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Serum Oncogene Proteins in Hazardous-Waste Workers

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Summary

Oncogene protein detection may be a valuable molecular epidemiological marker of commitment to the carcinogenic pathway in worker populations exposed to carcinogens. This paper reports the use of serum immunoblotting with monoclonal antibodies to oncogene proteins to screen hazardous waste workers with potential carcinogen exposure who employ state-of-the-art work practices and personal protective equipment to minimize real exposures. Two individuals in this cohort were found to be trace positive for the *ras* oncogene protein in their serum, but this may have been due to their cigarette smoking. These results are contrasted to previous results in hazardous waste workers with real workplace carcinogen exposures.

Introduction

The detection of activated oncogenes may play a major role in the molecular epidemiological study of cancer evolution in populations exposed to carcinogens in the workplace (Brandt-Rauf, 1988). We have recently reported the use of a newly developed immunoblotting technique (Niman et al., 1985) to detect oncogene-encoded proteins in the serum of individuals presumed to be at risk for the development of cancer due to their workplace exposure to hazardous waste containing carcinogens (Brandt-Rauf and Niman, 1988). In this study, a cohort of sixteen clinically healthy hazardous waste workers were found to have long histories of exposure to multiple carcinogens including asbestos, polychlorinated biphenyls and other chlorinated hydrocarbons; although precise quantitation of exposures was not possible, based on the histories of work practices and limited protective equipment use, it was presumed that this cohort had suffered significant occupational carcinogen exposure. In addition, 75 per cent of these individuals were cigarette smokers. Examination of their serum by immunoblotting with monoclonal antibodies to the peptide sequences of nine different oncogenes revealed detectable oncogene protein levels in half of the individuals (Brandt-Rauf and Niman, 1988). In order to evaluate further the significance of these findings, particularly in terms of a relationship between occupational exposure to carcinogens and serum oncogene protein expression, the same technique has been applied to screen the serum of another cohort of hazardous waste workers of similar sex, race and age distribution but for whom potential exposures had been minimized due to appropriate work practices and appropriate use of personal protective equipment.

Methods

A cohort of 17 workers was selected from the employees of a state environmental agency who were involved in the clean-up of various hazardous waste sites in the region. These workers were of similar age, sex, and race distribution (see *Table I*) and involved in clean-up operations with similar potential exposures as the previously described cohort (Brandt-Rauf and Niman, 1988), however, they were judged to have had much less actual exposure. Whenever in the field dealing with hazardous wastes, appropriate work practices and personal protective equipment were employed to ensure minimal carcinogen or other toxin exposure. This cohort therefore represented a model control group for the study of oncogene protein expression in comparison to those with significant carcinogen exposure.

Blood samples were collected from each of these individuals and the serum was separated and stored frozen at −70°C until analysed. These samples were

Table I. Comparison of exposed and protected cohorts of hazardous waste workers

	Exposed	*Protected*
No. of individuals	16	17
Average age, years	42	40
Sex, per cent male	100	100
Race, per cent white	82	82
Average cigarette smoking, pack-years	13·3	7·5
Serum oncogene proteins, no. of positive individuals		
fes	6	0
ras	3	2*
sis	1	0

*Both of these individuals were only trace positive and both were cigarette smokers.

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analysed for the presence of protein products encoded by nine different oncogenes (*ras, fes, sis, int*-1, β-TGF, *myb, src, myc, mos*) by the immunoblotting technique as previously described (Niman et al., 1985; Brandt-Rauf and Niman, 1988). Briefly, for this assay, 100 μl serum are mixed with 400 μl phosphate-buffered saline at pH 7·4, 25 μl 2-mercaptoethanol, and 475 μl sample buffer in deionized water (6·25 per cent sodium dodecyl sulphate, 6·25 per cent glycerol), and placed in a boiling water bath for 5 min. Samples are then loaded on a 5–17 per cent polyacrylamide gel and electrophoretically separated and transferred to nitrocellulose. After blocking with phosphate-buffered saline containing 3 per cent bovine serum albumin and 0·1 per cent Triton X-100, the nitrocellulose is incubated overnight at 4°C with the primary monoclonal antibody (ascites fluid diluted 1:2000). After exhaustive washing, the location of the oncogene protein-antibody bands on the nitrocellulose is determined colorimetrically using a secondary anti-mouse immunoglobulin G antibody and an avidin–biotin–peroxidase complex (Vectastain; Vector Labs, Burlingame, California, USA). Approximate quantification of the degree of elevated expression of the oncogene proteins in each case is achieved by serial dilution of the samples until no difference from normal samples is detectable.

