Aminopeptidase W (AP-W) activity was assayed using the method of Gee & Kenny (1987) with Glu-Trp (0.5 mM) as substrate. Carboxypeptidase M (CP-M) was assayed using Hip-Lys (1 mM) as substrate and hippuric acid release quantified using h.p.l.c. with elution conditions as described for that of the ACE assay. Specificity was determined by inhibition with 1,10-phenanthroline. For each of these peptidases, 2–10 μg of membrane protein was incubated with the peptide substrate for 16 h, before termination of the reaction by boiling and analysis by h.p.l.c. Aminopeptidase N (EC 3.4.11.2, AP-N), aminopeptidase A (EC 3.4.11.7. AP-A) and DPP-IV were assayed using the same quantity of membrane proteins by fluorimetric methods as described previously (Fulcher & Kenny, 1983). γ-Glutamyl transpeptidase (EC 2.3.2.2, γ-GT) was assayed using the method described by Szasz (1969). Alkaline phosphatase (AP) was assayed using *p*-nitrophenol phosphate as substrate, as described by Bessey *et al.* (1946). All enzyme units are expressed as nmol of substrate hydrolysed/min.

Other methods

Preparation of kidney microvillar membranes (P4) was as described previously (Booth & Kenny, 1974). The e.l.i.s.a. of E-24.11 was performed as described by Howell *et al.* (1991). Protein was assayed by the method of Lowry *et al.* (1951).

RESULTS

Membrane peptidase activities on Caco-2 cells

Cell membranes were prepared from Caco-2 cells (passages 83–88) at day 5 (when cells were confluent), and after 8, 15, 21 and 26 days in culture. The activities for eight peptidases in Caco-2 cells cultured for 26 days are shown in Table 1; the enrichment values of four peptidases (shown in parentheses) in the P2 fraction were in the range 4.3–7.9-fold. Fig. 1 shows the

Table 1. Comparison of peptidase activities in membranes from Caco-2 (P82–88) and HT-29 cells at 26 and 28 days in culture respectively with those of pig kidney microvilli

See the Experimental section for details. Kidney microvillar activities marked with an asterisk (*) were taken from Bourne *et al.* (1989). N.D., not detectable. The values in parentheses for Caco-2 membranes are the enrichment factors related to the activities in the homogenate.

	Specific activities (units/mg of protein)						
	P2 fraction				Relative activity of P2 as % of P4		
Enzyme	Caco-2	HT-29 (Std)	HT-29 (Glc^-,Glc^+)	P4 fraction (pig kidney)	Caco-2	HT-29 (Std)	HT-29 (Glc^-,Glc^+)
Aminopeptidase A	N.D.	N.D.	N.D.	126*	–	–	–
Aminopeptidase N	4.75 (4.3)	N.D.	N.D.	524*	1	–	–
Aminopeptidase P	3.61	N.D.	N.D.	68*	5	–	–
Aminopeptidase W	3.35	0.43	0.55	120*	3	0.3	0.5
Carboxypeptidase M	N.D.	1.09	0.75	5.3	–	20.6	14
Dipeptidyl peptidase IV	83 (6.2)	2.40	7.40	606*	14	0.4	1.2
Endopeptidase-24.11	2.12 (7.9)	N.D.	N.D.	283	1	–	–
γ-Glutamyl transpeptidase	28.8	N.D.	N.D.	297	10	–	–
Microsomal dipeptidase	2.17 (7.7)	N.D.	N.D.	121*	2	–	–
Peptidyl dipeptidase A (ACE)	6.55	N.D.	N.D.	11.7	56	–	–

