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# Time-dependent activity and expression of glutathione *S*-transferases in the human colon adenocarcinoma cell line Caco-2

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The human colon carcinoma cell line Caco-2 was examined for glutathione *S*-transferase (GST) composition and activity. Freshly seeded cells and cells until 4 days after confluency contain only the placental (Pi) form of glutathione transferase. Cells in culture for longer periods start to express class-Alpha GST isoenzymes. Confluent cells in culture for 20 days or longer contain up to 90% class-Alpha GST. Class-Mu GSTs are not detectable. GST activity gradually increases from $564 \pm 28$ to $5381 \pm 165$ nmol/min per mg of protein at day 0 and 32 after confluency respectively. With regard to GST composition, Caco-2 cells in culture for longer periods most resemble small-intestinal cells, whereas short-time cultures have characteristics of colonic cells. This cell line is very well suited for the study of both the *in vitro* properties and the expression of class Alpha and Pi GSTs.

Glutathione *S*-transferases (GSTs) are dimeric proteins with important functions in the detoxication and binding or transport of various compounds [1,2]. Multiple GST isoforms have been characterized, which can be divided into three subclasses. Class Alpha, Mu and Pi enzymes, each consisting of several isoforms, are basic, near-neutral and acidic proteins with intermediate, high and low molecular masses respectively [1–4].

Recent studies have revealed an increased activity of GST Pi in several tumours as compared with that in its surrounding normal tissue [5–9]. Since GST is involved in the detoxication of anti-cancer drugs [10,11], this increased activity could have a significant contribution to the drug resistance of such tumours [12–14].

We recently showed that, in normal human colon mucosa, GST Pi is by far the most abundant isoform present [15]. It was shown previously by Bauer & Wendel [16] and Siegers *et al.* [17] that the activity of total GST is higher in colon tumours than in normal tissue. Colon tumours are generally insensitive towards anti-cancer drugs.

To investigate further the possible relationship between GST activity and drug resistance, studies on human colon carcinoma cell lines *in vitro* could be very elucidative. In order to find a cell line suited for this purpose, we investigated the activity and composition of GSTs in the colon-adenocarcinoma-derived Caco-2 cell line.

## METHODS

### Cell culture

Caco-2 cells (passage 146) were maintained at 37 °C in Dulbecco's modified Eagle's medium, containing 20% (v/v) fetal-bovine serum, 1% non-essential amino acids, 1 mM-L-glutamine, 20mM-Hepes and penicillin/streptomycin (both 100 units/ml) in an atmosphere of $CO_2$/air (1:19) at 90–100% relative humidity. Cells grown in 75 $cm^2$ culture flasks (Costar, Cambridge, MA, U.S.A.) were supplied on 100 ml of culture medium, which was changed daily. Cells reach confluency at days 5–7 after seeding. Caco-2 cells were kindly donated by Dr. J. A. Fransen and Dr. L. A. Ginsel, Academic Hospital Leiden, The Netherlands.

### Isolation of cytosolic GSTs

Cells were washed three times with phosphate-buffered saline (PBS) and scraped from the bottom of the culture flask. Cells were diluted 1:5 in 20 mM-Tris/HCl buffer, pH 7.4, containing 0.25 M-sucrose and 1.4 mM-dithiothreitol and were subsequently homogenized in a glass/Teflon homogenizer [18]. Preparation of 150000 *g* supernatants and isolation of GSTs was performed as described [15,18].

### Miscellaneous

Protein was determined by the method of Lowry *et al.* [19], with bovine serum albumin as standard. GST activity with 1-chloro-2,4-dinitrobenzene as substrate was determined as described by Habig *et al.* [20]. SDS/polyacrylamide-gel electrophoresis and isoelectric focusing was performed by methods described previously [15, 21]. After staining with Coomassie Brilliant Blue, the gels were scanned at 600 nm with a laser densitometer (LKB 2202 Ultrascan; LKB, Bromma, Sweden). Western blotting and subsequent immunodetection with monoclonal antibodies against GST Pi and Mu (W. H. M. Peters, unpublished work) were performed as described previously [22].

