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INVITED COMMENTARY
POTENTIAL CONTRIBUTION OF THE GLUTATHIONE S-TRANSFERASE SUPERGENE FAMILY TO RESISTANCE TO OXIDATIVE STRESS

JOHN D. HAYES and RICHARD C. STRANGE

Biomedical Research Centre, Ninewells Hospital and Medical School, University of Dundee, Dundee DD1 9SY, Scotland and Centre for Pathology and Molecular Medicine, School of Postgraduate Medicine, Keele University, North Staffordshire Hospital Centre, Stoke-on-Trent, Staffordshire ST4 7QB England

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The glutathione S-transferase (GST) supergene family comprises gene families that encode isoenzymes that are widely expressed in mammalian tissue cytosols and membranes. Both cytosolic (particularly the isoenzymes encoded by the alpha, mu and theta gene families) and microsomal GST catalyse the conjugation of reduced glutathione (GSH) with a wide variety of electrophiles which include known carcinogens as well as various compounds that are products of oxidative stress including oxidised DNA and lipid. Indeed, several lines of evidence suggest certain of these isoenzymes play a pivotal role in protecting cells from the consequences of such stress. An assessment of the importance of these GST in humans is presently difficult however, because the number of alpha and theta class genes is not known and, the catalytic preferences of even identified isoforms is not always clear.

KEY WORDS: Glutathione S-transferase, supergene family, oxidant stress, reactive oxygen species.

PHYSIOLOGICAL AND PATHOLOGICAL EFFECTS OF REACTIVE OXYGEN SPECIES

Recent studies emphasise the critical role of the reactive oxygen species (ROS), superoxide radical and hydrogen peroxide (H_2O_2), in many physiological and pathological events.[1,2] These species have complex effects on cells; thus, apparently physiological concentrations of H_2O_2 (eg 10^{-6} M) have mitogenic effects including increasing DNA synthesis and expression of c-myc and c-fos and, appear to act as intra- and extra-cellular messengers.[3-5] Such concentrations of H_2O_2 also rapidly activate the NF-KB transcription factor.[6] Increased or inappropriate generation of ROS, often referred to as oxidative stress, occurs in many pathologies and can cause cell damage and even death via effects including glutathione depletion, perturbation of intracellular calcium homeostasis, loss of DNA integrity and peroxidation of membrane lipid.[7-9]

*Corresponding author.

Because of the potentially chaotic effects of ROS, cells use various mechanisms to modulate their concentration; these include iron sequestration and the widespread expression of antioxidant enzymes such as CuZn- and Mn-superoxide dismutase, catalase and Se-glutathione peroxidase. Together these regulate the levels of superoxide radical and H_2O_2 in cells and suppress formation of the dangerously reactive hydroxyl radical (HO·).[10] Gene transfection studies indicate the need for coordinated expression; CuZn-superoxide dismutase increases the sensitivity of mammalian cells to oxidative stress while catalase confers protection and cells transfected with both genes resemble wild type cells.

The coordinated expression of such antioxidant enzymes is essential for life in an aerobic environment and while clearly generally effective, many types of intra- and extra-cellular molecules demonstrate evidence of oxidative damage even under resting conditions. Much interest has focused on lipid and DNA.[1,2,9] Peroxidation of membrane lipid is continuous though in healthy tissues, levels of the products of the phenomenon, such as malondialdehyde and alkenals, are low indicating continuous detoxication of these potentially dangerous compounds. Increased lipid peroxidation has been found in various pathologies. While it can be a consequence rather than cause of injury, it is potentially harmful since it alters membrane fluidity and permeability. The process is initiated by abstraction of a hydrogen atom from a polyunsaturated fatty acid (PUFA) by active compounds such as HO·. The resulting radical reacts with oxygen to form peroxy radicals which abstract further hydrogen atoms forming lipid hydroperoxides. These decompose to form a complex mixture which includes aldehydes such as malondialdehyde, 2-alkenals and 4-hydroxyalkenals. The latter are cytotoxic with high reactivity towards particularly sulphydryl groups.[11] PUFA differ in their susceptibility to peroxidation and since the composition of membranes differs, preferred sites for peroxidation may exist within or between cells depending on pO_2 and antioxidant status.[12] Antioxidant defence systems therefore, would be expected to demonstrate appropriate subcellular and cellular location.