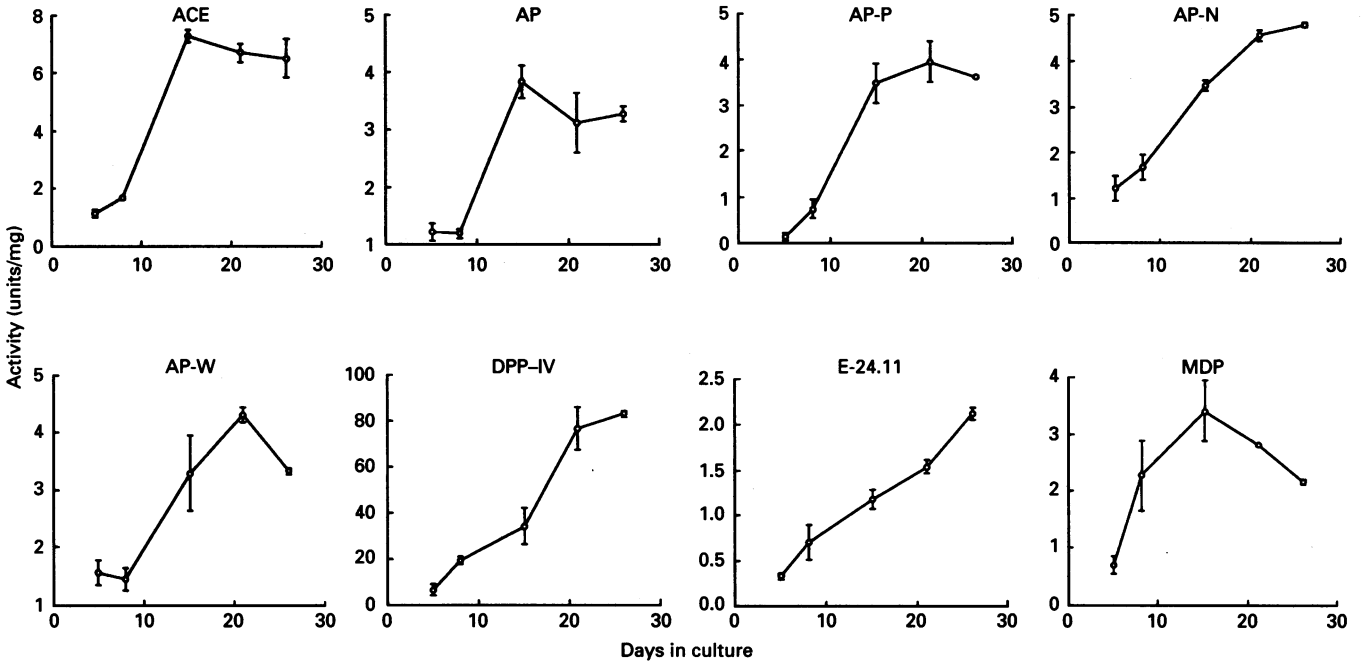

Fig. 1. Expression of membrane peptidases on Caco-2 cells over 26 days in culture

Caco-2 cells (passages 83–88) were grown in culture and membranes prepared as described in the Experimental section. Enzyme activities are from P2 fractions and are the means of three cell preparations. Error bars are ±S.E.M.

profiles of membrane peptidases expressed by Caco-2 cells over this period. All peptidase activities increased in the first 15 days in culture; however, AP-P and ACE activities plateaued whereas MDP activity was maximal at this period in culture. In contrast, AP-N, DPP-IV and E-24.11 activities were still rising after 26 days in culture. γ-GT activity was only detectable on Caco-2 cells after 15 days in culture (not shown).

E.l.i.s.a. of E-24.11 on the same samples gave results in close agreement with those determined by enzymic assay. On P2 fractions after 15 and 26 days in culture the e.l.i.s.a. predicted 21.9 and 33.7 ng of enzyme/mg of protein respectively [compared with 19.1 and 34.3 ng of enzyme/mg of protein determined using the h.p.l.c. enzyme assay, calculated from the $k_{cat.}$ value of 5645 min^{-1} (Matsas *et al.*, 1984)].

Membranes from Caco-2 cells of a higher passage number (P181–183) were also prepared and two peptidases assayed. DPP-IV activities were comparable with those from Caco-2 cells (passages 83–88), but E-24.11 was not detectable by enzymic assay or e.l.i.s.a.

