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,3 or have been shown to be involved in intracellular transport of xenobiotics by non-catalytic binding.3 GSTs are ubiquitous in mammals and are widely expressed in human tissues.9 The different classes of GSTs each consist of one or more isoenzymes with a specific, although sometimes partly overlapping substrate specificity.9 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 pre-malignant 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 carcinogenic. 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.8–10 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 described. 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.
**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 GST\(\alpha\) (b, f, j, n, r, v), GSTP1-1 (c, g, k, o, s, w) and GST\(\theta\) (d, h, l, p, t, x) and corresponding hematoxylin & eosin staining (a, e, i, m, q, u). (Magnification 100×)
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           | –    | –    | –    |
| Paneth cells           | –    | +    | +    |
| Endocrine cells        | –    | –    | –    |
| Duodenum               | +    | +    | +    |
| Enterocytes            | –    | –    | –    |
| Mucous cells           | +    | –    | –    |
| Crypts of Lieberkuhn   | +    | +    | +    |
| Paneth cells           | –    | –    | +    |
| Brunner glands         | –    | –    | –    |
| Endocrine cells        | –    | –    | –    |
| Pancreas               | +    | +    | +    |
| Centrocinar 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 |

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 activity1, 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.

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