DNA strand breaks and damage to bases and sugars are an early and significant result of oxidative stress. They result from HO· induced damage or activation of nucleases.[9] HO· induced damage to bases can be identified by end-products such as 5-hydroxymethyl uracil, 8-hydroxyadenine and 8-hydroxyguanine.[9]

The utilization of oxygen as an electron acceptor necessitates the coordinated expression of enzymes that decrease production of HO· or allow the repair/replacement of damaged molecules such as lipid and DNA. Although the processes whereby these molecules are repaired are poorly understood, peroxidase activity is central to the detoxication of lipid and DNA hydroperoxide.

THE GLUTATHIONE S-TRANSFERASES: A SUPERGENE FAMILY OF DETOXICATING ENZYMES

A growing body of evidence suggests the glutathione S-transferase (GST) supergene family is important in protection against oxidative stress.[13] The GST catalyse the conjugation of the reduced glutathione thiolate anion with a wide range of electrophiles, including drugs, chemotherapeutic agents and, known carcinogens. This relative non-specificity of the GST has resulted in their being studied as contributors to drug resistance and, as protectors against carcinogens. Thus, the ability of the GST to catalyse reactions with alkylating agents such as melphalan, chlorambucil as well

as redox-active drugs such as bleomycin, has lead to considerable interest in their role in drug resistance in cancer chemotherapy.[14] By contrast, the many studies showing for example, the protective effect of GST inducers in mice administered carcinogens as well as the ability of GST isoenzymes to prevent nuclear accumulation of carcinogens has led to the belief that this complex family of isoenzymes is important in the protection of cells from xenobiotics.

Substrate specificities, immunological identity and more recently, protein and DNA sequencing approaches have allowed the multiple isoforms expressed in mammalian cells to be classified into gene families and much work is underway to determine the *in vivo* function of these families.[15,16] The GST isoenzymes expressed in mammalian tissue cytosols comprise the alpha, mu, theta and pi gene families (Table 1). Each of these may include multiple genes, with active enzymes comprising homo- and heterodimeric combinations of monomers (monomer molecular weights 22 000–26 000) encoded by genes of the same family. Within each gene family, the GST isoenzymes share considerable sequence homology (>50%), between-family homology is much less (<25%).[17] A membrane-associated GST, originally described as the microsomal isoform, has also been identified. This enzyme shares little sequence homology with the forms expressed in cytosol and may have arisen by convergent evolution.[18]

Alpha, mu and pi class GST are present in animals and yeast but not bacteria or plants. The genes of these families share regions of sequence identity that suggest a common precursor that arose after the divergance of plants from animal/fungi. Theta GST share less homology with alpha, mu and pi and are more widely spread, being found in plants and bacteria as well as mammals, birds and fish. Pemble and Taylor[19] have proposed that eukaryotes obtained theta by the bacterial endosymbiosis that gave rise to mitochondria. According to this hypothesis, alpha, mu and pi precursors arose subsequently from duplications of the theta gene in the order, mu followed by divergence of alpha from pi.

Alpha Class GST

At least five human alpha GST enzymes have been identified.[17,20-24] Of these, A1-1, A1-2 and A2-2 represent distinct enzymes with cDNA available for the A1 and A2 subunits.[17,25-28] These isoenzymes are fairly widely expressed. Recent work indicates the presence of multiple genes and pseudogenes on chromosome 6p12; these include GSTA1 and GSTA2 as well as GST 6, a gene that apparently encodes a further func-

TABLE 1
Glutathione S-transferase gene families and gene loci

Gene families	Human genes	Rat genes
Theta	T1[1], T2	5, 12, 13
Mu	M1[2], M2, M3, M4, M5	3 (Yb1), 4 (Yb2), 6 (Yn1), 9 (Yn2) 11 (Yo)
Pi	P1[3]	7 (Yf),
Alpha	A1, A2[4], A	1 (Ya), 2 (Yc_1), 8 (Yk), 10 (Yc_2)

