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ALPHA, MU AND PI CLASS GLUTATHIONE S-TRANSFERASES IN HUMAN SYNOVIUM AND CULTURED SYNOVIAL FIBROBLASTS: EFFECTS OF INTERLEUKIN-1α, HYDROGEN PEROXIDE AND INHIBITION OF EICOSANOID SYNTHESIS

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We describe expression of alpha, mu and pi class glutathione S-transferases (GST) and, CuZn- and Mn superoxide dismutase (SOD) in human synovium and cultured synovial fibroblasts. Immunohistochemical and immunoblotting studies showed synovium and cultured cells expressed pi GST and both isoforms of SOD. Cellular localisation was largely perinuclear. No expression of alpha or mu GST was detected even though polymerase chain reaction analysis showed 4/6 subjects had positive genotypes at the polymorphic, mu class GSTM1 locus. Incubation of cultured synovial fibroblasts with H_2O_2, IL-1α and the cyclooxygenase and lipoxygenase inhibitor, Tenidap, did not induce expression of alpha, mu or pi GST though treatment with IL-1α caused a marked increase in the expression of Mn SOD.

KEY WORDS: glutathione S-transferase, superoxide dismutase, oxidant stress, synovium, polymerase chain reaction, interleukin-1α.

INTRODUCTION

Rheumatoid arthritis (RA) is a systemic disease of unknown aetiology characterised by inflammation of synovial joints and destruction of cartilage and bone. Chronic inflammation is associated with increased levels of reactive oxygen species (ROS) such as superoxide radical and hydrogen peroxide (H_2O_2) which in RA, may be produced by phagocytes in the synovial fluid and pannus and/or by synovial endothelial cells during hypoxia-reperfusion. Data describing products of oxidative stress in the serum, synovial fluid and breath of patients with inflammatory joint disease suggest the synovium is exposed to levels of these species that exceed its detoxication capacity[1].

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The effects of ROS are complex; low concentrations (eg H_2O_2 10^{-8}M) are mitogenic while high levels (eg H_2O_2 10^{-4}M) may cause cell damage or even death[2]. Consequently, intracellular levels of superoxide radical and H_2O_2 are modulated by the antioxidant enzymes, CuZn- and Mn superoxide dismutase (SOD), glutathione peroxidase and catalase thereby reducing formation of hydroxyl radical[3]. However, while co-ordinated enzyme expression protects cells, many molecules suffer ROS-induced damage during oxidative stress.

Recent data suggest a role for the glutathione S-transferase (GST) supergene family in the repair/detoxication of various products of oxidative stress. The cytosolic GST can be grouped into the alpha, mu, theta and pi classes[4,5]. While the number of genes encoding these isoforms is unclear, the alpha and mu classes appear to each comprise at least six genes[5]. The most widely expressed mu gene, GSTM1, is polymorphic with about 50% of individuals being homozygous for the deleted *GSTM1*O* allele[5-7]. These subjects express no GSTM1 enzyme.

Several lines of evidence implicate alpha, mu and theta GST in antioxidant defence; firstly, in vitro substrates include products of ROS attack on lipid and DNA[8]. Secondly, alpha and mu GST demonstrate coordinated expression and location to similar cell types as the superoxide dismutases and Se-glutathione peroxidase[9]. Thirdly, the finding of an antioxidant responsive element (ARE) in the rat alpha Ya gene that is activated by H_2O_2 and other oxidants[10] suggests expression of this gene can be altered by oxidant stress.

Synovium is a heterogeneous tissue with resident populations of fibroblasts, macrophages and endothelial cells which in RA is infiltrated by lymphocytes and phagocytes. Expression of GST and other antioxidant enzymes by synovial cells has not been described but may be critical in mediating the response to oxidative stress. Since the growth status of cells influences expression of mu and pi GST[11], inflammatory products that affect cell proliferation (eg ROS and cytokines) may also affect expression of these GST.

We now describe studies in human synovium to define expression of alpha, mu and pi GST and CuZn- and Mn SOD. Since a feature of RA is the marked increase in synovial fibroblasts, we have also examined the effects of H_2O_2 and/or IL-1α on expression of these enzymes in cultured fibroblasts. It is not known if GSTM1 phenotype reflects genotype in these cells, and we have compared genotype determinations made using the polymerase chain reaction (PCR) with phenotype determined using immunoblotting. Since increased synthesis of prostaglandins (PG), particularly PGE_2, is a prominent feature of RA and alpha GST isoforms A1-1 and A2-2 preferentially catalyse the formation of $PGF_{2\alpha}$ and PGD_2 respectively from PGH_2[12], we have also studied expression of alpha GST after inhibition of eicosanoid synthesis by Tenidap, an anti-rheumatic drug that inhibits lipoxygenase and cyclooxygenase[13,14].

MATERIALS AND METHODS

Patient Samples

Synovium was obtained, with the approval of the Ethics Committee of the North Staffordshire Hospital, at synovectomy or total joint replacement from the patients shown in Table 1.

