Jpn. J. Cancer Res. **91**, 310–316, March 2000

Expression of Glutathione S-Transferase α, P1-1 and T1-1 in the Human Gastrointestinal Tract

Wieke C. C. de Bruin, Muriel J. M. Wagenmans and Wilbert H. M. Peters[1]

Department of Gastroenterology, St. Radboud University Hospital, PO Box 9101, 6500 HB Nijmegen, The Netherlands

Glutathione S-transferases (GSTs) form a family of enzymes, which play an important role in the prevention of cancer by detoxifying numerous potentially carcinogenic compounds. GSTs catalyze the conjugation of glutathione to such harmful molecules, and enable their secretion. Human GSTs can be divided into five main classes. The θ class of isoenzymes was only recently identified and limited (immunohistochemical) data on these enzymes are available. In the present study, paraffin-embedded sections of different gastrointestinal tissues were analyzed immunohistochemically for GSTα, GSTP1-1 and GSTT1-1 expression using specific antibodies. GSTα, GSTP1-1 and GSTT1-1 were highly expressed in all gastrointestinal tissues examined, with a unique cellular distribution. GSTT1-1 is the first GST isoenzyme demonstrated in duodenal Paneth cells and glands of Brunner. The common expression of GSTα, GSTT1-1 and GSTP1-1 in many cell types along the human gastrointestinal tract suggests an important role in the protection against carcinogens and other xenobiotics.

Key words: Glutathione S-Transferase — Gastrointestinal tract — Immunohistochemistry

Human glutathione S-transferases (GSTs; EC 2.5.1.18) are a family of phase II detoxification enzymes that can be divided into at least five classes, α, μ, π, θ and ζ.[1, 2] Their main function is to catalyze the conjugation of glutathione (GSH) to an electrophilic site of a broad range of potentially toxic and carcinogenic compounds, thereby making them less biologically active and enabling their secretion.[1]

Some members of the GST family also exhibit GSH-peroxidase activity towards (lipid-) peroxides[1, 3] or have been shown to be involved in intracellular transport of xenobiotics by non-catalytic binding.[1] GSTs are ubiquitous in mammalians and are widely expressed in human tissues.[1] The different classes of GSTs each consist of one or more isoenzymes with a specific, although sometimes partly overlapping substrate specificity.[1] GSTs have an important role in the prevention of carcinogenesis.[1, 4] In this respect, high tissue levels of GSTs are protective against cancer.[4, 5] However, in an early phase of malignant transformation GST(P1-1) may be overexpressed in premalignant and malignant cells, where it may contribute to the phenomenon of anti-cancer drug resistance.[1, 4, 6, 7]

Via food consumption or otherwise, humans are daily exposed to a wide variety of xenobiotics of different chemical origin, some of which are carcinogens. Therefore, it is reasonable that the GSTs are abundantly expressed in the epithelium of tissues of the gastrointestinal tract, which functions as a first barrier. Because of the great variation in chemicals which have to be detoxified, knowledge of the GST isoenzyme patterns in the exposed tissues is very important. Expression of GSTα, μ and π in the gastrointestinal tissues has been described previously.[8–16] In humans, two θ class isoenzymes, GSTT1-1 and GSTT2-2 have been identified so far. They display high activity towards methyl halides, sulfate esters and lipid peroxides.[17–22] In addition GSTT1-1 is also a bioactivator of dihalomethanes.[23, 24] GSTT1-1 is expressed in several human gastrointestinal tissues, such as esophagus, stomach, liver and colon.[22, 24–26] For GSTT1-1 a genetic polymorphism has been decribed. Carriers of the genotype who have lost both functional GSTT1-1 alleles, are considered to be at higher risk for developing certain types of cancers.[1, 27–29]

Because of the protective properties of GSTT1-1 it is of interest to learn more about the cell-type-specific expression in the human gastrointestinal tract and the relative levels of this isoenzyme in different cell types, in order to get insight into the functional role of GSTT1-1 in the human gastrointestinal tract and its involvement in carcinogenesis of gastrointestinal tissues. In this study, the expression of GSTα, GSTP1-1 and GSTT1-1 was analyzed immunohistochemically in human esophagus, stomach, duodenum, pancreas, liver and colon.