Indirect immunofluorescent staining

Antibodies against E-24.11 and DPP-IV were found to react strongly with the cell-surface monolayer of Caco-2 (P83–88) (Fig. 2). The two antibodies gave similar staining patterns which showed punctuate surface staining typical for brush-border antigens. However, there was also significant DPP-IV staining seen at the site of cell–cell contacts (Fig. 3*b*), indicating that some antigen is present on the lateral membrane. E-24.11 staining was

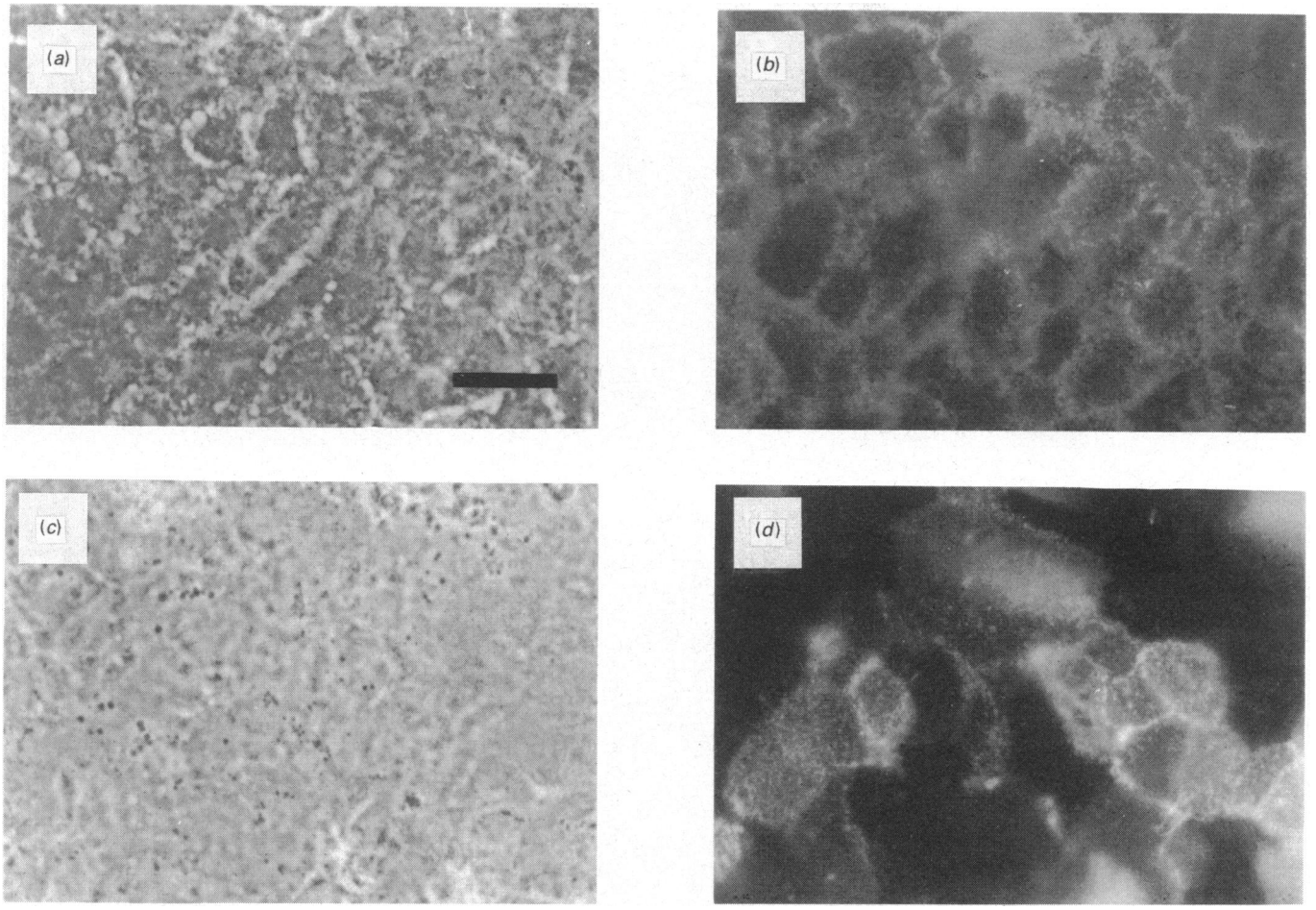

Fig. 2. Indirect immunofluorescent staining of Caco-2 cells (P88) grown on coverslips in culture

