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# Serum growth factors and oncoproteins in firefighters

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Firefighters are potentially at increased risk for cancer and non-malignant respiratory disease due to their toxic exposures on the job. Growth factors and oncogene proteins are thought to play a role in the development of various malignancies and pulmonary fibrotic diseases. Therefore, a cohort of firefighters and matched controls have been screened for the presence of nine different growth factors and oncoproteins using an immunoblotting assay. Fourteen of the firefighters were found to be positive for β-transforming growth factor (β-TGF) related proteins compared to no positives in the controls ($P = 0.0017$). These results suggest that β-TGF may be a possible biomarker for monitoring firefighters and other exposed workers for the potential development of cancer or non-malignant respiratory disease.

## INTRODUCTION

With the increasing use of synthetic chemical-based materials in building construction, there has been growing concern over the health hazards to firefighters from inhalation of the toxic components of smoke generated from the burning of these materials as well as from the presence of other hazardous building materials such as asbestos[1]. We have previously demonstrated that firefighters are routinely exposed to significant ambient concentrations of a wide range of toxic materials[2] and that they suffer significant acute physiological effects (such as decrements in pulmonary function) as a result of these exposures[3]. Furthermore, morbidity and mortality studies of firefighters have raised the possibility of their increased risk of chronic respiratory disease and cancers of various sites which may be related to their exposure to the toxic components of smoke[1].

In order to investigate further the potential adverse health effects of toxic exposures in firefighters in relation to their risk of cancer and chronic respiratory disease, we have examined the levels of various oncogene proteins and growth factors in the serum of firefighters as compared to unexposed controls. Various oncogene proteins and growth factors have been implicated in the development of human cancers, and certain growth factors have also been implicated in the development of pulmonary fibrosis due to exogenous agents[4,5]. Thus, these proteins may serve as useful biomarkers for the risk of development of cancer and/or non-malignant respiratory disease in cohorts of exposed workers.

## MATERIALS AND METHODS

A cohort of 226 New York City firefighters were enrolled in a medical surveillance programme at Mount Sinai Medical Center. The programme included complete medical and occupational histories, physical examinations, pulmonary function tests and chest radiographs. The programme was undertaken to evaluate the prevalence of pleural and pulmonary fibrosis due to asbestos exposure among long-term firefighters. It was found that 14 per cent of those firefighters with no known exposure to asbestos demonstrated radiographic evidence of asbestos exposure, a rate that was considerably higher than expected based on chest X-rays of the general population[6]. Since asbestos is a known cause of both malignant (lung cancer, mesothelioma) and non-malignant (asbestosis) respiratory disease, this cohort of firefighters was considered to be good candidates for the study of expression of growth factors and oncoproteins as possible biomarkers of malignant and non-malignant

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respiratory disease. As an initial attempt to investigate this possibility (and limited by cost constraints), 33 of these firefighters were selected for study. The 33 firefighters studied were a consecutive series drawn from the cohort who had agreed to participate in the medical screening. These 33 volunteers had no prior history of cancer and were representative of the entire firefighter cohort, and, in particular, had a similar prevalence of radiographic evidence of pulmonary disease (i.e., 6 out of 33 had X-ray abnormalities characteristic of asbestos exposure). Blood samples were obtained from these volunteers by routine venipuncture, the serum was separated by centrifugation and stored at −70°C until the time of analysis. A comparison group of 16 individuals was recruited from healthy medical centre volunteers (office workers and medical staff with no known toxic exposures) matched to the firefighters (approximtely 1:2) on age (±5 years), smoking status (smoker vs. non-smoker), race and sex. Lack of older smokers in the comparison group (as well as cost constraints) limited the number of subjects in this group. Histories and blood samples were obtained as above.