MATERIALS AND METHODS

Tissue specimens Tissue specimens were selected from formalin-fixed, paraffin-embedded blocks of normal gastrointestinal tissues obtained from patients who were operated on for gastrointestinal cancer at the Department of Surgery, St. Radboud University Hospital, Nijmegen, The Netherlands. The study was approved by the local committee on human experimentation.

[1] To whom correspondence should be addressed.
E-mail: w.peters@gastro.azn.nl

Immunohistochemistry From each specimen 4 μm thick slices were cut. The sections were deparafinized, rehydrated and washed with phosphate-buffered saline (PBS). To block endogenous peroxidase activity the slides were incubated for 10 min in 2% hydrogen peroxide in methanol. Afterwards the slides were washed in PBS and incubated with 4% bovine serum albumin (BSA, Boehringer, Mannheim, Germany)/0.1% Triton X-100 (Merck, Darmstadt, Germany) in PBS to block non-specific binding. The slides were incubated overnight at 4°C with mouse monoclonals against GSTα (recognizing the GSTA1 and A2 subunits),[30] and GSTT1-1 (not recognizing the GSTT2-2 isoenzyme)[31] and rabbit polyclonal anti-GSTP1-1 (Biotrin International, Dublin, Ireland) diluted 1:10000, 1:500 and 1:1750 in blocking buffer, respectively. The slides were washed with PBS-0.05% Tween 20 (PBST). Then the slides were incubated with peroxidase (PO)-conjugated rabbit anti-mouse (1:100), biotin-conjugated rabbit anti-mouse (1:400) and PO-conjugated swine anti-rabbit (1:40), respectively. Thirdly, the slides were incubated with swine anti-rabbit PO-conjugated (1:40), streptavidine PO-conjugated (1:100) and rabbit anti-mouse PO-conjugated (1:100) antibodies, respectively. Finally, the slides were stained with diaminobenzidine (DAB; Merck) and counterstained with hematoxylin. Incubations omitting the primary antibody were used as negative controls. Slides were examined by two independent observers. Specimens of each tissue from at least 3 patients were analyzed. Hematoxylin and eosin staining was performed according to standard procedures.

Because no appropiate antibodies were available for GSTT2-2 and μ class GSTs these enzymes were not examined in this study.

GSTT1-1/chromagranin double staining The dual labeling for GSTT1-1 and chromagranin A on tissue sections of human stomach, duodenum and pancreas was performed essentially as described by Yong Xue *et al.*[32] GSTT1-1 immunoreactivity was stained with DAB as a chromagen, whereas chromagranin A positive cells were visualized with Fast Blue (Sigma, St. Louis, MO). The slides were analyzed for co-localization of the two antigens.

RESULTS

Representative sections of different organs of the human gastrointestinal tract analyzed immunohistochemically for the expression of GSTα, GSTP1-1 and GSTT1-1 and corresponding hematoxylin and eosin staining are shown in Fig. 1. Results are also summarized in Table I.

In human esophagus, the squamous epithelium stained positive for GSTP1-1 and GSTT1-1 in a similar way: strong reactivity was seen in the columnar mucus-secreting cells and the parabasal compartment of the mucosa. In addition, the submucosal esophageal glands (not shown) were stained for both classes of GSTs. No immunoreactivity for GSTα (Fig. 1b) was seen in the esophagus.

In stomach, a strong staining for GSTα was seen in mucous cells and parietal cells, while the chief cells were only moderately positive. For GSTP1-1 extensive staining was seen only in the mucous and parietal cells of the upper part of the mucosa, while chief cells were negative. GSTT1-1 displayed staining in the mucous and parietal cells and to a lesser extent in the chief cells. Like GSTα, cells positive for GSTT1-1 were identified throughout the mucosa.