Caco-2 cells (P88) were grown in culture for 26 days and then immunofluorescently stained as described in the Experimental section. (*a*) and (*c*) are micrographs viewed by phase-contrast optics of (*b*) and (*d*) respectively. (*b*) Cell-surface expression of DPP-IV. (*d*) Cell-surface expression of E-24.11. Note here the patchy staining of E-24.11; this contrasts with the uniform staining of DPP-IV seen in (*b*). (Bar, 5 μM.)

not uniform across the whole cell monolayer. The number of cells staining positively for E-24.11 and DPP-IV appeared to increase during culture, but even at 26 days not all cells stained positive for E-24.11. Caco-2 cells of a higher passage number (passages 181–183) were also stained for E-24.11 and DPP-IV. It can be seen from Fig. 3 that DPP-IV staining resembles that of the lower passages of Caco-2 cells (Fig. 2), but no E-24.11 staining was apparent.

PI-PLC-released membrane enzymes from Caco-2 (P87) P2 membranes

Supernatants containing PI-PLC-released proteins were assayed for the following enzyme activities: ACE, AP, AP-P, MDP and E-24.11 (Fig. 4). AP, AP-P and MDP were all released from Caco-2 membranes by PI-PLC in a concentration-dependent manner. ACE and E-24.11 were not released from P2 membranes.

Membrane peptidase activities on HT-29 cells

Three membrane peptidases were identified on HT-29 (std) and (Glc^-,Glc^+) cells at 28 days in culture, and the activities are shown in Table 1.

Fig. 5 shows how the three membrane peptidase activities, identified on HT-29 (Glc^-,Glc^+) cells, change over 28 days in culture. AP-W activity is seen to be still rising at 28 days in culture. However, DPP-IV activity rose steeply for 14 days and then began to level off. By enzymic assay of eight other peptidases, only one, CP-M, was detected. Tissue culture media, collected after HT-29 (Glc^-,Glc^+) cells had been in culture for 5, 7, 14, 21 and 28 days, were assayed for all ten membrane peptidases; none were detectable.

Comparison of membrane peptidase activities in Caco-2 and HT-29 cells with those in renal microvilli

All of the ten peptidases present in pig kidney microvilli, except for AP-A and CP-M, were identified in membrane preparations from Caco-2 cells. In contrast, only three peptidases were detected in the membranes from both HT-29 cell populations. Table 1 compares the specific activities of the membrane peptidases found in P2 fractions from Caco-2 and HT-29 cells with those from pig kidney P4 fractions. ACE and DPP-IV activities were abundant in Caco-2 cell membranes, being about 56% and 14% of the specific activities of the renal membranes, whereas the remaining membrane peptidases were present in P2 fractions at levels 1–5% of those in renal membranes. In the two HT-29 cell populations, CP-M was relatively abundant, but the activities of AP-W and DPP-IV were very low.

DISCUSSION

The great interest in Caco-2 and HT-29 cell lines derives from their resemblance to enterocytes of the small intestine, even

