A POSSIBLE ROLE FOR GLUTATHIONE TRANSFERASE MU IN SUSCEPTIBILITY TO ASBESTOS RELATED PULMONARY DISEASE

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Summary

Glutathione-S-transferases are Phase II conjugating enzymes that detoxify a variety of electrophilic agents. GST-1, the gene encoding glutathione-S-transferase mu (GST-mu) is polymorphic in the population being deleted in approximately 45% of Caucasians examined to date. This polymorphism was previously reported to be associated with increased susceptibility to smoking-induced lung cancer. We hypothesize that GST-mu deficiency may confer susceptibility to asbestos-induced pulmonary disease, due to a reduced ability of GST-mu deficient cells resident in the lung to detoxify reactive electrophiles associated with asbestos exposure.

In this preliminary study, individuals deficient in GST-mu were more frequently observed in a cohort of workers suffering from non-malignant asbestos-related disease than expected on the basis of the distribution of this genotype in control populations. Although of borderline statistical significance ($p=0.085$) this finding suggests that GST-mu status may be related to susceptibility to asbestos-related pulmonary disease.

Key Words

Asbestos, glutathione-S-transferases, pulmonary disease, susceptibility.

Introduction

While asbestos related disorders are among the most well studied occupational diseases, relatively little is known about host determinants which may affect individual susceptibility to these conditions.

A better understanding of such factors may ultimately lead to improved screening programs to identify individuals at risk of developing pulmonary problems as a result of previous asbestos exposures, may shed light on the mechanisms of asbestos toxicity and may suggest new avenues for therapeutic interventions to ameliorate or prevent the development of asbestos related disease.

The mechanism whereby asbestos leads to pulmonary fibrosis and lung cancers is a matter of debate. One theory implicates asbestos-related oxygen free radicals in the pathogenesis of these diseases (1-5). Alternatively, asbestos fibers may serve to carry other exogenous toxins into pulmonary cells, perhaps enhancing their ability to interact with critical cellular targets (6). The cytotoxic and genotoxic damage caused by electrophilic compounds which result from oxygen free radical generation and/or the metabolism of certain asbestos associated exogenous compounds (e.g., PAH's derived from cigarette smoke) may be involved in the pathogenesis of asbestos induced pulmonary fibrosis and tumorigenesis.

Because many reactive electrophiles are susceptible to glutathione mediated detoxification reactions (7,8), we investigated whether glutathione-S-transferase mu (GST-mu) activity, which is known to be polymorphic in humans, is associated with asbestos induced lung disease. GST-mu is deficient in a high percentage of Caucasians (9-12, 16) and this deficiency has previously been reported to be associated with increased sensitivity to smoking induced lung cancers (9,10). GST-mu deficiency is also associated with increased sensitivity to the induction of genetic damage by mutagens that are known substrates for this enzyme (13). The results of the current study provide preliminary evidence which suggests that individuals deficient in this enzyme may also be susceptible to the development of asbestos related non-malignant pulmonary disease.

Materials and methods

Study population. The study cohort consisted of 87 adult workers, either self-presenting or referred by their primary physicians to the Center for Occupational and Environmental Medicine at the Massachusetts Respiratory Hospital, Braintree, MA. Smoking, medical and occupational histories were recorded. Most subjects had asbestos related diseases (ARD), however, several individuals

without ARD were included to ensure that genotypic analyses were always done blindly.

Diagnostic protocol and criteria. Primary disease classifications included asbestos related pulmonary disease (ARPD) and asbestosis. Diagnoses were based upon physical examination, pulmonary function tests (PFTs), and chest radiography (CXR), all blind as to GST-mu status. Criteria for diagnosis of asbestosis included the presence of interstitial changes on CXR (using the International Labor Office classification, radiographs rated 1/0 or higher), evidence of restrictive changes on PFTs, and/or clinical findings consistent with interstitial disease. The finding of rales on chest auscultation in the absence of other evidence of interstitial disease was not sufficient criteria for the diagnosis of asbestosis.

Exposure classification. As no air or personal sampling data were available, a rough asbestos exposure index, adapted from a protocol previously described (14), was constructed. Briefly, all subjects provided their previous employment histories. Prior to data analysis, all occupations listed were classified as involving either no, low, or high asbestos exposures. These were assigned weights of 0, 1 and 5, respectively. To account for temporal changes in asbestos use, pre-1965 exposures were assigned a relative exposure value of 4, 1972-75 a value of 2, and those occurring after 1972 a value of 1. Summary exposure scores were obtained by multiplying job classification weights by the appropriate temporal weights and summing for all years worked.