In duodenum, strong reactivity for GSTα was seen in the enterocytes of the villi and the crypts of Lieberkühn. More basally in the duodenal mucosa, staining of the enterocytes was less intense. Immunoreactivity for GSTP1-1 was very low in the enterocytes throughout the mucosa. Staining for GSTT1-1 was seen at the apical side of the enterocytes but not in the mucous cells. In addition, positivity for GSTT1-1 was seen in the cells of Paneth and the submucosal glands of Brunner.

In the pancreas, centroacinar cells and the ducts displayed reactivity for all three GSTs examined. Only GSTα reactivity was seen in the acinar cells. In the islets of Langerhans no GST-positive cells could be detected. The reactivity in the pancreatic tissue was strongest for GSTα, followed by GSTP1-1, whereas the staining intensity and the number of positive cells was lower for GSTT1-1 than for the other two classes of GSTs investigated here.

Intense reactivity for GSTα was seen in the liver in the hepatocytes, bile ducts and the endothelial cells. For GSTP1-1 reactivity was found only within the bile ducts. No expression of GSTP1-1 was observed in the hepatocytes. GSTT1-1 expression was detectable in the hepatocytes, although to a lesser extent than GSTα, whereas the bile duct epithelium was also positive for GSTT1-1.

In the colon, enterocytes displayed low reactivity for GSTα, while no staining was seen in the goblet cells. Expression of GSTP1-1 was seen in the enterocytes and goblet cells. In addition, enterocytes and goblet cells throughout the mucosa were positive for GSTT1-1. In the colon, the intensity of the staining for the three GST isoenzymes was low.

In all tissues investigated, connective tissue components were consistently negative. For GSTα, reactivity in the nuclei of positively stained cells was seen in all tissues. Nuclei were positively stained for GSTP1-1 in the parietal cells of the stomach. For GSTT1-1, nuclear staining was not seen in either of the investigated tissues.

In the duodenum, GSTT1-1 immunoreactivity was found in the cells of Paneth and the glands of Brunner. Because expression of GSTs was never noted in these cells, we wondered whether GSTT1-1 was also expressed in duodenal endocrine cells. To identify the enteroendocrine cells, an immunohistochemical staining for chroma-