[1] polymorphic with at least two common alleles; *GSTT1*A*, *GSTT1*0*
[2] polymorphic with at least three common alleles; *GSTM1*A*, *GSTM1*B*, *GSTM1*0*
[3] possibly polymorphic
[4] RFLP described

tional GST. So far no cDNA or protein sequence data is available for this enzyme indicating it is either weakly expressed, only expressed under particular conditions or, only expressed in specific tissues (e.g. skin, see below).[17,28]

In humans, expression of alpha GST is subject to marked variation. Thus, levels of A1-1 and A2-2 vary considerably between-tissues and -individuals.[29] In some tissues (e.g. liver, kidney, adrenal), alpha isoenzymes are strongly expressed in almost all subjects although a few individuals have been identified who fail to express this class of GST even in these organs (other GST classes were expressed in these specimens).[30] In other tissues expression of alpha genes is either weak (e.g. lung), weak or absent (e.g. brain) or absent (e.g. erythrocytes).[21-33] The importance of tissue-specific expression of alpha GST is suggested by reports of a highly basic alpha class isoenzyme in human skin that is apparently unique.[22,33] Though this isoenzyme remains to be characterised thoroughly, it is attractive to speculate that expression of this alpha GST mediates the adaptive response of skin to ultraviolet light (UV). UV represents a significant stress since many compounds (e.g. tryptophan, cysteine and NADH) can absorb UV and generate H_2O_2 and superoxide or singlet oxygen.[34] Significantly, UV causes a dose-dependent increase in lipid peroxidation in liposomal membranes and membrane sensitivity to UVA appears to be related to the level of unsaturated lipid.

While an apparently common *Hgi* A1 restriction fragment length polymorphism (RFLP) of A2 has been reported,[35] the molecular basis of inter-individual and tissue-specific differences in alpha expression and, its significance against oxidative stress is unknown.

Mu Class GST

To date, 5 mu class genes, M1, M2, M3, M4 and M5 have been identified in humans.[17,36,37] Evidence suggests these genes occur as a cluster on chromosome 1.[36,37] Those studied so far also demonstrate between-tissue and -individual variation. The pattern of tissue expression of the most widely expressed mu gene, GSTM1, is similar to that of alpha class genes. Expression of M2 is restricted to skeletal muscle. M3 is found in brain, lung and testis, as well as skeletal muscle. M4 has been found in a human lymphoblastoid cell line and M5 has been identified in human brain.[21,38,39]

GSTM1 is of particular interest because it is polymorphic with 4 common phenotypes resulting from homo- and heterozygotic combinations of the *GSTM1*0*, *GSTM1*A* and *GSTM1*B* alleles.[29,36,38-42] About 50% of most populations are homozygous for the deleted *GSTM1*0* allele (GSTM1 null polymorphism) and express no GSTM1 enzyme.[29,30,40,41] A rare allele, *GSTM1*3*, has also been proposed on the basis of starch gel zymogram studies but this putative, further gene has not been characterised.[44-45]

The possibility that other mu class genes also demonstrate polymorphism has not been studied though a minority of subjects (about 10%) fail to express GSTM3 in brain. The molecular basis for this phenomenon is unknown.[21]

Theta Class GST

This is the most recent GST gene family to be identified and studied. The widespread expression of theta GST has been interpreted as indicating a fundamental role for these enzymes. Two cytosolic theta GST, T1-1 and T2-2, have been isolated from

human liver.[46,47] While some amino acid sequence data have demonstrated that these enzymes are genetically distinct, cDNA clones have not been isolated for both enzymes; thus, a cDNA for T1 has been isolated by Pemble *et al.*[48,49] but, to date the cDNA encoding T2 has not been cloned. In the rat, two cytosolic (subunits 5 and 12)[19,50] and one mitochondrial (subunit 13)[51] theta class GST exist. It is not known whether humans also express a mitochondrial enzyme.