GST-mu assay. GST-mu assays were performed, in duplicate, on approximately 2.5 x 10^6 sonicated lymphocytes using radiolabelled trans-stilbene oxide (TSO) (final specific activity 1.5-3 μCi/μM) (Chemsyn Science Laboratories, Lenexa, KA) essentially as previously described (13). Controls, consisting of boiled samples, were included with each assay batch. Background levels ranged from 10-15% of those obtained with samples form GST-mu active individuals. Enzymatic activity was expressed as pmol conjugate formed/min/10^7 cells.

GST-mu polymerase chain reaction (PCR) based assay. A PCR based assay has been developed which allows for the detection of the gene (GST-1) encoding GST-mu activity. This assay is a modifica-

tion of that described by Comstock, et al. (15). Briefly, using appropriate primers, a 273 base pair fragment is amplified from carriers of GST-1. Since the poor metabolizer phenotype is due to a deletion of GST-1 no fragment is amplified from individuals expressing this phenotype. Amplifications were performed on sonicated samples equivalent to approximately 50,000 lymphocytes using TAQ DNA polymerase (Perkins Elmer Cetus, Norwalk, CN). Controls, consisting of known GST-mu positive and GST-mu negative individuals (previously determined by enzymatic analysis), were included in each run.

Statistical analysis. GST-mu status was compared between asbestos related disease groups and control populations (including historical controls) using Chi square (c^2) analysis. Asbestos exposure and smoking histories were compared by GST-mu status by Wilcoxon Rank Sum analysis using the Krushal-Wallis Test (c^2 approximation). Analyses were performed using an established computer software program (Statistical Analysis Software, 604 SAS Institute, Cary, NC).

Results

Study population. 77 Individuals with asbestos related disease and 10 individuals with other pulmonary diseases were enrolled in this study. Summary statistics of the population are presented in Table 1. Disease subjects included 40 individuals (52%) with pleural disease and 37 (48%) with asbestosis. The remaining 10 members of the cohort (11%) had pneumonia, emphysema, or other respiratory complaints with no history of asbestos exposure or asbestos related disease. The distribution of smoking (pack/years) and asbestos exposure (qualitative exposure scores) were compared between GST-mu subgroups using the Wilcoxon Rank Sum test. No significant differences in either smoking ($p = 0.65$) or exposure scores ($p = 0.93$) were observed.

Glutathione transferase-mu status. GST-mu phenotype was determined biochemically, using the TSO-glutathione conjugation assay, on the first 57 individuals enrolled in the study. The GST activities observed in this group ranged from 0-1,864 pmoles product/min/10^7 cells. Twenty-eight exhibited activities of less than 75 pmoles product/minute/10^7 cells, a value within the experimental

error of this assay, and twenty-eight had activities of greater than 200, with only one falling between these values.

In order to compare GST phenotype to genotype, the PCR based assay was employed to determine the presence or absence of the GST-1 gene in all members of the study group. Figure 1 presents a photograph of a representative agarose gel, stained with ethidium bromide, displaying the diagnostic 273 bp PCR generated GST-1 fragment for several individuals previously phenotyped. Excellent overall agreement was observed between the phenotypic and genotypic analyses, with only two discrepancies out the 57 individuals assayed both ways. These two individuals were dropped from the subsequent analysis.

GST-mu status and asbestos disease. Several groups have looked at the distribution of GST-mu within various populations using techniques similar to those relied upon in this investigation. In these studies the prevalence of GST-mu positive (non-deleted) individuals was observed to range from 43% to 59% (Table 2). In a second cohort of Caucasians previously studied in this laboratory, the prevalence of GST-mu positive was 53% (13). In the current study 6 out of the 10 individuals who did not suffer from asbestos related disease were GST-mu positive.

Among those exhibiting asbestos related pulmonary disease in this study, 44 (57%) were GST-mu negative. Although not statistically significant comparisons of the prevalence of GST-mu in this cohort with various control groups (Table 3) suggest that the GST-mu deletion may be over represented among those with asbestos related disease; the prevalence of GST-mu was lower in this cohort than in the entire historical control population presented in Table 2 ($p=0.085$); from that of U.S. historical controls only ($p= 0.147$); and from that of intra-lab controls ($p = 0.187$). No difference in GST-mu status was observed between individuals exhibiting asbestos pleural disease only vs. those with parenchymal asbestosis ($p = 0.87$) (Table 3).