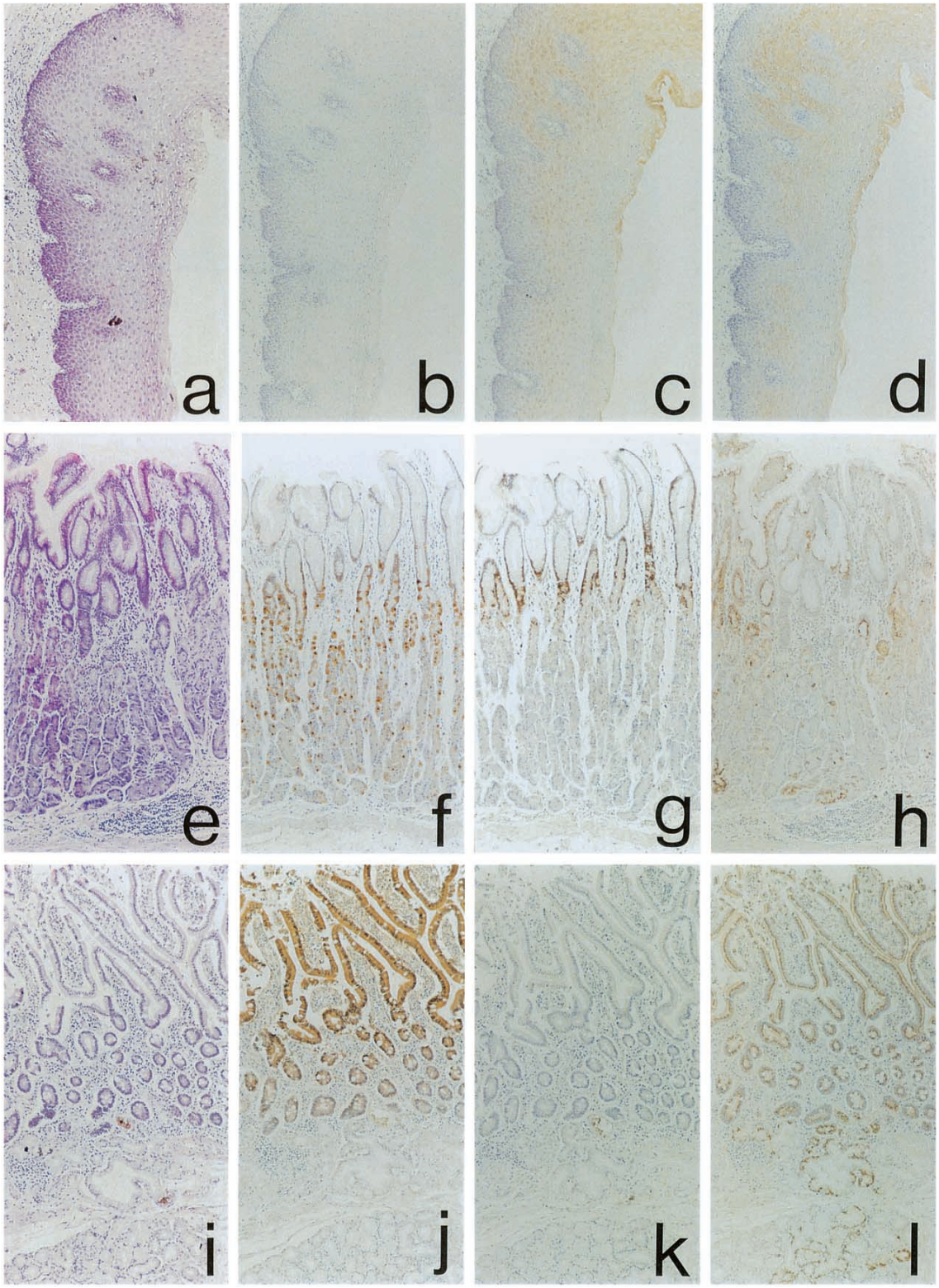

Fig. 1. Sections of normal human esophagus (a–d), stomach (e–h), duodenum (i–l), pancreas (m–p), liver (q–t) and colon (u–x) were stained for GSTα (b, f, j, n, r, v), GSTP1-1 (c, g, k, o, s, w) and GSTθ (d, h, l, p, t, x) and corresponding hematoxylin & eosin staining (a, e, i, m, q, u). (Magnification 100×)