The human GSTT1 enzyme has attracted much recent interest as Ketterer *et al.*[48] and Pemble *et al.*[49] have recently identified a null allele at this locus. Since the enzyme catalyses the detoxication of monohalomethanes and ethylene oxide *in vitro*, lymphocytes from expressors of the gene appear protected against the sister chromatid exchange induced by these compounds. It is possible that like individuals with the GSTM1 null genotype, homozygotes for the GSTT1 null allele (*GSTT1*0*) will have an altered cancer risk. The consequences of the genotype however, are still unclear as the rat GSTT1 homologue (GST5-5), possesses dehalogenase activity against dichloromethane giving rise to S-chloromethyl GSH which produces formaldehyde. Expression of rat GST5-5 in the Ames test system is mutagenic.[52] The influence of GSTT1 null on susceptibility to particular cancers is presently therefore, unpredictable.[48,49]

Initial estimates of the frequency of GSTT1 null (30–40%) now appear high and in further studies, Pemble and Taylor (unpublished results) found a lower frequency of the genotype (20%). This figure is similar to the frequency found in the authors laboratory (16%) in control women (L. Yengi, C. Redman, A. Warwick and R. Strange unpublished results).

Microsomal GST

A unique GST that is activated by N-ethylmaleimide is present in the endoplasmic reticulum and outer mitochondrial membrane.[18] The enzyme has been termed microsomal GST and appears to have a distinct evolutionary origin from the presumably soluble isoforms expressed in cytosol. The microsomal GST comprises 3 subunits each of 154 amino acids (excluding the initiating methionine). The cDNA for this enzyme has been cloned by De Jong *et al.*[53] and, in the human, it is encoded as a single copy gene on chromosome 11.

The activity of microsomal GST towards the model GST substrate, 1-chloro-2,4-dinitrobenzene, can be increased *in vitro* by the sulphydryl agents, iodoacetamide, diethylmaleate, cystamine, dithiopyridyl, cystine and glutathione disulphide. Modification of Cys49 appears to represent the activation mechanism. It is noteworthy that the enzyme in rat liver can also be activated *in vivo* by CCl_4 and phorone, both of which can produce ROS. In addition, treatment of rats with acrolein, a product of oxidative stress, also increases the activity of the hepatic microsomal GST *in vivo*.[54,55]

In humans, microsomal GST is strongly expressed in liver. There is no quantitative evidence for inter-individual differences in expression. However, in human lung, immunohistochemical studies showed marked qualitative inter-individual differences in the levels of the microsomal isoenzyme (D. Lamb, D.J. Harrison and J.D. Hayes unpublished observations).

EVIDENCE LINKING THE GST WITH PROTECTION AGAINST OXIDATIVE STRESS

While the GST originally attracted attention because of their activities towards xenobiotics including known carcinogens, their real function is unclear. With increasing interest in ROS, more attention has focused on the putative importance of the GST in the detoxication of the products of oxidative stress. Both *in vitro* and *in vivo* studies have contributed to the view that these enzymes are important in this context. It should also be recognised that because different GST isoenzymes apparently display unique subcellular localisation, their individual contribution to protection against oxidative stress may be distinct in terms of site of activity. Thus, microsomal GST may function within membranes to reduce peroxidised lipid directly (i.e. without the action of phospholipase A). Mitochondrial-specific theta class GST (subunit 13) and the nuclear specific GST (subunit 5*)[56] may provide unique protection against oxidative stress. Similarly, the distribution of isoenzymes of the GST gene families expressed in cytosol can be different. Thus, immunofluorescence studies in cultured rat glomerular mesangial and epithelial cells show that both express alpha class Ya (subunit 1) and mu class Yb1/Yb2 (subunits 2 and 3) though their subcellular distributions differ[57]; Ya is distributed in cytosol while Yb1/Yb2 is predominantly perinuclear. These data support reports of nuclear staining for GST and the view that mu GST catalyse the reduction of peroxidised DNA.