## RESULTS AND DISCUSSION

The protein contents of Caco-2 cells cultured for different periods of time are given in Fig. 1. At approximately day 16 after being confluent, a steady state is reached between cell renewal and cell death. The specific activity of GST in the 150000 *g* supernatant steadily increased at least until day 32 after confluence (Fig. 2).

Abbreviations used: GST, glutathione *S*-transferase; PBS, phosphate-buffered saline (8 mM-sodium phosphate/0.14 M-NaCl, pH 7.4).

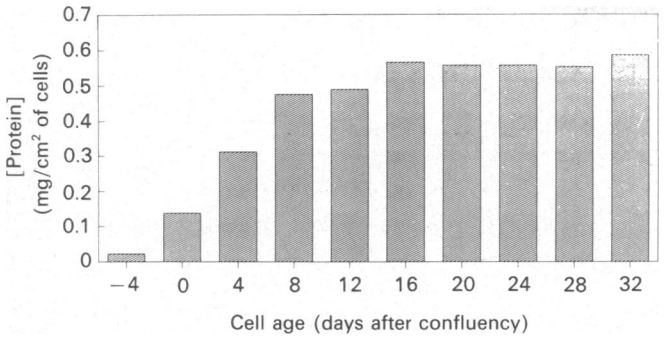


**Fig. 1. Protein content of Caco-2 cells**

In each experiment, ten flasks with Caco-2 cells were cultured as described in the Methods section. Every 4 days one flask was taken and cells were harvested and treated as described in the Methods section. The protein content was determined in the whole homogenate and is expressed as mg of protein/cm² of cells. The cells were confluent at day 0.

Cytosolic GSTs were purified from different batches of cells by affinity chromatography on GST–agarose. These purified preparations were subjected to SDS/polyacrylamide-gel electrophoresis and isoelectric focusing, and the results are shown in Figs. 3 and 4 respectively. Until day 4, only one GST isoform was present in the Caco-2 cells. This isoform co-migrates with GST Pi from human placenta (Figs. 3 and 4). This protein band on immunoblot gives a positive staining with a monoclonal antibody against GST Pi (Fig. 5). Thus it can be concluded that this isoform represents GST Pi. As Figs. 3–5 reveal, this isoenzyme is also present in older cells.

GST Pi was quantified, and increasing amounts of this isoform could be observed until day 8 after confluency (Table 1). When GST Pi expression stabilized (around day 8), expression of class-Alpha GST could be observed (Figs. 3 and 4). Since total GST activity steadily increases (Fig. 2), whereas the expression of GST Pi remains constant, this suggests that the relative content of the class-Pi transferases decreases in cells from 8 days and older. This is exactly what is seen in Fig. 6, where GST isoenzyme distribution is presented. In older cells, expression of class-Alpha enzymes increases, and ultimately these isoforms contribute to about 90% of the total GST protein.

On immunoblot, with a monoclonal antibody against

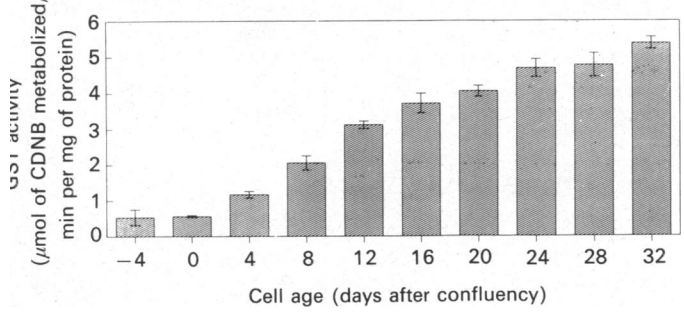


**Fig. 2. GST activity of Caco-2 cells**

The specific activity was measured with 1-chloro-2,4-dinitrobenzene (CDNB) as substrate and is plotted against the age of the cells.