Discussion

Factors which might play important roles in individual susceptibility to asbestos-induced disease include nutritional status, smoking history, and genetic variability in host defense mechanisms. We

have begun to investigate this latter possibility, focusing our initial efforts on glutathione-S-transferase mu, one of the Phase II conjugating enzymes. Glutathione transferases are a complex group of enzymes that mediate a conjugation reaction between an electrophilic substrate and reduced glutathione (7,8). Although GST mediated conjugations can lead to the activation of some compounds, notably the low molecular weight dihaloalkanes, most such reactions lead to a reduction in cytotoxicity and genotoxicity (7,8).

Many Caucasians are deficient in GST-mu type enzymatic activity (9-12). This deficiency appears to result, in most cases, from a homozygous deletion of the GST-1 locus, which encodes GST-mu (17). Individuals deficient in GST-mu have been reported to be at increased risk for smoking-induced lung cancer (9,10) and exhibit an increased sensitivity of peripheral lymphocytes to genotoxicity mediated by trans-stilbene oxide and cigarette smoke (13).

The results presented here suggest that GST-mu status may also be associated with increased susceptibility to asbestos induced lung disease. Individuals genetically deficient in this Phase II detoxifying enzyme were more frequently observed in a cohort of workers suffering from asbestos related interstitial fibrosis and pleural plaques than expected on the basis of the distribution of this genotype in the general population (Tables 2 and 3). Historical controls were used in this analysis because few non-diseased individuals were seen at the enrolling clinic. We believe that the comparison with historical controls is valid since the prevalence of the GST-mu negative phenotype has been quite consistent in previous investigations (Table 2). In fact, it is likely that the historical control populations included at least some individuals suffering from asbestos related disease. This would tend to bias the results from this analysis toward the null hypotheses. None-the-less, we cannot rule out the possibility that the observed effect is due to uncontrolled differences in the populations compared. Confirmation awaits the analysis of a larger cohort of asbestos exposed individuals, which is underway.

The over representation of GST-mu negative individuals among those with ARD did not appear to be due to differences in asbestos exposure or smoking histories. This allows for the possibility that a causal relationship may exist between GST status and ARD. This possibility is consistent with proposed mechanisms of asbestos

toxicity. Reactive electrophilic products resulting from free radical generation or Phase I metabolism of exogenous compounds associated with asbestos fibers (e.g., cigarette smoke, PAHs from ambient air pollution) are likely to be susceptible to Phase II enzymatic conjugation reactions (7,8,18). The cytotoxic damage caused by these reactive electrophiles may, in part, be responsible for asbestos induced pulmonary fibrosis. These products may also be genotoxic, raising the interesting possibility that GST-mu status might be a determinant of susceptibility to asbestos induced lung cancer. Notably, asbestos exposure was not controlled for in the studies reporting an association between GST-mu status and sensitivity to smoking related lung cancer (9,10). It is very likely that the populations studied in these investigations included cases of asbestos related lung cancer. Thus, it will be of great interest to confirm the association suggested herein and to investigate whether it applies to asbestos induced lung cancer as well.

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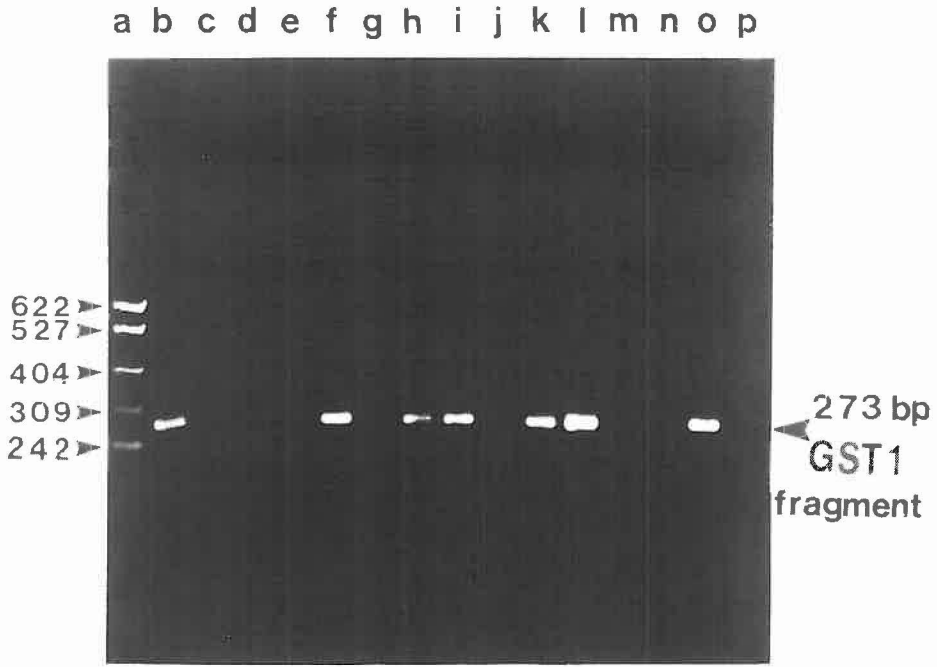