The serum samples were assayed simultaneously and blind for the presence of two different growth factors, β-transforming growth factor (β-TGF) and platelet derived growth factor (PDGF), and seven oncogene proteins (*ras, fes, myc, myb, mos, src, int*) by the immunoblotting technique as previously described[7–9]. Briefly, for this assay, 100 μl serum are mixed with 400 μl phosphate buffered saline (PBS), 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 minutes. Samples are then loaded on a 5–17 per cent polyacrylamide gel and electrophoretically separated and transferred to nitrocellulose. After blocking with PBS containing 3 per cent bovine serum albumin and 0.1 per cent Triton X-100, the nitrocellulose is incubated overnight at 4°C with monoclonal antibodies directed against synthetic peptides representing predicted growth factor and oncogene sequences (ascites fluid diluted 1:2000). After exhaustive washing, the location of the bands on the nitrocellulose is determined colorimetrically using a secondary anti-mouse IgG antibody and an avidin–biotin–peroxidase complex (Vectastain; Vector Labs, Burlingame, California, USA). Approximate quantification of positive results is achieved by serial dilution of samples until no difference from normal is detectable; on the basis of prior studies in cancer patients, an a priori criterion for positivity was selected to be a fivefold increase in protein expression. The primary antibodies used were directed against the following protein sequences: β-TGF (hybrid 100-30C05 and hybrid 100-34E06, sequence ALDTNYCFSSTEKNC); PDGF (hybrid 112-09B10, sequence SLGSLTIAEPAMIAEC); *fes* (hybrid 127-42C11 and 127-50D04, sequence LMEQCWAYEPGQRPSF, and hybrid 121-14C09, sequence IGRGNFGEVFSGL(C)); *int* (hybrid 222-35C08 and hybrid 222-37F04, sequence LHNNEAGRTTVFS(C)); *myb* (hybrid 133-10F06, sequence LGEHHCTPSPPVDHG); *src* (hybrid 203-07D10, sequence (C)GSSKSKPKDPSQRRHS); *myc* (hybrids 155-11C07, 155-08G01 and 155-09F06, sequence CSTSSLYLODLSAAASEC); *mos* (hybrid 165-35F02, sequence LGSGGFGSVYKA(C)); *ras* (hybrid 142-24E05, sequence YREQIKRVKDSDDVPMVLVGNKC and hybrid 143-03E04 sequence YTLVREIRQHKLRKLNPPDESGPGC). In this immunoblotting system, these antibodies have been found to give specific, sensitive and reproducible results. The specificity of the antibodies has been demonstrated by the blocking of the activity by preincubation with the specific peptide for the protein of interest and the failure of blocking of the activity with peptides of other proteins. The assay system is capable of detecting proteins in the nanogram range and is found to give reproducible results when repeated on the same sample.

Statistical significance of differences between the groups was determined using the Fisher exact test because of the small numbers involved, and a $P < 0.05$ was considered significant. Because for the serum tests nine independent markers were determined, it was necessary to adjust for multiple comparisons, since there is a probability of $(1-0.95^9) = 0.37$ that one or more of these 9 tests will be significant at the 0.05 level purely because of sampling variation; a conservative adjustment (the 'Bonferroni correction')[10] was employed by dividing the α-level by the number of tests (9) to obtain an adjusted α-level of 0.0056. In addition, for positive findings, the strength of the association (i.e., the degree to which being a firefighter increases the risk of having a five-fold elevation of marker) was determined by the prevalence odds ratio (POR) with 95 per cent and 99 per cent confidence intervals.

## RESULTS

The results are presented in *Table 1*. The firefighters and unexposed controls are seen to be similar in terms of age, sex, race and smoking status. In terms of serum growth factors or oncogene proteins, no positive bands for eight of the proteins (PDGF, *ras, fes, myc, myb, mos, src, int*) were detectable in any of the specimens. However, fourteen of the firefighters were positive for β-TGF related proteins, whereas, none of the unexposed controls was positive. The β-TGF positive firefighters were quite similar to the firefighters who were negative in terms of age (average = 55 years for both groups)

**Table 1.** Comparison of firefighters and unexposed controls

| | *Firefighters* | *Controls* |
|---|---|---|
| Number | 33 | 16 |
| Age, years | 55 | 54 |
| Sex, per cent male | 100 | 100 |
| Race, per cent white | 100 | 100 |
| Cigarette smoking, pack-years | 26 | 21 |
| Serum proteins, number positive | | |
| β-TGF | 14 | 0 |
| all others | 0 | 0 |

and time firefighting (average = 31 years for both groups); positives were slightly more likely than negatives to have abnormal chest X-rays (21 vs. 16 per cent) and a prior history of asbestos exposure (36 vs. 26 per cent), but positives were slightly less likely than negatives to complain of pulmonary symptomatology (36 vs. 42 per cent) and had lower cigarette smoking exposure (71 per cent smokers vs. 95 per cent smokers; 19 pack-years vs. 32 pack-years). Nevertheless, none of these differences was statistically significant.

However, the finding of 14/33 β-TGF positive firefighters compared to 0/16 controls was statistically significant. Fisher's exact test gives a 2-sided *P*-value of 0.0017 compared to an adjusted α-level of 0.0056. This result is thus unlikely to be due to sampling variation or multiple comparisons. In addition, in this case, the point estimate of the POR is infinite with an exact 95 per cent confidence interval of (2.9, ∞) and an exact 99 per cent confidence interval of (1.7, ∞). Thus, we can be 99 per cent confident that the true POR is 1.7 or larger, suggesting a moderate to strong association between firefighting and an elevated level of β-TGF.

## DISCUSSION

The statistically significant finding of such a high percentage of firefighters with abnormal expression of particular growth factor related proteins in their serum compared to negative results in otherwise similar, unexposed, healthy controls raises the possibility that this growth factor may be related to pathological processes induced by firefighting exposures. As noted previously, firefighters are known to be routinely exposed to a whole host of respiratory toxins and carcinogens that may contribute to an increased incidence of malignancies and non-malignant respiratory disease. Furthermore, β-TGF may be implicated in both types of processes.