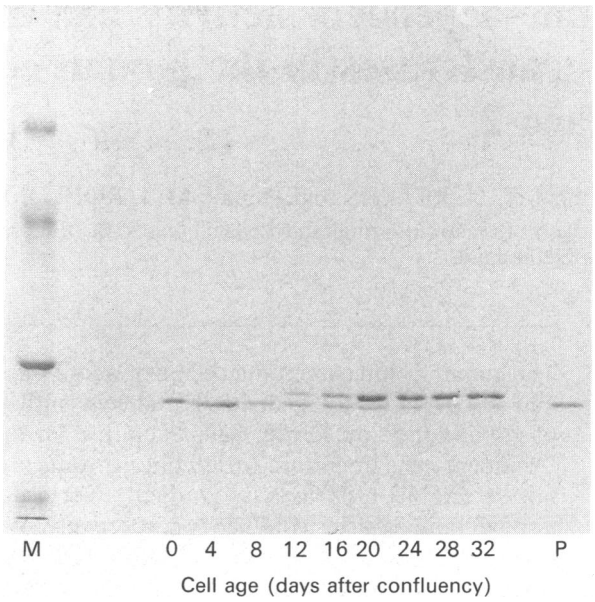


**Fig. 3. GST–agarose-purified GSTs from Caco-2 cells separated by SDS/polyacrylamide-gel electrophoresis**

Slot M contains marker proteins with molecular masses of 68, 43, 29 and 20 kDa respectively (from top to bottom; the lowest band indicates the tracking-dye front). GSTs (2–10 μg) were applied in the slots indicated by 0, 4, 8 etc., which indicates the age of the cells (days after reaching confluency). Slot P contains GST Pi from placenta (2 μg).

GST Mu, the presence of this isoenzyme could be demonstrated neither in freshly seeded nor in older cells. In human colon, GST Mu is expressed in approx. 60% of the individuals investigated (W. H. M. Peters, unpublished work).

Until now we have analysed normal mucosa from nine

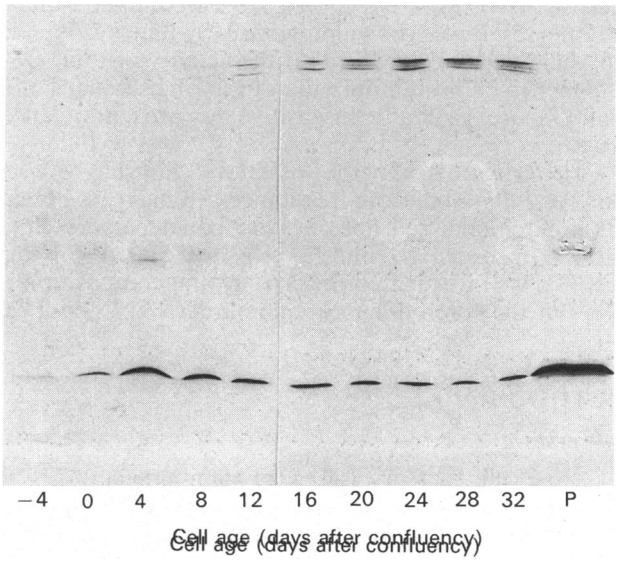


**Fig. 4. GST–agarose-purified GSTs from Caco-2 cells separated by isoelectric focusing**

Transferases (2–20 μg) were separated on isoelectric-focusing gel (pH range 3–9; basic proteins are on top of the gel). For further details, see the legend to Fig. 3.