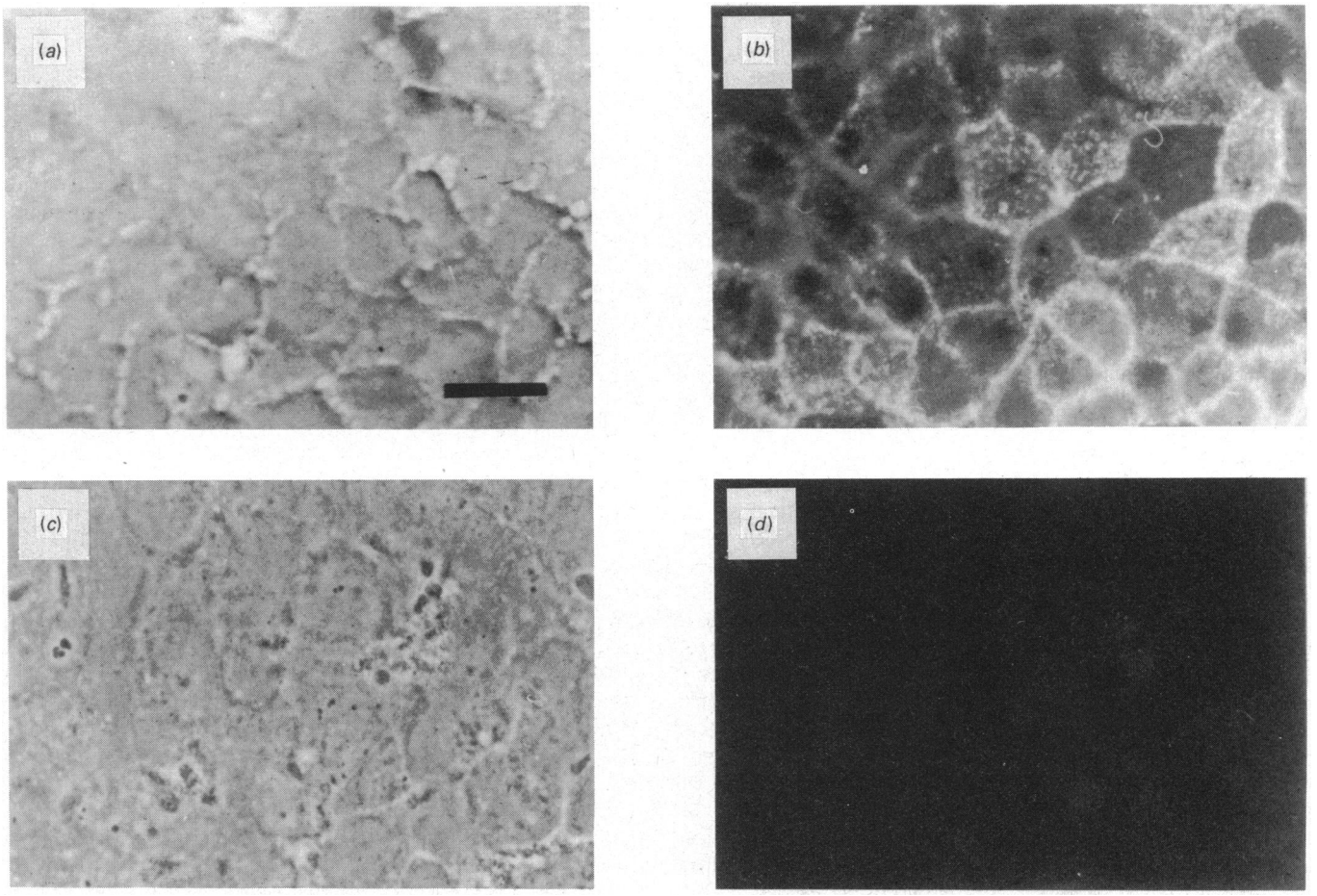

Fig. 3. Indirect immunofluorescent staining of Caco-2 cells (P183)

Caco-2 cells (P183) were grown and immunofluorescently stained as described previously. (*a*) and (*c*) are micrographs viewed by phase-contrast optics of (*b*) and (*d*) respectively. (*b*) Cell-surface expression of DPP-IV. (*d*) Cell-surface expression of E-24.11. The lack of E-24.11 staining is apparent, whereas DPP-IV staining is retained in this high-passage Caco-2 cell monolayer. (Bar, 5 μM.)

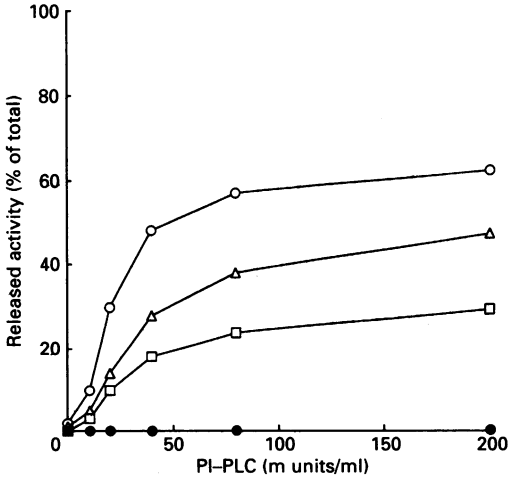

Fig. 4. Release of membrane hydrolases from Caco-2 (P87) cell membranes by PI-PLC

See the Experimental section for details. A P2 fraction was prepared from Caco-2 cells cultured for 26 days and incubated with PI-PLC. Released activities were assayed in the supernatant fraction: ○, AP; △, AP-P; □, MDP; ●, ACE and E-24.11.