TABLE 1
Patient Details

	disease	joint	MnSOD	CuZnSOD	GSTM1/mu		alpha	pi	culture
1	RA	knee	+*	+	G+	P−	−	+	+
2	RA	knee	+	+		P−	−	+	+
3	RA	wrist	+*	+			−	+	+
4	RA	hip	+	+			−	+	+
5	RA	shoulder	+*	+		P−		+	+
6	OA	knee	+	+			−	+	+
7	OA	knee	+	+	G+	P−	−	+	+
8	OA	knee	+	+	G−	P−	−	+	+
9	OA	knee	+	+	G+	P−	−	+	+
10	OA	knee	+	+			−	+	+
11	OA	knee	+	+		P−		+	
12	OA	hip	+	+				+	+
13	OA	hip	+	+				+	
14	ReA	hip	+	+	G−	P−	−	+	+
15	AS	knee	+	+				+	+
16	PVNS	knee	+	+			−	+	+
17	CTS	wrist	+	+		P−		+	
18	TS	ext pol	+	+				+	+
19	Men. Tear	knee	+	+	G+	P−		+	+
20	Men. Tear	knee	+	+		P−		+	
21	Men. Tear	knee	+	+		P−		+	

Abbreviations: RA, rheumatoid arthritis. OA, Osteoarthritis. ReA, Reactive arthritis. AS, Ankylosing Spondylitis. PVNS, Pigmented villonodular synovitis. CTS, idiopathic carpal tunnel syndrome. TS, tenosynovitis. Men. Tear, meniscal tear. *, Mn SOD mol. wt. 16500. G, GSTM1 genotype. P, GSTM1 phenotype.

Immunohistochemical Localisation Of Enzyme Expression

Localisation of enzyme expression in paraffin wax sections of synovial tissue from 3 subjects (Table 1; patients 1, 14 and control) was performed using an immunoperoxidase method[15]. Primary antibodies were used at a dilution of 1/75 and positive control sections (liver, kidney) were also studied.

Immunoblotting

For immunoblotting, blocks of synovium (2–3 mm^3) were snap frozen in liquid N_2 immediately after removal. Before electrophoresis, frozen tissue was ground to a fine powder, dissolved in sample buffer (see below) at 100°C and centrifuged (10 500 g, 5 min, 20°C) to remove insoluble material. In experiments with synovial fibroblasts, cells were trypsinised from culture flasks, cell numbers counted in an haemocytometer and equal numbers (about 4×10^5) of cells from each flask diluted with sample buffer comprising 125 mM Tris-HCl (pH 6.80) containing 4% (w/v) SDS, 1% (v/v) 2-mercaptoethanol, 20% (v/v) glycerol and 0.02% (w/v) bromophenol blue. The mixture was heated (100°C, 5 min) and 10 μl portions (2×10^4 cells) loaded onto 12.5% SDS discontinuous polyacrylamide gels and immunoblotted after electrophoresis. Blots were incubated with sheep primary antisera to human CuZn- and Mn SOD, the alpha A1 monomer and pi GST and, rabbit primary antisera to GSTM1 and the rat alpha class Ya and Yc monomers. Each GST antibody cross-reacted on immunoblots with other isoforms of the same class. There was no reaction

with GST isoforms from other classes[16]. Antisera to the SOD isoforms were only recognised by their own antigens[17]. A horseradish peroxidase detection system was routinely used. Weak or negative signals were checked using an enhanced chemiluminescence detection system (ECL, Amersham International plc.). To quantify changes in enzyme expression following incubation with recombinant IL-1α (British Biotechnology Products, Ltd, Oxon), H_2O_2 and Tenidap (Pfizer Central Research, Kent), samples were equalised for cell number, immunoblotted and the grey levels of bands compared by image analysis (Confocal Technologies, U.K.).

Isolation Of Synovial Fibroblasts

Synovium was dissected free from surrounding tissue, cut into 2–3 mm^3 pieces and digested (3–4 h, 37°C) with occasional shaking in Earles Balanced Salt Solution containing 2 mg/ml collagenase (Sigma Type 1A). The suspension was filtered through 100 micron mesh, centrifuged (600 g, 5 min) and resuspended in Dulbeccos Modified Eagles Medium (DMEM) containing 10% heat inactivated foetal calf serum (FCS), 2 mM L-glutamine, penicillin (100 U/ml), streptomycin (100 μg/ml) and fungizone (0.25μg/ml). Cells were seeded into 25 cm^2 plastic culture flasks and cultured (37°C) in a humidified incubator (5% CO_2: 95% air). Adherent synovial fibroblasts were grown to confluence and passaged with 0.25% (w/v) trypsin containing 1 mM EDTA. Cells were cultured further in 75 cm^2 flasks and passaged at a ratio 1:2. Cells were studied up to passage 12.

Immunofluorescence Microscopy

Subconfluent and confluent synovial fibroblasts grown on 13 mm diam. glass coverslips were fixed (30 min, 20°C) in 3.5% formaldehyde in phosphate buffered saline (PBS). Cells were washed (X3) and permeabilised with 95% ethanol at –20°C for 2 min. After washing in PBS the coverslips were incubated (30 min, 20°C) with primary antibodies to Mn- and CuZn SOD and alpha, mu and pi GST and washed X3 (15 min) with PBS. Fluorescein conjugated secondary antibodies were incubated with the cells for a further 30 min, followed by extensive washing with PBS. Coverslips were mounted in PBS/glycerol (1:9) containing 25 mg/ml 1,4-diazobicyclo-(2.2.2) octane to prevent quenching of fluorescence. Slides were examined using a microscope with an epi-fluorescence attachment and photographed.