Figure 1. GST1 Genotypic Analysis of 14 Subjects Using a PCR Based Assay. Lane a, MSP1 digested pBR322 DNA (fragment sizes, in base pairs, are indicated to the left); lanes b-p, PCR amplified samples with diagnostic 273 base pair fragment indicated to the right. Lane b, subject 1 (576); lane c, subject 2 (120), lane d, subject 3 (0), lane e, subject 4 (ND); lane f, subject 5 (868); lane g, subject 6 (0), lane h, subject 7 (456), lane i, subject 8 (868); lane j, subject 9 (ND); lane k, subject 10 (ND), lane l, subject 11 (736), lane m, subject 12 (ND); lane n, subject 13 (0); lane o, subject 14 (776), lane p, blank (no cells added). Values in parentheses are GST1 enzymatic activities in pmoles TSO conjugate/min/10 million cells. ND = not determined.

Table 1. Demographics of the study population

N	87
Age	
Mean (yrs)	66 ± 10[1]
Range	42 - 91
GST-1 Status:	
Not deleted (positive)	39
Deleted (negative)	48
Asbestos Exposure	
Mean Exposure Score	67 ± 44[1]
Range	0 - 185
Smoking Status:	
Ever Smoked	68
Never Smoked	17
Unknown	2
Mean Pack-years	40 ± 27
Gender	
Male	86
Female	1

[1]Standard Deviation

Table 2. The prevalence of GST-mu phenotype or genotype in various control populations

	Wiencke 1990 (ref 13)	Seidegard 1990 (ref 9)	Bell 1992 (ref 16)	Smith 1992 (current work)	Heckbert 1992 (ref 19)	Van Poppel 1992 (ref 11)	Zhong 1991 (ref 12)	Seidegard 1986 (ref 10)	Hussey 1987 (ref 20)	Seidegard 1985 (ref 21)
GST-mu positive	24 (53%)	66 (58%)	59 (55%)	6 (60%)	51 (43%)	120 (55%)	131 (58%)	46 (59%)	23 (55%)	115 (46%)
GST-mu negative	21	48	49	4	69	100	94	32	19	133
Population Characteristics										
Nationality	USA	USA	USA	USA	USA	Netherlands	UK	Sweden	UK	Sweden
Mean Age (years)	39 ± 11	60 ± 3	NA	56 ± 9	64	38 ± 9.6	NA	63.5 ± 7.4	NA	NA
Gender (% Male)	42	47	NA	100	NA	100	NA	94	48	NA

NA = not available

Table 3. Comparison of the distribution of GST-1 status by study population

	All ARD[1,2]	Pleural[2] Disease Only	Asbestosis[2]	All Historical Controls	US Historical Controls	Historical Controls from this lab
GST-mu positive[3]	33[4]	18	15	641	206	30
GST-mu negative	44	22	22	569	191	25

[1]Asbestos-related disease
[2]This study
[3]Determined enzymatically and/or with PCR
[4]Number of individuals

Mantel-Haenszel Chi-Square Test Statistics
Pleural Disease Only Compared to Asbestosis; Chi-Square = 0.03; p = 0.87
All Controls (Table 2 all) Compared to ARD; Chi-Square = 2.97; p = 0.085
US Controls (Table 2) Compared to ARD; Chi-Square = 2.105 p = 0.147
Lab Controls (Table 2) Compared to ARD; Chi-Square = 1.744; p = 0.187

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