GST Substrates Identified In Vitro

The GST catalyse the conjugation with GSH of a variety of electrophiles that result from ROS activity (Table 2). Substrates include α, β-unsaturated aldehydes such as 4-hydroxyalkenals and base propenals that are the products of ROS reactions and lipid peroxidation.[58] Amongst α, β-unsaturated compounds acrolein and crotonaldehyde are detoxified by pi class GST as are cytosine propenal, thymine propenal and uracil propenal.[58] By contrast, conjugation of 4-hydroxyalkenals with GSH is catalysed primarily by alpha and mu class GST11.[59]

In addition to their conjugating activity certain GST demonstrate *in vitro* peroxidase activity against substrates that include lipid hydroperoxide as well as, products of oxidative damage to DNA such as DNA hydroperoxide and 5-hydroxymethyluracil.[13,48,56] Thus, alpha and theta class enzymes demonstrate activities towards linoleic acid hydroperoxide via the reaction:

$$FAO_2H + 2GSH \rightarrow FAOH + GSSG + H_2O$$

TABLE 2
Relative activities of GST families towards products of oxidative stress
Data show relative levels of reactivity of cytosolic GST towards different substrates. As data for each gene family may include results from studies in different species the relative levels shown are approximate

	alpha	mu	theta	pi
cumene hydroperoxide	350	20	230	1
linoleic acid hydroperoxide	15	1	25	8
4-hydroxy nonenal	2	5	-	1
cholestereol oxide	1	0	0	0
DNA hydroperoxide	0	3	350	1
trans stilbene oxide	1	2600	-	1

These cytosolic GST can catalyse the reduction of fatty acid hydroperoxides but not esterified fatty acid hydroperoxides (cf below activities of microsomal GST). It is believed that phospholipase activity is required to release the fatty acid hydroperoxide moiety. The effectiveness of cytosolic GST isoenzymes in inhibiting lipid peroxidation *in vitro* in rat liver microsomes, initiated by ADP-Fe^{3+} and NADPH has been shown by experiments in which the formation of malondialdehyde was reduced by addition of rat alpha class GST $YaYc_1$ and Yc_1Yc_1 (1-2 and 2-2 isoenzymes) to preparations of isolated microsomes.[13,60] Mu class GST also demonstrate peroxidase activities towards DNA hydroperoxide and 5-hydroxymethyluracil. In the rat, one alpha isoform has high activity towards 5-hydroperoxymethyluracil and one theta GST can reduce DNA hydroperoxide.[13]

It is emphasised that less is known of the catalytic properties of human GST towards either lipid or DNA hydroperoxides although it is clear that within both human and rat gene families differences exist in catalytic efficiencies; for example, A2-possesses 4-fold greater peroxidase activity against lipid hydroperoxides than A1-1.[27] Similarly in the rat, the YaYa (1-1) and YkYk (8-8) isoenzymes have markedly different activities towards various lipid substrates; YaYa catalyses the formation of prostaglandins E_2 and F_{2a} from PGH_2 and, the reduction of linoleic acid hydroperoxide while YkYk has no activity towards these substrates but has high activity towards 4-hydroxy non-2-enal.[13] Amongst the human enzymes, GSTM1, M2 and M3 are active against 4-hydroxyalkenals, GSTM1 contributes, quantitatively the greatest protection against these products of lipid peroxidation because of the high levels of expression of the *M1*A* and *M1*B* alleles in the tissues of those who possess these genes.

An unidentifed GSH-dependent inhibitor of *in vitro* lipid peroxidation has been reported by several groups. This inhibitor appears to be a protein as protection against lipid peroxidation is abolished by heat and treatment with trypsin. As the microsomal GST has been shown to reduce phospholipid hydroperoxide and 4-hydroxyalkenals, it has been suggested that it is the factor responsible for the inhibition of lipid peroxidation. Mosialou *et al.*[62] showed that activation of microsomal GST in rat liver microsomes undergoing non-enzymic peroxidation results in a marked decrease in malondialdehyde equivalents. These workers have also shown that protection against lipid peroxidation has an absolute requirement for glutathione or αL-Glu-L-Cys-Gly and thiol-containing glutathione analogues that are not substrates for microsomal GST do not protect against lipid peroxidation. Significantly, Mosialou *et al.*[62] used reconstructed proteoliposomes to show that microsomal GST can function to reduce phospholipid hydroperoxides within artificial membranes. Moreover, the enzyme appears active with both mono- and di-substituted phospholipids. These data suggest the microsomal GST prevents the propagation of lipid peroxidation *in situ*.