Effects Of H_2O_2, IL-1α And Tenidap On Enzyme Expression In Synovial Fibroblasts

To study the effects of these treatments on expression of GST and SOD, a suspension of about 5×10^5 cells was added to 75 cm^2 culture flasks. Growth medium was removed after 72 h, replaced with maintenance medium (medium containing 0.5% FCS) and the cells returned to the incubator for a further 24 h. The maintenance media were replaced (day 0) with growth media containing H_2O_2 (10^{-6}M) with/without human IL-1α (1 ng/ml) or, Tenidap (5 μg/ml) and effects on enzyme expression were studied after up to 72 h.

Determination Of GSTM1 Genotype

GSTM1 genotype was determined in 6 samples of synovium using PCR performed in duplicate using 1 μg DNA and primers to exon 4 and exon 5 of GSTM1[18]. Since

GSTM1 is deleted, *GSTM1*O/GSTM1*O* homozygotes can be differentiated from *GSTM1*A* and *GSTM1*B* homo- and heterozygotes by the absence of a 273 bp fragment (exon 4, intron 4, exon 5) on agarose gels after ethidium bromide staining. Primers to β globin were used as positive control.

Analytical Methods

Cell numbers were determined using a crystal violet colourimetric assay[19], numbers determined by this method correlated well with those determined using a haemocytometer. PGE_2 levels in media were determined in duplicate using radioimmunoassay (New England Nuclear, MA, USA).

Statistical Methods

Wilcoxon signed rank matched pairs tests were used to compare means of cell numbers determined by optical density measurements.

RESULTS

Immunohistochemical Localisation Of Antioxidant Enzymes In Synovial Tissue

GST and SOD isoenzymes in synovial tissue were localised using immunohistochemistry. Synovial tissue is heterogeneous and shows marked inter- and intra-sample variation in cellularity, fat and connective tissue content. These differences may depend on the site of removal and type and extent of disease. Data therefore, were interpreted as showing the presence/absence of expression rather than quantitative differences in level.

a) Alpha GST. No staining detected (Figure 1a).
b) Mu GST. No staining detected.
c) Pi GST. Strong positivity was seen in fibroblast and macrophage type cells of the synovial lining layer (Figure 1b), as well as many infiltrating lymphocytes. While most of the lining layer was positive, some regions of lymphocytic infiltration around blood vessels showed little or no staining.
d) Mn SOD. Intense staining was observed in the fibroblasts and macrophages of the synovial lining layer, in endothelial cells and in some lymphoblasts.
e) CuZn SOD. The staining pattern for CuZn SOD was similar to that for Mn SOD.

Expression Of Antioxidant Enzymes In Synovial Tissue

To confirm the immunohistochemical results, immunoblotting was used to detect expression of SOD and GST isoenzymes in synovium.

a) Alpha GST. Alpha GST was not detected in 11 synovia examined even after overloading the gels and prolonged exposure of the X-ray film using the ECL method. Lanes containing alpha GST standard demonstrated strong overloading.
b) Mu GST. No expression of mu isoenzymes was detected in 12 synovia examined even though lanes containing mu GST standard demonstrated strong overloading. The GSTM1 genotype of 6 subjects was examined by PCR; a 273 bp fragment of DNA was detected in 4 subjects indicating they had positive GSTM1 genotypes.