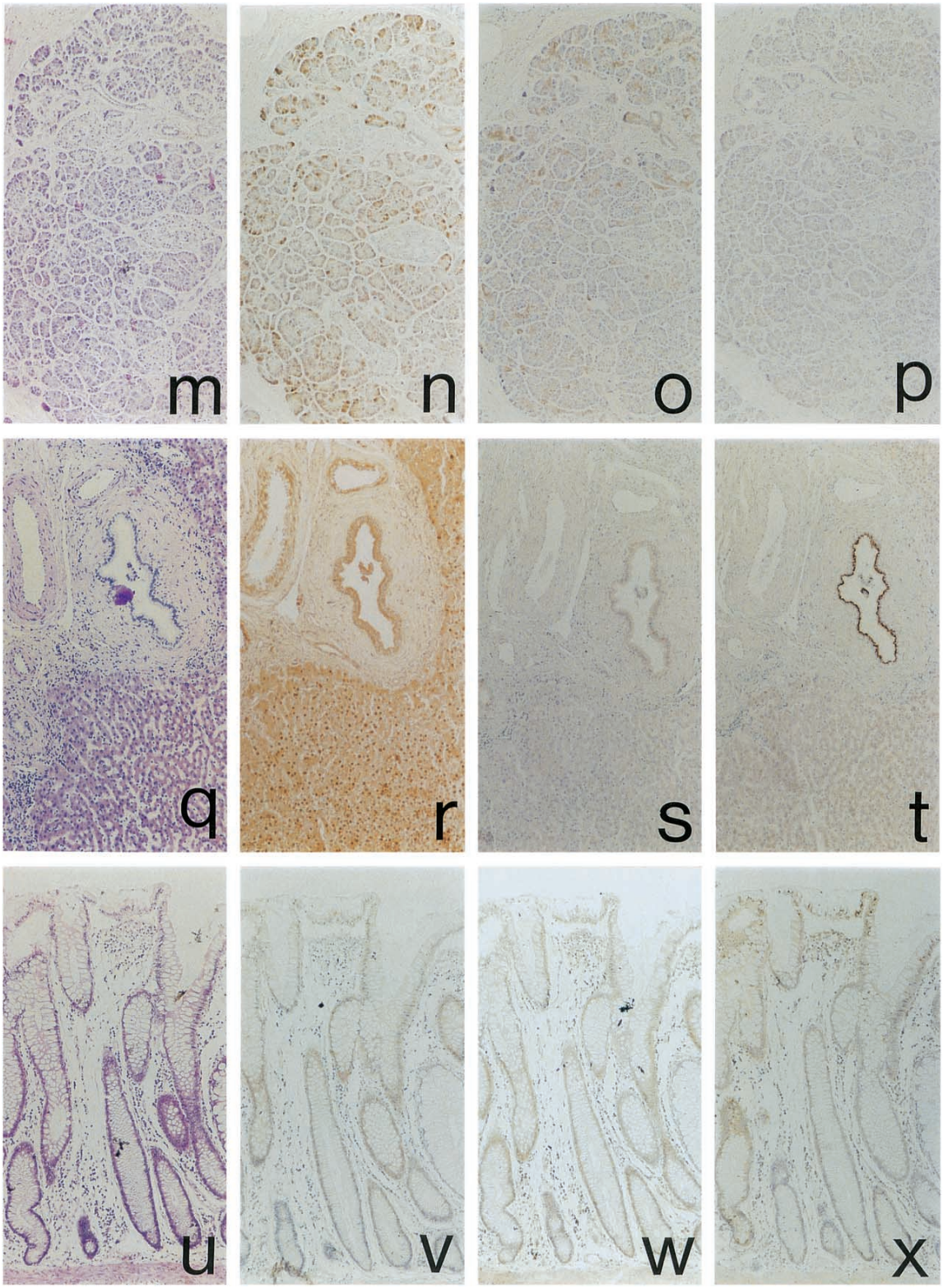
m
n
o
p
q
r
s
t
u
v
w
x

Table I. Cell-type-specific Expression of GSTα, GSTP1-1 and GSTT1-1 in Human Gastrointestinal Tissues

Tissue and cell types	GSTα	GSTπ	GSTθ
Esophagus			
Squamous epithelium	−	+	+
Submucosal glands	−	+	+
Stomach			
Chief cells	+	−	+
Mucous cells	+	+	+
Parietal cells	+	+	+
Endocrine cells	−	−	−
Duodenum			
Enterocytes	+	+	+
Mucous cells	−	−	−
Crypts of Lieberkühn	+	+	+
Paneth cells	−	−	+
Brunner glands	−	−	+
Endocrine cells	−	−	−
Pancreas			
Centroacinar cells	+	+	+
Acinar cells	−	−	−
Duct cells	+	+	+
Endocrine cells	−	−	−
Liver			
Hepatocytes	+	−	+
Bile duct epithelium	+	+	+
Endothelial cells	+	−	−
Colon			
Enterocytes	+	+	+
Goblet cells	−	+	+
Number of positive cell types	11/21	11/21	15/21

granin A was performed on sections of stomach and duodenum, with pancreatic tissue as a positive control. To investigate a possible co-localization of chromogranin A and GSTT1-1, a double staining for these antigens was performed on tissue sections of human stomach, duodenum and pancreas. However, no dual expression of GSTT1-1 and chromagranin A was seen in these tissues (result not shown), indicating that GSTT1-1 is not expressed in enteroendocrine cells.

DISCUSSION

GST isoenzymes are expressed in a tissue- and cell-type-specific manner. Presumably this has a function in providing optimal protection against potentially toxic compounds. The epithelium of the human gastrointestinal tract is an important first site of contact with harmful compounds from food, drugs or medication. In previous studies the cell-type-specific expression of GSTα, μ and π in the human gastrointestinal tract was investigated.[8–16] The presence of GSTT1-1 in human esophagus, stomach, pancreas, liver and colon was shown before on immunoblots.[15, 22, 24, 25] However, no data on its cell-type-specific expression in normal gastrointestinal tissues in comparison to other classes of GSTs were available.