The active form of β-TGF is a homodimeric peptide of molecular weight 25 kDa with interchain disulphide linkages in which each chain contains 112 amino acids and has a molecular weight of 12.5 kDa; β-TGF is actually synthesized as a much larger molecule and the monomeric forms are derived proteolytically from these larger precursor polypeptides which may be secreted, which may explain the range of molecular weight bands identified in serum. The biological activity of β-TGF was originally defined in terms of its ability to induce fibroblasts to express a transformed phenotype and stimulate anchorage-independent growth. However, β-TGF has since been shown to have an effect on nearly every tissue and cell type. β-TGF apparently normally plays essential roles in embryogenesis, particularly during periods of morphogenesis, and some of these same β-TGF-derived embryological mechanisms can be reiterated in the adult during the normal processes of tissue remodelling and repair as well as aberrantly in various pathological processes, including possibly carcinogenesis and pulmonary fibrosis[4,5,11].

The action of β-TGF on cells is actually bifunctional; in certain instances as noted, β-TGF stimulates anchorage-independent growth (oncogenic), but in others, it acts as a growth inhibitor (anti-oncogenic). When functioning oncogenically, it may act in concert with other growth factors such as PDGF or oncogenes such as *ras* and *myc*[5]. Nevertheless, it seems clear that under the appropriate conditions, β-TGF is somehow intimately related to the carcinogenic process. For example, β-TGF has been found to be over-expressed in many different types of human tumour cell lines and human cancers, including lung cancer, stomach cancer, breast cancer, renal cancer, brain cancer, prostate cancer, hepatoma, melanoma and haematopoietic malignancies[12]. Evidence suggests that firefighters may be at increased risk for many of these types of cancer[1]. In addition, it seems possible that chemical carcinogens to which firefighters are exposed are capable of producing cell transformation accompanied by over-expression of β-TGF. For example, mouse embryo cells in culture that have been chemically transformed by exposure to polycyclic aromatic hydrocarbons (PAHs) have been shown to over-express transforming growth factors that appear identical to β-TGF[13]. As noted previously, firefighters are known to be exposed to a variety of carcinogens including high levels of PAHs[14,15] and to have evidence of biological effects (eg, increased PAH–DNA serum antigenicity) of these exposures[16]. Thus, it is possible that exposure of firefighters in the current cohort to workplace carcinogens has resulted in the production of neoplastic changes as evidenced by the increase in serum β-TGF related peptides. Individuals in this cohort with these elevated levels may thus be at increased risk for the development of work-related cancers.

Alternatively, or in addition, the elevated levels of β-TGF in this cohort may be reflective of some non-malignant pathological process. As noted, firefighters may be at increased risk of non-malignant respiratory disease[17,18] and are known to be exposed to non-carcinogenic respiratory toxins[2] and particularly, in this cohort, there is radiographic evidence of an increase in non-malignant respiratory disease related to asbestos exposure. Asbestos is known to be fibrogenic in the lung, although the exact mechanisms of development of chronic pulmonary fibrosis from asbestos or other causes is unclear. However, it is likely that β-TGF plays a role in human pulmonary fibrosis as indicated by the observation that it is constitutively expressed by alveolar macrophages that will secrete β-TGF upon activation[19]. The secreted β-TGF is a powerful chemoattractant for monocytes[20] and fibroblasts[21]. β-TGF stimulates monocytes to increase production of IL-1, which is a potent fibroblast mitogen[20], and β-TGF directly stimulates the growth of immature fibroblasts[22]. Furthermore, β-TGF has the combined effect of enhancing the synthesis of collagen by lung fibroblasts[23] while suppressing protease secretion[24]. In the rat model of bleomycin-induced pulmonary fibrosis, which closely resembles chronic human fibrotic lung disease, β-TGF was found to be significantly elevated following exposure, particularly in the alveolar interstitium

in areas of increased cellularity and tissue organization, and β-TGF levels correlated with maximum fibroblast collagen synthesis; this temporal and spatial relationship supports the importance of β-TGF in the pathogenesis of exogenously induced pulmonary fibrotic disease[25]. Finally, in studies of humans with pulmonary fibrotic conditions, increased mRNA for β-TGF has been found by *in situ* hybridization in early inflammatory exudates and increased extracellular β-TGF protein has been found by immunohistochemical staining in subsequent fibroblast-rich alveolar buds[26]. Thus, it is possible that elevated levels of β-TGF in this firefighter cohort reflects the early effects of their workplace exposure to respiratory toxins that can induce non-malignant pulmonary disease such as pulmonary fibrosis.

Further follow-up of this and other exposed cohorts will be necessary to confirm these hypotheses. However, these results suggest that the immunological detection of serum β-TGF related proteins may be a potential biomarker for the monitoring of pathological changes related to non-malignant respiratory disease and/or cancer in firefighters and possibly other exposed worker cohorts.

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