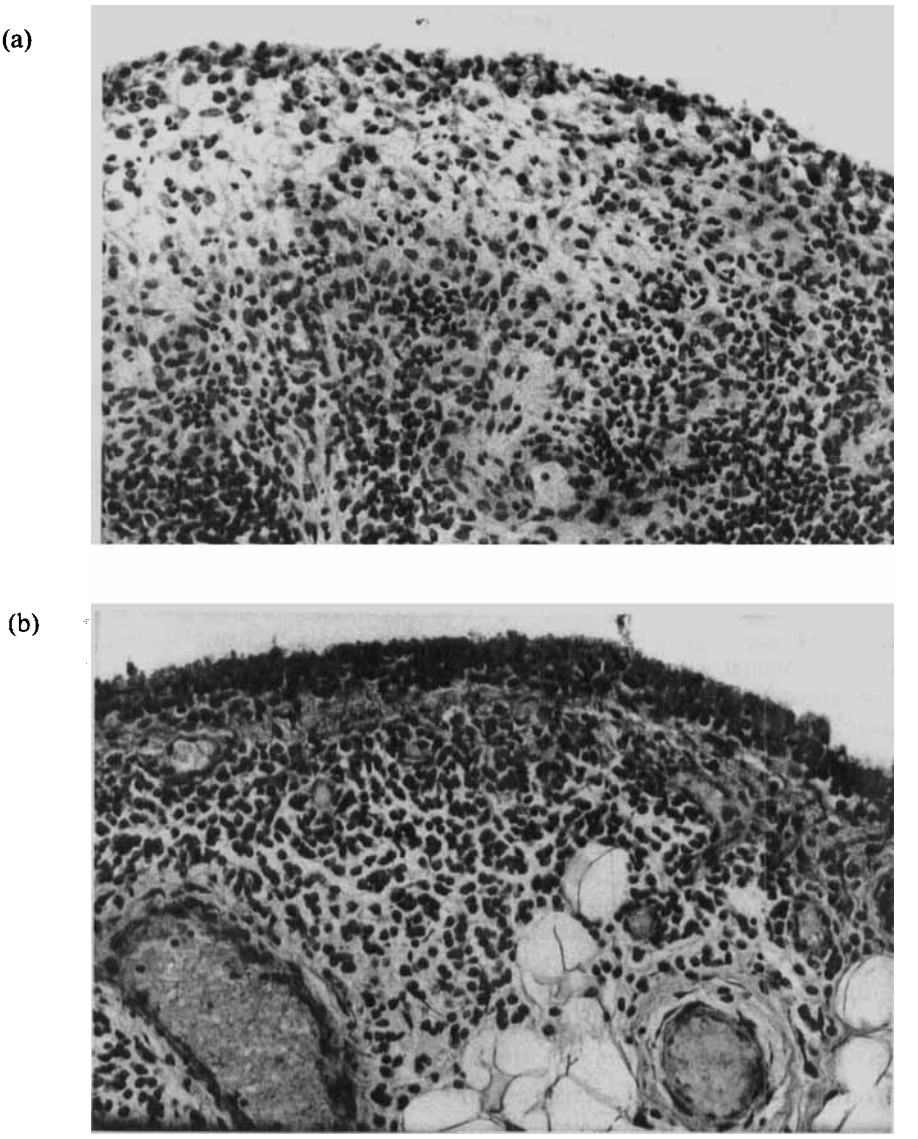

FIGURE 1 *Localisation of GST in synovial tissue.* Sections of synovium from a patient with RA stained by an indirect immunoperoxidase method. Staining for alpha GST (a) was negative. Pi GST (b) was strongly positive, especially in the fibroblast and macrophage cells of the lining layer. The sections were counterstained with haematoxylin. Mag X250.

c) Pi GST. A single band (mol. wt. 23000) was detected in all 21 samples.

d) Mn SOD. Mn SOD was detected in all 21 synovia examined irrespective of clinical condition. While a protein with the expected monomer mol. wt. of 22000 was identified in all cases, a further band of lower mol. wt. (approx. 16500) was seen in 3/5 RA patients. In 2 cases the 16500 band was the most prominent (Figure 2a). To determine if expression of this protein was

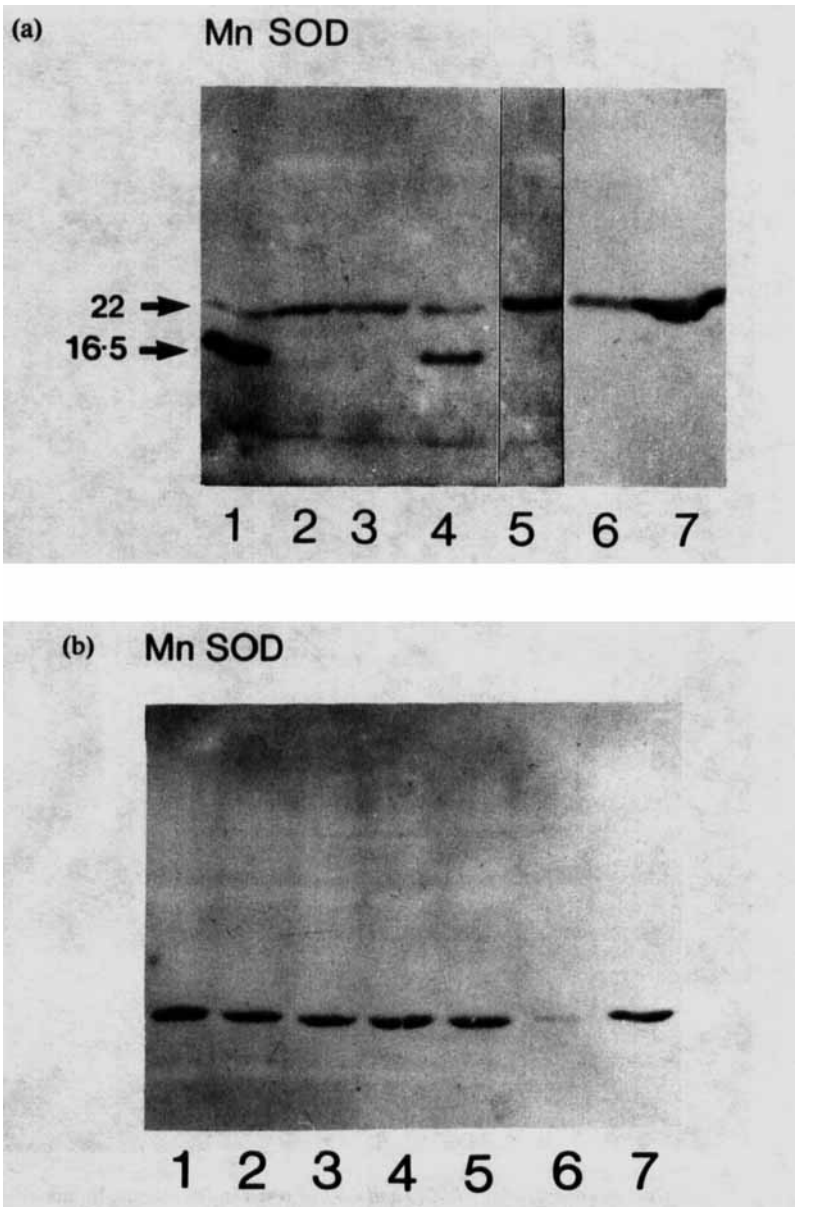

FIGURE 2 *Expression of Mn SOD in synovium and blood leucocytes.* Expression of Mn SOD in synovium (a) from subjects with RA (lanes 1,4,7), osteoarthritis (lane 2,3,5) and reactive arthritis (lane 6) was studied using immunoblotting. The molecular weight ($X10^3$) of peptides is indicated. Figure 2b shows expression of Mn SOD in blood leucocytes from 7 RA patients.