Results and Discussion

The results for this cohort are presented in *Table I* in comparison to the previous results on corresponding workers with significant carcinogen exposure. As noted, the present cohort was similar in terms of age, sex, race and job to the prior cohort but had less exposure to cigarette smoke and less real exposure to workplace carcinogens. Potential carcinogen exposures in both cohorts were quite diverse, but, unfortunately, precise quantitative data on actual levels of ambient exposure of the cohorts are not available. Although half of the prior cohort was positive for at least one serum oncogene protein (*ras, fes* or *sis*), in the present case, only trace positive bands (less than two-fold elevation) for the *ras* proteins were detected in two individuals and no positive bands for the proteins of any of the other eight oncogene proteins were detected in any of the specimens. The two positive individuals in this cohort were remarkable only for their relatively heavy cigarette consumption (14 pack-years and 20 pack-years, respectively). These results would tend to suggest that the prior positive findings were indeed related to real carcinogen exposures and that the current cohort, by minimizing their exposures to the hazardous wastes dealt with, had managed to avoid activation of oncogenes.

It is interesting to note that most of the prior positive results were for the *fes* and *ras* oncogenes and the two current positive results were for the *ras* oncogene and that most of the exposures in both cases were presumably by the respiratory route; in addition, in the prior cohort most of the positive individuals were cigarette smokers (7 out of 8) and only 1 out of 4 non-smokers was positive, and, as noted, in the current cohort both positives were smokers. *Ras* gene activation has been found frequently in human lung cancers, particularly of the non-small-cell variety (Kurzrock et al., 1986; Rodenhuis et al., 1987). In our own studies of serum oncogene protein levels in non-small-cell lung cancer patients, 15 out of 18 patients had increased expression of the *ras* gene product and all of these individuals were cigarette smokers (Brandt-Rauf et al., 1989). In another study of tissue from lung cancer patients, 50 per cent of adenocarcinomas (5 out of 10) had an activated *ras* gene; two of these tumours were less than 2 cm in size and had not yet metastasized, and, thus, the authors concluded that *ras* gene activation may be an important early event in the pathogenesis of adenocarcinoma of the lung (Rodenhuis et al., 1987). Furthermore, all five patients with *ras*-positive adenocarcinomas in this study were heavy cigarette smokers, whereas, two of the patients with *ras*-negative adenocarcinomas had never smoked and a third had stopped smoking 13 years prior to the development of his lung cancer; this provides additional support for a possible smoking-related link to particular oncogene activation (in this case *ras*) and the development of lung cancer (Rodenhuis et al., 1987). This would also be consistent with the results of studies *in vitro* showing that exposure of cells to one of the major known carcinogenic constituents of cigarette smoke, benzo(a)pyrene, can produce activation of the *ras* oncogene (Marshall et al., 1984). The *fes* gene has also been found to be expressed in human lung cancer tissue (Slamon et al., 1984). In our study of serum oncogene protein levels in lung cancer patients, 11 out of 18 were positive for *fes* products and all of these individuals were cigarette smokers (Brandt-Rauf et al., 1989). Furthermore, it is known that during development, the protein product of the *fes* gene family is expressed in a very limited tissue-specific fashion, primarily in the bone marrow, liver and lung (Pimentel, 1986; Mathey-Prevot et al., 1982). Thus in pulmonary carcinogenesis, the expression of the *fes* gene product may correspond to a process of pulmonary cellular de-differentiation. Therefore, the expression of *ras* and *fes* gene products in workers with respiratory carcinogen exposure, particularly if they are also cigarette smokers, may be indicative of a commitment of pulmonary cells to the oncogenic pathway.

It is tempting to speculate that the oncogene protein positive individuals in these cohorts are the ones most at risk for the development of malignancy, particularly cancer of the lung. The two positive individuals in the present cohort may be at risk for the development of malignancy, particularly lung cancer, primarily due to their relatively heavy cigarette smoking history which was the only distinguishing characteristic of these two individuals in this cohort. The other negative results in the present cohort may attest to the reduction of carcinogen dose in this group due to, among other things the adequacy of work practices and use of personal protective equipment to minimize exposures. Obviously, long-term follow-up of these cohorts will be necessary before any definitive statements about the predictive value of serum oncogene proteins in cancer prevention can be made. However, it should be noted that one of the oncogene protein positive individuals in the prior cohort has already gone on to develop a potentially pre-malignant lesion. In this case, one of the *ras* positive workers with a particularly long history of asbestos exposure developed rectal bleeding and was found to have a tubulo-villous adenoma of the colon. Asbestos exposure is thought to be associated with colonic neoplasia (Wylie et al., 1987), and colonic adenomas are known to be capable of over-expression of the *ras* gene (Spandidos and Kerr, 1984). Furthermore, upon removal of the

adenoma, the individual's serum oncogene protein expression reverted to normal. Thus, this technique of serum oncogene protein detection may prove to be a valuable molecular epidemiological marker for the development of cancer in individuals environmentally or occupationally exposed to carcinogens.

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