Little evidence exists to suggest that cytosolic GST can protect membranes against lipid peroxidation. However, the breakdown of membrane lipids that occurs following oxidative stress can result in the release into cytoplasm of aldehydes and peroxidised fatty acids, potential substrates for cytosolic GST.

Coordinated Expression of GST and Antioxidant Enzymes

Interesting data indicating coordinated expression of GST and antioxidant enzymes such as the superoxide dismutases and glutathione peroxidase have been obtained from immunohistochemical studies of human and animal tissues. For example,

expression of alpha GST and CuZn- and Mn-superoxide dismutase is located to similar cells; hepatocytes but not biliary epithelium, large but not small airway epithelia.[63-66]

The distribution of these enzymes in kidney is also interesting. Thus, light microscopy studies of human kidney have shown glomerular expression of the superoxide dismutases and alpha and mu class GST is weak compared to that in the proximal tubule.[67] In the rat, immunoperoxidase staining also showed weak glomerular expression with localisation of these enzymes to deep proximal and distal tubules and medullary collecting ducts.[57] The pattern of alpha expression therefore, differs somewhat from that in humans where these isoforms are limited to the proximal tubule. Interestingly, alpha and mu GST and CuZn SOD, enzymes with a presumed antioxidant function, are expressed in segments of the nephron with high metabolic activity and, known to be vulnerable to oxidant damage. Presumably constitutive expression of antioxidant enzymes is inadequate when faced with sudden increases in ROS.

Immunoblotting studies of alpha GST expression in isolated rat glomeruli showed expression of Ya (and mu Yb1/Yb2) but only very weak expression of Yc and Yk.[57] The significance of these different patterns of expression may relate to the recognised importance of glomerular prostaglandin production. Furthermore, tubular cells which express Yc and Yk more strongly, are metabolically active and may undergo high rates of lipid peroxidation and/or be particularly sensitive to the genotoxic effects of alkenals.

High concentrations of ROS (e.g. approx. 10^{-4}M H_2O_2) are among many compounds (e.g. eicosanoids and cytokines) produced by both infiltrating and resident glomerular cells following renal insult.[8,68] These may influence, via effects on cell growth/death, the processes of mesangial cell proliferation and/or hypertrophy with expansion of the mesangial matrix that characterise the pathogenesis of glomerulosclerosis. Apart from directly protecting against oxidative stress, the particular importance of alpha GST in determining the response of mesangial cells to oxidative stress is also suggested by data showing that the A1-1 and A2-2 isoforms preferentially catalyse the formation of prostaglandin F_2 and prostaglandin D_2 respectively from prostaglandin H_2.[27] Prostanoids appear to play a critical role in mediating the glomerular response to many pathologic challenges and, significantly their synthesis is influenced by oxidative stress.[69] Recent studies also show them to be involved in the regulation of cell proliferation/hypertrophy.[70] In the scenario of glomerulosclerosis, production of PGF_{2a} would be considered harmful as it is a mitogen and a vasoconstrictor and, increases the permeability of the glomerular basement membrane. Both GST A1 and A2 monomers have been detected in human kidney.

The relatively low levels of expression of alpha and mu GST and CuZn SOD in glomeruli implies filtration requires less metabolic work than tubular reabsorption, and/or supports the suggested physiological role for ROS, particularly H_2O_2 within the glomerulus.[67] It also suggests glomeruli are particularly vulnerable to oxidant injury, especially when the endothelium is disrupted allowing infiltration of inflammatory cells which release ROS, a phenomenon characteristic of many forms of glomerulonephritis.

Expression of the GST During Development

Studies in developing human tissues also suggest the importance of alpha and mu GST in protection against oxidative stress.[63-66] Firstly, alpha class GST and GSTM1

demonstrate time- and tissue-coordinated expression with CuZn-superoxide dismutase; thus, these enzymes are up-regulated during development in liver, a tissue that is metabolically active in the perinatal period but not in tissues such as lung with lower levels of oxygen consumption.