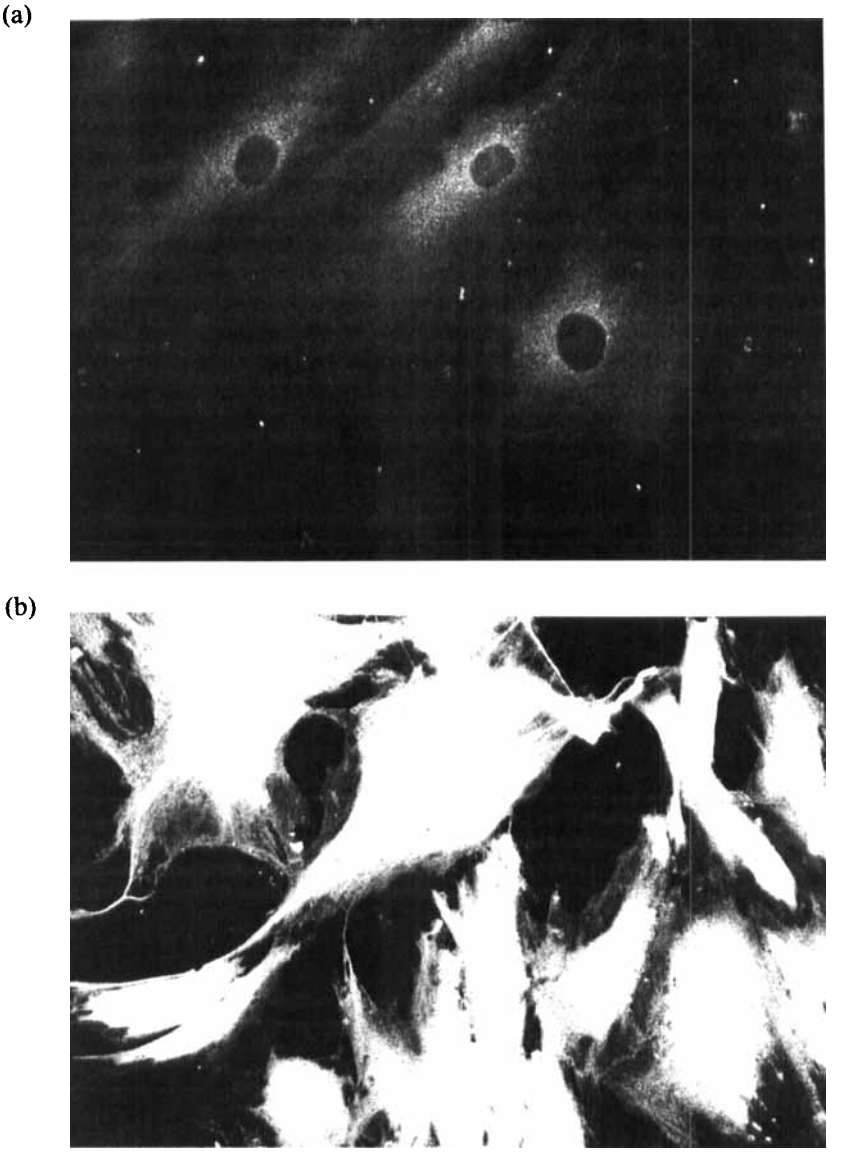

FIGURE 3 *Immunofluorescence staining of SOD and GST in synovial fibroblasts.* Immunofluorescence microscopy was used to determine the subcellular distribution of a) alpha GST, b) pi GST, c) Mn SOD in cultured synovial fibroblasts. Mag X500.

inherent to RA patients, we also examined expression of Mn SOD in peripheral mononuclear cells. In 16 RA patients (including the 3 subjects demonstrating the 16500 mol. wt. band) only one band (mol. wt. 22 000) was seen (Fig. 2b).

e) CuZn SOD. A single band with the expected mol. wt. of 18000 was observed in the 21 synovia examined.

(c)

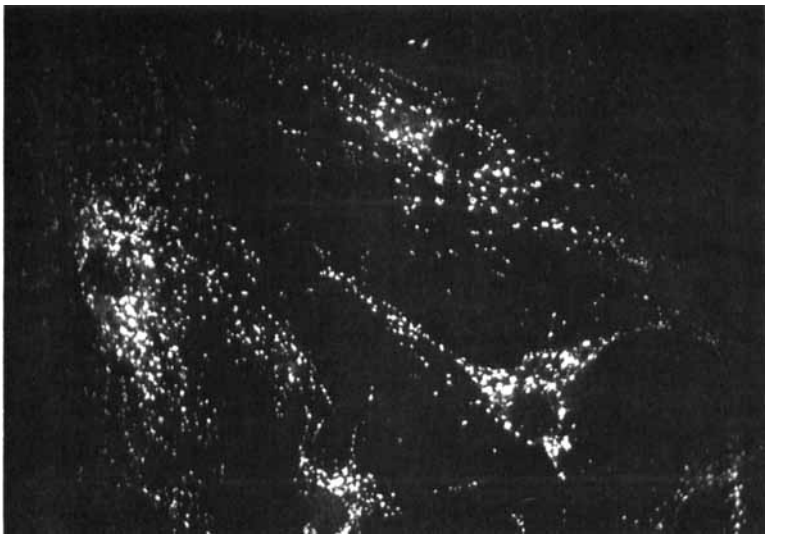

FIGURE 3 *continued*

Immunofluorescence Microscopy

The distribution of Mn- and CuZn SOD, alpha, mu and pi GST within synovial fibroblasts was studied by immunofluorescence. Examples of the staining patterns are shown in Figure 3abc.