In Table I, an overview of the cell-type-specific expression of the GST isoenzymes is presented. Except for human esophagus, the epithelial cells of the digestive systems express the three GSTs investigated here. Besides the intestinal epithelial cells, gastric parietal cells, centroacinar and duct cells of the exocrine pancreas and hepatic bile duct cells all display reactivity for the three GSTs.

A striking feature of this study was the presence of GSTT1-1 in duodenal Paneth cells and Brunner glands, whereas the other GSTs isoenzymes investigated here were not observed in these particular cells. The precise function of the Paneth cells and the Brunner glands is unknown, but a role in mucosal immunity has been suggested.[33, 34] Whether GSTT1-1 is functionally involved in this process has yet to be elucidated.

Another interesting finding is the frequent expression of GSTT1-1, which is present in most cell types lining the gastrointestinal tract (15 out of the 21 types of cells versus 11 out of 21 for GSTα and GSTP1-1: see Table I). Clearly the role of GSTT1-1 and the consequences for gastrointestinal functioning of the GSTT1-1 null genotype, which can be found in approximately 20% of Caucasians and even higher percentages in other ethnic groups,[1] has to be investigated in more detail.

In accordance with the results for GSTα and GSTP1-1, no GSTT1-1 could be detected in pancreatic acinar cells or in the endocrine cells of the gastrointestinal tissues investigated. These cell types therefore may lack part of the protection against toxic and carcinogenic compounds, which might have implications for the cancer susceptibility of these cells.

Nuclear staining for GSTα was seen in all tissues examined, whereas nuclear staining for GSTP1-1 was seen in gastric parietal cells only. These results confirmed previous data.[14] For GSTT1-1, which, like GSTα, displays (lipid) peroxidase activity[1, 18] no nuclear staining was demonstrated. The reason for this differential nuclear staining is unclear, but there may be implications for protection of nuclear components against (oxidative) damage.

In summary, this study demonstrates that GSTT1-1 and to a lesser extent GSTα and GSTP1-1 are widely distributed in the human gastrointestinal tract, showing cell-type-specific expression. GSTT1-1 is the only isoenzyme found in duodenal Paneth cells and glands of Brunner.

ACKNOWLEDGMENTS

We thank Dr. M. Pruszczynski (Department of Pathology, St. Radboud University Hospital, Nijmegen) for his help with the

interpretation of the data. This study was supported by the Dutch Cancer Society (grant 95-913).

(Received November 1, 1999/Revised December 25, 1999/ Accepted January 13, 2000)