Immunohistochemical studies show complex changes in the expression of particularly alpha class isoenzymes in human developing kidney. Between 12–36 weeks gestation, primitive nephrons demonstrate positivity for alpha and pi GST along their entire lengths. At about 36 weeks however, alpha GST is down-regulated in the distal tubule and collecting ducts while expression in the proximal tubule is initially restricted to the part of the tubule next to the glomerulus. The distal part of the proximal tubule is negative. At this gestational time, the entire nephron is positive for pi. After 40 weeks, alpha GST is expressed along the entire proximal tubule while pi is restricted to the distal tubule and collecting ducts.[67,71,72] Expression is maintained in the proximal tubule after 40 weeks gestation suggesting the function of these isoforms is associated with the onset of mature proximal tubular function. We suggest that proximal tubular cells, adjacent to the glomerulus, become functional first. Cells in the elongating, distal portion of the proximal tubule divide and do not express alpha GST. When elongation is complete and the tubule becomes functional, alpha expression is up-regulated and these GST are present along the entire proximal tubule.[67,71]

GST Expression is Coordinated with Se-glutathione Peroxidase

Studies by Stone & Dratz[73] showed the importance of antioxidant diets in GST expression. Thus, rats fed diets deficient in vitamin E and selenium demonstrated increased GST activities in all tissues examined except the retina. Since only total GST activity towards 1-chloro-2,4-dinitrobenzene was measured it is not clear which GST class was affected by these dietary manipulations. More recent studies show that reducing Se-glutathione peroxidase activity in rat livers by administration of Se-deficient diets causes a compensatory increase in expression of alpha (Ya,Yc) and mu (Yb1) GST.[74]

The Antioxidant Response Element

The finding of a responsive element (ARE) in the 5′-flanking region of the rat Ya_2 gene that is also activated by H_2O_2 and other oxidants provides the first molecular evidence that expression of this GST class is altered by oxidative stress and supports the view that these isoforms are part of an antioxidant defence system that has evolved to meet a need for tissue- and time-specific protection.[75] Analysis of the 5′-flanking region of the Ya_2 gene has provided compelling evidence for the close interrelationship between detoxication of xenobiotics and resistance to oxidative stress. Regulation of GST is complex and in addition to the ARE, the Ya_2 subunit gene also contains glucocorticoid and xenobiotic responsive elements (XRE).[75] The DNA sequence of the ARE is distinct from that of the XRE but a similar sequence has been found in the rat NAD(P)H:quinone reductase gene. The functional core sequence of the ARE, 5′-ggTGACaaaGC-3′, is responsive to H_2O_2 although the concentration of the oxidant needed to increase expression of transfected chloramphenicol acetyltransferase (CAT)-ARE constructs in cells is rather high (10^{-3}M).

The possibility that the element is regulated via a redox mechanism similar to that described for the bacterial oxyR protein[76] in which binding of the protein to DNA depends on its oxidised/reduced state has been studied *in vitro* but no such difference

in the ARE-binding protein has been detected. Examination of the recognition motif of the ARE binding protein shows substantial similarity with the AP-1/12-0-tetradecanoylphorbol-acetate responsive element suggesting the involvement of c-jun, a transcription factor that is both activated and up-regulated by H_2O_2 and UV.[77-79] Rushmore *et al.*[75] have suggested several possible mechanisms for the regulation of Ya; these include the binding of the xenobiotic (e.g. polycyclic aromatic hydrocarbon) to the Ah receptor followed by activation of the cytochrome P450 CYP1A1 gene by the resulting complex. Metabolism of the xenobiotic in a CYP1A1-catalysed reaction could result in the formation of a reactive oxygenated intermediate that could interact with the ARE to effect transcription. Alternatively, phenolic antioxidants (e.g. tert-butylhydroquinine) or oxidants such as H_2O_2 could cause oxidative stress in the cell thereby activating the ARE without involvement of the Ah receptor or CYP1A1.

The role of the ARE in protection against H_2O_2-induced stress however, is still unclear. Thus, we studied the effects of H_2O_2 on expression of alpha class enzymes in cultured human synovial fibroblasts, a cell type that does not appear to constitutively express this GST class. Even though the cells were exposed to concentrations of H_2O_2 similar to those used by Rushmore *et al.*[75] (i.e. $>10^{-3}$M), we found no detectable expression of alpha class GST comprising the A1, A2, Ya or Yc monomers following treatment with H_2O_2 (10^{-6}, 10^{-4} or 10^{-3}M). Expression of mu and pi GST as well as SOD isoforms was also found to be unaffected.[80]

There are no corresponding studies describing expression of theta class GST.