a) Alpha GST. No positivity for alpha GST was detected (Figure 3a).

b) Mu GST. No positive staining for mu class GST was detected.

c) Pi GST. Staining for pi GST was particularly bright around the nucleus (Figure 3b). Staining was detected in all cells although the intensity varied between individual cells in any population. Similar variations were found in quiescent and growing cells.

d) Mn SOD. Figure 3c shows the network of bead-like structures within the cytoplasm that demonstrated positivity for Mn SOD. Staining was particularly intense around the nucleus but was also found at the periphery of the lamellipodia. Bright staining was found in all cells examined from fibroblast cultures prepared from 16 subjects. There were no obvious differences between quiescent and growing cells.

e) CuZn SOD. The staining pattern for CuZn SOD was similar to that of pi GST but differed from that of the Mn-isoform; localisation was mainly associated with the cytoplasm surrounding the nucleus. The staining intensity varied between individual cells in any population but the isoform was expressed in all cells examined from the 16 separate cultures.

Effects of H_2O_2, IL-1α And Tenidap On Expression Of Antioxidant Enzymes

To determine if expression of GST and SOD by synovial fibroblasts was altered by H_2O_2 (10^{-6}M), IL-1α or Tenidap, enzyme expression was studied 24 h, 48 h and 72 h after treatment. Synovial fibroblast numbers were determined 72 h after incubation with H_2O_2, IL-1α or Tenidap.

After 72 h, there was a significant increase ($p < 0.001$) of 59% in cell numbers in control wells compared with numbers at day 0 ($n = 7$). Compared with the control wells at 72 h, cell growth was unaffected by treatment with H_2O_2. Treatment with IL-1α or, IL-1α and H_2O_2 (10^{-6}M) resulted in significant increases ($p < 0.05$) in cell numbers of 19% above that in 72 h control wells and 21% above that in 72 h wells treated with the oxidant alone.

After 72 h incubation, PGE_2 concentrations in control wells were 0.04–0.05 ng/ml ($n = 3$). After treatment with IL-1α, PGE_2 levels were 2–3 ng/ml. In the presence of Tenidap, both with and without IL-1α, no PGE_2 was detected.

a) Alpha GST. No expression of alpha GST was detected 72 h after treatment with Tenidap, IL-1α and/or H_2O_2 (10^{-5}, 10^{-6}M). Higher levels of H_2O_2 (10^{-3} and 10^{-4}M) for up to 48 h also did not effect expression of alpha GST.

b) Mu GST. No expression of mu GST was detected after 72 h treatment with Tenidap, IL-1α and/or H_2O_2. Treatment with higher concentrations of H_2O_2 (10^{-3} and 10^{-4}M) for up to 48 h also did not effect expression of mu isoforms.

c) Pi GST. Expression of pi GST was unchanged 72 h after treatment in control, oxidant stressed and IL-1α treated cells.

d) Mn SOD. Expression of Mn SOD was unchanged in control cells after 72 h. Following treatment with IL-1α, both with and without H_2O_2, expression of this enzyme was markedly increased compared with the control cells, after 24 h, 48 h and 72 h (Figure 4). H_2O_2 and Tenidap had no apparent effect on expression.

e) CuZn SOD. Expression of CuZn SOD was not affected by any of the treatments.

DISCUSSION

We have studied expression of alpha, mu and pi GST and CuZn and Mn SOD in human synovium. Since the tissue contains fibroblasts, macrophages, endothelial cells and, in inflammatory conditions various types of leucocytes, immunohistochemistry was first used to identify which cells expressed these enzymes. We then studied the effects of H_2O_2, IL-1α and Tenidap on the expression of GST and SOD in cultured synovial fibroblasts. These cells were selected because an outstanding feature of RA is the increase in synovial fibroblast numbers and their response to oxidative stress and cytokine stimulation may be critical.

Immunohistochemical studies showed widespread expression of pi GST and, CuZn and Mn SOD. Expression of alpha and mu GST was not detected. These results were supported by immunoblotting; while expression of pi GST and both SOD isoforms was found in all synovia, no subjects expressed alpha or mu GST.

Interestingly, immunoblotting provided evidence that Mn SOD might be susceptible to oxidant stress. Previous studies have shown H_2O_2 causes inactivation and even fragmentation of CuZn SOD[20] and, while we found no evidence of altered

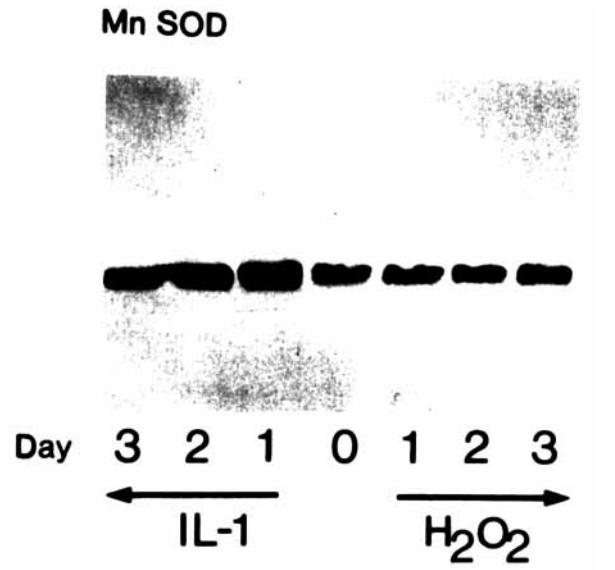

FIGURE 4 *Effects of IL-1α and H_2O_2 on expression of Mn SOD in synovial fibroblasts.* Immunoblotting was used to determine the effects of IL-1α and H_2O_2 (10^{-6}M) on expression of Mn SOD in cultured synovial fibroblasts.

forms of CuZn SOD, evidence of fragmentation of the Mn-isoform was found in 3/5 subjects with RA; it is possible that the immunoreactive protein with a molecular weight 16500 could be a ROS-modified form of Mn SOD (mol. wt. 22000). Interestingly, this phenomenon was not found in peripheral blood leucocytes even in RA patients who demonstrated two Mn SOD bands in synovium.