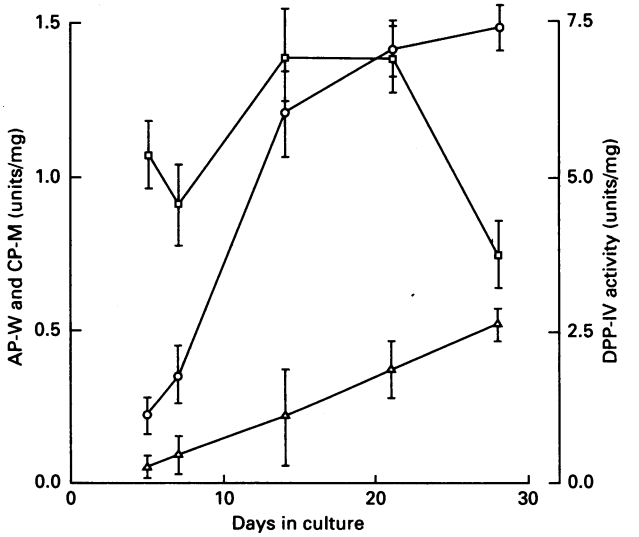

Fig. 5. Expression of membrane peptidases on HT-29 (Glc^-,Glc^+) cells over 28 days in culture

HT-29 (Glc^-,Glc^+) cells were grown and membranes prepared as described in the Experimental section. Enzyme specific activities are from P2 fractions and are the means of three cell preparations. Error bars are ±S.E.M. △, AP-W; □, CP-M; ○, DPP-IV.

though their true origins were from colonic cells, which normally lack a well-developed brush border. Enterocytes, like their counterpart in the kidney proximal tubule, express many microvillar peptidases. In this paper we have surveyed ten of this group of enzymes in both cell lines. Pinto *et al.* (1982, 1983) first reported the presence of AP-N in HT-29 and Caco-2 cells. Hauri *et al.* (1985) applied immunochemical methods to Caco-2 cells and demonstrated the presence of AP-N and DPP-IV, but failed to reveal ACE and 'PABA-peptide' hydrolase. The AP-N staining was much weaker than that of DPP-IV. Chantret *et al.* (1988) assayed microvillar membrane-enriched fractions of 20 cell lines, including Caco-2 and HT-29 cells, for five hydrolases, including these two peptidases. Both DPP-IV and AP-N were identified, but the activities were much higher in the Caco-2 cells than in HT-29 cells. However, the present paper is the first comprehensive survey of microvillar peptidases in these cell types. Caco-2 cells expressed eight membrane peptidases, which in addition to AP-N and DPP-IV, included ACE, AP-P, AP-W, MDP, E-24.11 and γ-GT. In contrast, only three activities were observed in both HT-29 (std) and (Glc^-,Glc^+) cells, namely DPP-IV, AP-W and CP-M. The last enzyme was not detected in Caco-2 cells and the first two were present at much lower activity than in Caco-2 cells. AP-N was not detected by our fluorimetric assay, in line with the low activity reported by Chantret *et al.* (1988) for HT-29 (Glc^-,Glc^+) cells. Our identification of ACE on Caco-2 cell membranes by enzyme assay contrasts with its reported absence by immunofluorescent staining of these cells (Hauri *et al.*, 1985). The use of a long incubation time enabled the sensitivity of the assay to be extended, and the inclusion of a specific inhibitor, 10 μM-enalaprilat, served to establish the activity as that of ACE. Similarly the use of 1 μM-phosphoramidon, 0.1 mM-cilastatin, 10 μM-amastatin and 0.1 mM-Dip-F served to confirm the specificity of the assays for E-24.11, MDP, AP-N, AP-A, AP-W and DPP-IV respectively. Immunofluorescent staining with polyclonal antibodies specific for E-24.11 and DPP-IV was also confirmatory evidence for the presence of these two peptidases.