GST Polymorphisms and Response to Oxidative Stress

Although a variety of putative substrates for the different GST gene families have been proposed, there remains uncertainty as to the true function of these enzymes *in vivo*. A potentially useful way to clarify this problem is to study the relationship between genetic polymorphism in GST genes and susceptibility (or prognosis) to pathologies which involve ROS.

Polymorphism at GSTM1 is most amenable to this approach as the molecular basis of the phenomenon is well understood and there is sufficient DNA sequence data available to allow identification of the most common variants. Of particular interest are homozygotes for *GSTM1*0* because they express no protein and might be expected to demonstrate impaired detoxication of substrates that include potential carcinogens such as benz(a)pyrene epoxides and the products of ROS attack on DNA such as 5'hydroxymethyl uracil. The GSTM1 null polymorphism might therefore identify individuals who are at increased risk of cancer or, who are less effective in the repair of oxidatively damaged molecules and therefore, are more susceptible to inflammation-induced damage.[81] These possibilities are linked as in some cases chronic inflammation may progress to malignancy particularly following phagocyte-mediated activation of potential carcinogens such as polycyclic aromatic hydrocarbons and aromatic amines.

There is accumulating evidence that the GSTM1 null polymorphism is associated with impaired detoxication although so far data indicates these individuals suffer an increased risk of cancers including those of lung, bladder, skin and gastrointestinal tract as well as pituitary adenomas.[81-84] In the context of inflammatory pathologies, GSTM1 null might be expected to be significant in liver disease as the gene is strongly expressed in hepatocytes, contributing about 50% of total GST activity in GSTM1 expressors. There are some data suggesting *GSTM1*O* homozygotes are more

susceptible to certain inflammatory liver diseases such as alcoholic hepatitis than homo- and heterozygotes for *GSTM1*A* and *GSTM1*B*.[85,86] Studies in British subjects suffering primary biliary cirrhosis and French subjects with alcoholic liver disease however, did not show any association between the GSTM1 null phenotype and susceptibility to these diseases.[85,45] There do not appear to be data in other inflammatory pathologies.

These studies have largely focused on the belief that the presence/absence of GSTM1 is a determining factor in disease susceptibility, that the products of *GSTM1*A* and *GSTM1*B* are equally protective and, disease risk in *GSTM1*0* heterozygotes and, homo- and heterozygotes for *GSTM1*A* and *GSTM1*B* is the same. These assumptions may not be justified since phenotyping studies in gastric, colorectal and brain cancers suggest individuals with two positive alleles are better protected than *GSTM1*0* heterozygotes[9,11] and recent studies in basal cell carcinoma of skin show the heterozygote GSTM1 A/B genotype is protective.[87]

The possibility that multiple allelism at alpha or theta class loci might determine susceptibility to oxidative stress has not been investigated, though studies investigating an association between the GSTT1 null genotype and susceptibility to various pathologies are underway in several laboratories.

GENERAL OVERVIEW

Oxidative stress is a feature of many pathologies. Because tissues are exposed to different types of oxidant stress and have differing availabilities of the metal ions that catalyse the Haber-Weiss reaction, resultant damage is likely to vary and cell-specific patterns of expression of repair enzymes are likely to be appropriate. Thus, a unique alpha isoform may be required to protect against the ROS generated in skin by exposure to UVA and UVB while different GST isoforms may protect glomerular, synovial and pancreatic cells against ROS generated by infiltrating blood-borne cells during inflammation. Similarly brain may require specific GST as it appears especially vulnerable to oxidant stress via reperfusion-hypoxia or trauma since it is rich in PUFA and iron and, a relatively weak expressor of antioxidant enzymes. A growing body of evidence suggests that GST isoenzymes are part of antioxidant defences though the confusing number of potential substrates, some resulting from ROS action, others from potential carcinogens makes assessment of the *in vivo* function of these enzymes difficult.

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