REFERENCES

1) Hayes, J. D. and Pulford, D. J. The glutathione S-transferase supergene family: regulation of GST and contribution of the isoenzymes to cancer chemoprevention and drug resistance. *Crit. Rev. Biochem. Mol. Biol.*, **30**, 445–600 (1995).
2) Board, P. G., Baker, J. T., Chelvanayagam, G. and Jermin, L. S. Zeta, a novel class of glutathione transferases in a range of species from plants to humans. *Biochem. J.*, **328**, 929–935 (1997).
3) Hurst, R., Bao, Y., Jemth, P., Mannervik, B. and Williamson, G. Phospholipid hydroperoxide glutathione peroxidase activity of human glutathione transferases. *Biochem. J.*, **332**, 97–100 (1998).
4) Tsuchida, S. and Sato, K. Glutathione transferases and cancer. *Crit. Rev. Biochem. Mol. Biol.*, **27**, 337–384 (1992).
5) Peters, W. H. M., Roelofs, H. M. J., Hectors, M. P. C., Nagengast, F. M. and Jansen, J. B. M. J. Glutathione and glutathione S-transferases in Barrett's epithelium. *Br. J. Cancer*, **67**, 1413–1417 (1993).
6) Sato, K. Glutathione transferases as markers of preneoplasia and neoplasia. *Adv. Cancer Res.*, **52**, 205–255 (1989).
7) Tew, K. D. Glutathione-associated enzymes in anticancer drug resistance. *Cancer Res.*, **54**, 4313–4320 (1994).
8) Hayes, P. C., Harrison, D. J., Bouchier, I. A. D., McLellan, L. I. and Hayes, J. D. Cytosolic and microsomal glutathione S-transferase isoenzymes in normal human liver and intestinal epithelium. *Gut*, **30**, 854–859 (1989).
9) Hayes, P. C. and Harrison, D. J. Immunohistochemical analysis of pancreas and gastrointestinal tract in man. *In* "Glutathione S-Transferases and Drug Resistance," ed. J. D. Hayes, C. B. Pickett and T. J. Mantle, pp. 441–450 (1990). Taylor & Francis, London.
10) Mulder, T. P. J., Roelofs, H. M. J., Peters, W. H. M., Wagenmans, M. J. M., Sier, C. F. M. and Verspaget, H. W. Glutathione S-transferases in liver metastases of colorectal cancer. A comparison with normal liver and primary carcinomas. *Carcinogenesis*, **15**, 2149–2153 (1994).
11) Ranganathan, S. and Tew, K. D. Immunohistochemical localisation of glutathione S-transferases α, μ and π in normal tissue and carcinoma from human colon. *Carcinogenesis*, **12**, 2383–2387 (1991).
12) Collier, J. D., Bennett, M. K., Hall, A., Cattan, A. R., Lendrum, R. and Bassendine, M. F. Expression of glutathione S-transfersases in normal and malignant pancreas: an immunohistochemical study. *Gut*, **35**, 266–269 (1994).
13) Terrier, P., Townsend, A. J., Coindre, J. M., Triche, T. J. and Cowan, K. H. An immunohistochemical study of pi class glutathione S-transferase expression in normal human tissue. *Am. J. Pathol.*, **137**, 845–853 (1990).
14) Schipper, D. L., Wagenmans, M. J. M., van Haelst, U., Peters, W. H. M., Wobbes, T., Verhofstad, A. A. J., Lange, W. P. H. and Wagener, D. J. T. Glutathione S-transferase in gastric carcinomas and in adjacent normal gastric epithelium: immunohistochemical and biochemical analyses. *Anticancer Res.*, **16**, 565–572 (1996).
15) De Bruin, W. C. C., Wagenmans, M. J. M., Board, P. G. and Peters, W. H. M. Expression of glutathione S-transferase theta class isoenzymes in human colorectal and gastric cancer. *Carcinogenesis*, **20**, 1453–1457 (1999).
16) Van Lieshout, E. M. M., van Haelst, U. J. G. M., Wobbes, Th. and Peters, W. H. M. Immunohistochemical localization of glutathione S-transferase α and π in human esophageal squamous epithelium, Barrett's epithelium and carcinoma. *Jpn. J. Cancer Res.*, **90**, 530–535 (1999).
17) Tan, K. L. and Board, P. G. Purification and characterization of a recombinant human theta-class glutathione S-transferase (GSTT2-2). *Biochem. J.*, **315**, 727–732 (1996).
18) Hussey, A. J. and Hayes, J. D. Characterization of a human class-theta glutathione S-transferase with activity towards 1-menaphthyl sulfate. *Biochem. J.*, **286**, 929–935 (1992).
19) Webb, G., Vaska, V., Coggan, M. and Board, P. Chromosomal localization of the gene for the human theta class glutathione S-transferase (GSTT1). *Genomics*, **33**, 121–123 (1996).
20) Tan, K. L., Webb, G. C., Baker, R. T. and Board, P. G. Molecular cloning of a cDNA and chromosomal localization of a human theta-class glutathione S-transferase gene (GSTT2) to chromosome 22. *Genomics*, **25**, 381–387 (1995).
21) Pemble, S., Schroeder, K. R., Spencer, S. R., Meyer, D., Hallier, E., Bolt, H. M., Ketterer, B. and Taylor, J. B. Human glutathione S-transferase theta (GST1-1): cDNA cloning and the characterization of a genetic polymorphism. *Biochem. J.*, **300**, 271–276 (1994).
22) Juronen, E., Tasa, G., Uusküla, M., Pooga, M. and Mikelsaar, A.-V. Purification, characterization and tissue distribution of human class theta glutathione S-transferase T1-1. *Biochem. Mol. Biol. Int.*, **39**, 21–29 (1996).
23) Sherratt, P. J., Manson, M. M., Thomson, A. M., Hissink, E. A. M., Neal, G. E., van Bladeren, P. J., Green, T. and Hayes, J. D. Increased bioactivation of dihaloalkanes in rat liver due to induction of class theta glutathione S-transferase T1-1. *Biochem. J.*, **335**, 619–630 (1998).
24) Sherratt, P. J., Pulford, D. J., Harrison, D. J., Green, T. and Hayes, J. D. Evidence that human theta glutathione S-transferase T1-1 can catalyze the activation of dichloromethane, a liver and lung carcinogen in the mouse. *Biochem. J.*, **326**, 837–846 (1997).
25) Mainwaring, G. W., Williams, S. M., Forster, J. R., Tugwood, J. and Green, T. The distribution of theta-class glutathione S-transferase in the liver and lung of mouse, rat and human. *Biochem. J.*, **318**, 297–303 (1996).