The alpha class of GST genes is complex and remains poorly understood[5]. Thus, while the A1-1 and A2-2 isoenzymes are relatively widely expressed, other genes such as that encoding the putatively skin-specific enzyme that appears to correspond with the rat YcYc isoform demonstrate restricted expression[21]. Expression of these alpha GST was not detected in synovium. Studies in various tissues have shown considerable inter-subject variation in the level of expression of alpha GST. For example, in brain, many subjects do not express this GST class[22]. Our examination of 11 synovia indicates this GST class is not expressed in this tissue. The significance of this variation in terms of inter-tissue differences in protection against oxidative stress is unclear but since recent data suggest the importance of this class, synovia might be considered to be relatively vulnerable to ROS. Immunohistochemical and immunoblotting studies similarly showed that mu isoforms such as GSTM1 are not expressed in synovium even in genotypically positive subjects. The importance of GSTM1 in the protection of cells from ROS-induced damage is also unclear and it is not known whether tissues that constitutively fail to express the locus are more sensitive than expressing tissues.

As expected, immunofluorescence studies in cultured fibroblasts showed expression of SOD and pi GST but not alpha or mu GST. Enzyme expression was strongest around the nucleus; staining for Mn SOD was bead-like as expected for a mitochondrial enzyme while pi GST and CuZn SOD were dispersed in cytosol.

Although cultured synovial fibroblasts did not constitutively express alpha GST, oxidative stress might be expected to affect expression since Rushmore *et al.*[10] showed induction of chloramphenicol acetyl-transferase (CAT) activity in cells transfected with constructs of the rat Ya monomer ARE and CAT following stress with concentrations of H_2O_2 similar to those we used (2.5×10^{-4}–10^{3}M). We found however, no detectable expression of alpha class GST comprising the A1, A2, Ya or Yc monomers following treatment of synovial fibroblasts with H_2O_2 (10^{-6}, 10^{-4} or 10^{-3}M). Expression of mu and pi GST and SOD isoforms was also unaffected.

We also attempted to effect expression of alpha GST in cultured synovial fibroblasts by treatment with IL-1α and Tenidap. Among the many cytokines present in synovial fluid and synthesised by synovial fibroblasts, IL-1α is of interest because it induces bone and cartilage degradation and can initiate chronic erosive arthritis in rabbits. The cytokine has pleiotrophic effects on diverse cell types including fibroblasts and it has been argued that exposure to IL-1α constitutes an oxidant stress[23]. While induction of Mn SOD appears a common effect, there was no effect on CuZn-isoform or alpha, mu or pi GST. While other studies have shown reciprocal changes in the expression of mu and pi GST in confluent cells and, cells in the log-phase of growth[11], we found no effects of IL-1α on mu and pi even though the cytokine significantly increased the growth rate of synovial fibroblasts. Similarly treatment with Tenidap, an inhibitor of PG synthesis had no effect even though alpha GST have different effects on the synthesis of $PGF_{2\alpha}$ and PGD_2.

Our studies suggest synovium may not be well protected against oxidant stress. Furthermore, since studies in transfected cells and transgenic animals have shown that isolated, increases in CuZn SOD expression can be deleterious[24], the implications for synovial fibroblasts of up-regulating Mn SOD but not other enzymes during oxidative stress are unclear.

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References

1. D.R. Blake, P. Merry, J. Unsworth, J. Outhwaite, C.J. Morris, L. Gray and L. Lunec (1989) Hypoxic reperfusion injury in the inflamed human joint. *Lancet*, 289–293.
2. B. Halliwell and J.M.C. Gutteridge, (1989) In: Free Radicals in Biology and Medicine, Clarendon Press, Oxford.
3. D. Touati (1989) The molecular genetics of superoxide dismutase in *E. coli*. An approach to understanding the biological role and regulation of SODs in relation to other elements of the defence system against oxygen toxicity. *Free Radical Research Communications*, **8**, 1–9.
4. B. Mannervik, P. Alin, C. Guthenberg, H. Jensson, M.K. Tahir, M. Warholm and J.H. Jornvall (1985) Identification of three classes of cytosolic glutathione S-transferase common to several mammalian species: Correlation between structural data and enzymatic properties. *Proceedings National Academy Sciences USA*, **82**, 7202–7206.
5. P.G. Board, M. Coggan, P. Johnston, V. Ross, T. Suzuki and G. Webb (1991) Genetic heterogeneity of the human glutathione S-transferases: A complex of gene families. *Pharmacology Therapeutics*, **48**, 357–369.
6. R.C. Strange, G.C. Faulder, B. Davis, R. Hume, J.A.H. Brown, W. Cotton and D.A. Hopkinson