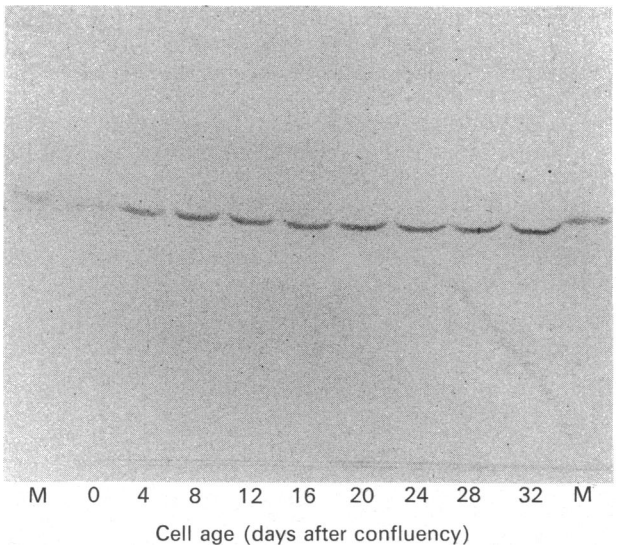


**Fig. 5. Immunoblot of 150000 *g* supernatants from Caco-2 cells treated with a monoclonal antibody against GST Pi**

Supernatants (150000 *g*; 50 $\mu$g) from Caco-2 cells were subjected to SDS/polyacrylamide-gel electrophoresis and subsequent Western blotting. GST Pi was detected by a monoclonal antibody. Slot M contained 0.5 $\mu$g of purified GST Pi from placenta. For further details, see the legend to Fig. 3.

**Table 1. Time-dependent expression of class-Pi GST in Caso-2 cells**

Values are means ± S.E.M. for at least three determinations. The GST Pi content was obtained by scanning the immunoblot of Fig. 5 with a laser densitometer (see the Methods section). Purified enzyme from placenta (Fig. 5, slots M) served as a standard protein.

| Cell age (days after confluency) | GST Pi content ($\mu$g/100 $\mu$g of cystolic protein) |
|---|---|
| 0 | 0.30±0.03 |
| 4 | 0.95±0.05 |
| 8 | 1.36±0.16 |
| 12 | 1.35±0.17 |
| 16 | 1.77±0.31 |
| 20 | 1.63±0.15 |
| 24 | 1.35±0.12 |
| 28 | 1.26±0.14 |
| 32 | 1.61±0.17 |

different human colon specimens obtained from both kidney donors without intestinal pathology and from patients with colon carcinomas. We could not detect class-Alpha transferases in any of these normal colon tissues [15], nor could they be demonstrated in colon carcinomas (W. H. M. Peters, unpublished work). In contrast, normal mucosa from human small intestine contains large amounts of class-Alpha GSTs [15,18]. In

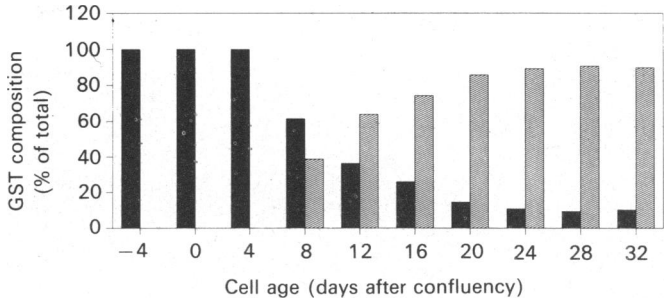


**Fig. 6. GST subunit distribution of Caco-2 cells**

The gels of Figs. 3 and 4 were scanned as indicated in the Methods section. The subunit distribution obtained from each type of gel was averaged and plotted against the age of the cells. ■, class-Pi GST; ▨, class-Alpha GST.

the cells studied here, expression of class-Alpha GSTs coincides with the development of microvilli [23]. Thus, with regard to the GST composition and in agreement with morphological characteristics, Caco-2 cells, although derived from a colon adenocarcinoma, may very much resemble normal small-intestinal epithelial cells [23]. Cells up to 4 days after confluency, however, have the properties of colonocytes.

Since these cells can be grown as a monolayer on different types of membranes [23], they are excellently suited for the study of both the transport and detoxicating properties of the different classes of GSTs. In addition, this cell line may be used to study the expression mechanism of the various class-Alpha transferases.

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