26) Van Lieshout, E. M. M., Tiemessen, D. M., Witteman, B. J. M., Jansen, J. B. M. J. and Peters, W. H. M. Low glutathione and glutathione S-transferase levels in Barrett's esophagus as compared to normal esophageal epithelium. *Jpn. J. Cancer Res.*, **90**, 81–85 (1999).
27) Chen, H., Sandler, D. P., Taylor, J. A., Shore, D. L., Liu, E., Bloomfield, C. D. and Bell, D. A. Increased risk for myelodysplastic syndromes in individuals with glutathione transferase theta 1 (GSTT1) gene defect. *Lancet*, **347**, 295–297 (1996).
28) Chenevix-Trench, G., Young, J., Coggan, M. and Board, P. Glutathione S-transferase M1 and T1 polymorphisms: susceptibility to colon cancer and age of onset. *Carcinogenesis*, **16**, 1655–1657 (1995).
29) Deakin, M., Elder, J., Hendrickse, C., Peckham, D., Baldwin, D., Pantin, C., Wild, N., Leopard, P., Bell, D. A., Jones, P., Duncan, H., Brannigan, K., Alldersea, J., Fryer, A. A. and Strange, R. C. Glutathione S-transferase GSTT1 genotypes and susceptibility to cancer: studies of interactions with GSTM1 in lung, oral, gastric and colorectal cancers. *Carcinogenesis*, **17**, 881–884 (1996).
30) Peters, W. H. M., Boon, C. E. W., Roelofs, H. M. J., Wobbes, Th., Nagengast, F. M. and Kremers, P. G. Expression of drug metabolizing enzymes and P-170 glycoprotein in colorectal carcinoma and normal tissue. *Gastroenterology*, **103**, 448–455 (1992).
31) Juronen, E., Tasa, G., Uusküla, M., Pooga, M. and Mikelsaar, A.-V. Production and characterization of monoclonal antibodies against class theta glutathione S-transferase T1-1. *Hybridoma*, **15**, 77–82 (1996).
32) Yong Xue, M. D., Lange, W. B., Smedts, F., Debruyne, F., de la Rosette, J., Schalken, J. and Verhofstadt, A. A novel immunoenzymatic technique to demonstrate multiple antigens in cells based on the selective destaining of substrate deposits and its application in characterizing the immunophenotype on neuroendocrine cells in the human prostate epithelium. *Appl. Immunohistochem.*, **6**, 69–76 (1999).
33) Quelette, A. J. Paneth cells and innate immunity in the crypt microenvironment. *Gastroenterology*, **113**, 1779–1784 (1997).
34) Coutinho, H. B., Robalinho, T. I., Coutinho, V. B., Amorin, A. M., Filho, J. T., Walker, E., King, G., Sewell, H. F. and Wakelin, D. Immunocytochemical demonstration that human duodenal Brunner's glands may participate in intestinal defence. *J. Anat.*, **189**, 193–197 (1996).