(1984) The human glutathione S-transferases: studies on the tissue distribution and genetic variation of the GST1, GST2 and GST3 isoenzymes. *Annals of Human Genetics*, **48**, 11-20.
7. J. Seidegard, W.R. Vorachek, R.W. Pero and W.R. Pearson, W.R. (1988) Hereditary differences in the expression of the human glutathione S-transferase activity on trans-stilbene oxide are due to a gene deletion. *Proceedings National Academy Sciences USA*, **85**, 7293-7297.
8. B. Ketterer, D.J. Meyer, J.B. Taylor, S. Pemble, B. Coles and G. Fraser (1990) GSTs and protection against oxidative stress. In *Glutathione S-transferases and drug resistance*, (ed. J.D. Hayes, C.B. Pickett and T.J. Mantle) Taylor and Francis, London, pp. 97-109.
9. R.C. Strange, A.A. Fryer, C. Hiley, J. Bell, D. Cossar and R. Hume (1990) Developmental expression of GST in human tissues. In *Glutathione S-transferases and drug resistance*. (ed J.D. Hayes, C.B. Pickett and T.J. Mantle) Taylor and Francis, London. pp. 262-271.
10. T.H. Rushmore, M.R. Morton and C.B. Pickett (1991) The antioxidant responsive element. Activation by oxidative stress and identification of the DNA consensus sequence required for functional activity. *Journal of Biological Chemistry*, **266**, 11632-11639.
11. G. Batist, A. Woo and M-S. Tsao (1991) Effect of proliferative state on glutathione S-transferase isoenzyme expression in cultured rat liver epithelial cells, *Carcinogenesis*, **12**, 2031-2034.
12. J.R. Burgess, N-W. Chow, C.C. Reddy and C-P.D. Tu (1989) Amino acid substitutions in human glutathione S-transferase confer different specification in the prostaglandin endoperoxide conversion pathway, *Biochemical and Biophysical Research Communications*, **158**, 497-501.
13. E. Moilanen, J. Alanko, M.Z. Asmawi and H. Vapaatalo (1988) CP-66, 248 a new anti-inflammatory agent is a potent inhibitor of leukotriene B_4 and prostanoid synthesis in human polymorphonuclear leucocytes *in vitro*. *Eicosanoids*, **1**, 35-39.
14. W.D. Blackburn, L.W. Heck, L.D. Loose, J.D. Eskra and T.J. Carty (1991) Inhibition of 5′ lipoxygenase product formation and polymorphonuclear cell degranulation. *Arthritis and Rheumatism*, **34**, 204-210.
15. C. Hiley, A.A. Fryer, J. Bell, R. Hume and R.C. Strange (1988) The human glutathione S-transferases: Immunohistochemical studies of the developmental expression of Alpha- and Pi-class isoenzymes in liver. *Biochemical Journal*, **254**, 255-259.
16. G.J. Beckett, A.F. Howie, R. Hume, B. Matharoo, C. Hiley, P. Jones and R.C. Strange (1990) Human glutathione S-transferases: radioimmunoassay studies on the expression of alpha-, mu- and pi-class isoenzymes in developing lung and kidney. *Biochimica et Biophysica Acta*, **1036**, 176-182.
17. R.C. Strange, W. Cotton, A.A. Fryer, P. Jones, J. Bell and R. Hume (1990) Lipid peroxidation and expression of copper-zinc and manganese superoxide dismutase in lungs of premature infants with hyaline membrane disease and bronchopulmonary dysplasia. *Journal of Laboratory and Clinical Medicine*, **116**, 666-673.
18. M.H. Davies, E. Elias, S. Acharya, W. Cotton, G.C. Faulder, A.A. Fryer and R.C. Strange (1993) GSTM1 null polymorphism at the glutathione S-transferase M1 locus: phenotype and genotype studies in patients with primary biliary cirrhosis. *Gut*, **34**, 549-553.
19 W. Kueng, E. Silber and U. Eppenberger (1989) Quantification of cells cultured in 96-well plates. *Analytical Biochemistry*, **182**, 16-19.
20. D.C. Salo, R.E. Pacifici, S. Lin, C. Giulivi and K.J.A. Davies (1990) Superoxide dismutase undergoes proteolysis and fragmentation following oxidative modification and inactivation. *Journal of Biological Chemistry*, **265**, 11919-11927.
21. G. Del Boccio, C. Di Ilio, P. Alin, H. Jornvall and B. Mannervik (1987) Identification of a novel glutathione S-transferase in human skin homologous with class alpha glutathione transferase 2-2 in the rat, *Biochemical Journal*, **244**, 21-25.
22. R.C. Strange, A.A. Fryer, B. Matharoo, L. Zhao, J. Broome, D. Campbell, P. Jones, I.C. Pastor and V.P. Singh (1992) The glutathione S-transferases: comparison of isoenzyme expression in normal and astrocytoma brain. *Biochimica et Biophysica Acta*, **1139**, 222-228.
23. J. Le and J. Vilcek (1987) Tumour necrosis factor and interleukin 1: cytokines with multiple overlapping biological activities. *Laboratory Investigation*, **56**, 234-248.
24. Y. Groner, O. Elroy-Stein, K.B. Avraham, Y. Yarom, M. Shickler, H. Knobler and G. Rotman (1990) Down syndrome clinical symptoms are manifested in transfected cells and transgenic mice overexpressing the human Cu/Zn-superoxide dismutase gene. *Journal of Physiology (Paris)*, **84**, 53-77.

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