The membrane preparation on which the assays were performed was the P2 fraction after Ca^{2+} precipitation. Our preparation is similar to, though not identical with, the P2 membrane preparations of these cells studied by Stieger *et al.* (1988) and Chantret *et al.* (1988). The use of bivalent metal ions, Ca^{2+} or Mg^{2+}, to aggregate membranes other than those of the brush border (Schmitz *et al.*, 1973; Booth & Kenny, 1974) is a well documented technique when applied to various tissues. The approach appears to be valid for differentiated Caco-2 cells for which high enrichments of microvillar markers have been noted, but may be inadequate for HT-29 cells which are less homogeneous in terms of their differentiated phenotype, as indicated by a low enrichment for DPP-IV and a value of less than unity for AP-N (Chantret *et al.*, 1988). Our enrichment values for Caco-2 cells ranged from 4.3 to 7.9 for four peptidases at 26 days; the highest value was for E-24.11 which is consistent with its predominantly apical membrane localization by immunostaining (Fig. 2*d*). The lower values may indicate that these peptidases exist in other intracellular compartments, as suggested by immunofluorescent staining of DPP-IV, which was also seen on basolateral membranes (Fig. 2*b*). Recycling of cell-surface DPP-IV by endocytosis and the presence of the enzyme in a lysosomal fraction has been reported in Caco-2 cells by Matter *et al.* (1990).

Nine peptidases that have previously been characterized in both renal (Kenny *et al.*, 1987) and small-intestinal (Norèn *et al.*, 1986) microvillar membrane preparations were thus identified in these colonic carcinoma cell lines. With regard to the nature of colonic epithelial cell, there is a notable lack of information on surface peptidase activities; indeed no enzymic marker has yet been defined for colonic apical membranes (Hauri *et al.*, 1986). Table 1 compares the specific activities of this group of peptidases in a P4 fraction from pig kidney with those in the P2 fraction at 26 or 28 days in culture of Caco-2 and HT-29 cells. Although the fractions are not truly comparable in homogeneity, the P4 fraction being more homogeneous with regard to microvillar membranes than the P2 fraction, the renal P4 fraction is a useful reference standard with which to compare other apical membrane preparations. It is notable that three peptidases, ACE, DPP-IV and γ-GT, in Caco-2 cells achieved specific activities comparable with those of kidney microvilli, as did CP-M in HT-29 cells. For the rest, the activities in the cell lines were in the range, 0.3–5%, of those in the P4 fraction of kidney.

Both cell types achieved confluence at day 5–7 in culture. All the peptidases in Figs. 1 and 5 increased in activity (expressed per mg of protein) after achieving confluence, but the detailed patterns differed. Some continued to increase over the period studied [AP-N, DPP-IV, E-24.11 in Caco-2 cells and AP-W in HT-29 (Glc^-,Glc^+) cells]. Others reached a plateau (ACE and AP-P in Caco-2 cells) and some (AP, MDP and AP-W in Caco-2 cells) appeared to decline in the last week or so of culture. The reasons for these differences must await further studies. At present, we cannot distinguish between recycling of the enzymes into internal pools, changes in rates of synthesis or degradation, relating to the state of differentiation, or shedding from the surface [although in the case of HT-29 (Glc^-,Glc^+) cells, none of the peptidases were detected in the medium before the cells were harvested]. Yoshioka *et al.* (1991) reported that the increase in DPP-IV activity seen in cultured Caco-2 cells is primarily due to an increase in enzyme synthesis rather than decreased degradation. Two of the peptidases, MDP and AP-P, and the esterase, AP, have been shown to be glycolipid-anchored ectoenzymes in pig kidney (Hooper *et al.*, 1987, 1990) and their release by PI-PLC from Caco-2 cells confirms a similar anchorage in these cells. The patterns of expression of these three glycolipid-anchored hydrolases did not suggest any characteristic features of their activity profiles compared with enzymes known to be peptide-anchored.

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