SHORT COMMUNICATION

Antibodies to collagen IV in the serum of workers exposed to hydrocarbons and volatile organic chemicals

A. J. Stevenson, H. J. Mason, P. Pai, M. Yaqoob and G. M. Bell

Significant numbers of workers (14%) chronically exposed to volatile organic chemicals commonly found in spray paints had elevated levels of uncharacterized antibodies to collagen IV, a basement membrane protein. No increased frequency of subjects with positive results for anti-glomerular basement membrane antibodies (anti-GBM) was found in this group. These anti-GBM antibodies are directed against a specific epitope on the non-collagenous domain (NC1) of the α3 chain of collagen IV. Anti-GBM antibodies are diagnostic for Goodpasture's syndrome that has been reported to occur following acute inhalation of volatile substances. In another group of workers, exposed both dermally and by inhalation to petroleum-based oil mists, 5% had positive results for anti-GBM antibodies. We conclude that the measurement of general, uncharacterized antibodies to collagen IV may be a useful indicator of basement membrane damage in workers occupationally exposed to volatile organic chemicals.

Keywords: *occupational exposure, basement membrane, collagen IV antibodies.*

Introduction

Immunological mechanisms have been postulated to play a role in the development of renal disease following exposure to hydrocarbons (HCs) and volatile organic chemicals (VOCs) by several authors (Beirne and Brennan 1972, Nelson *et al.* 1990, Roy *et al.* 1991). Perhaps the most well documented case, where immune mechanisms are known to be involved, is that of Goodpasture's syndrome which has been reported to develop following acute inhalation of VOCs. This disease is characterized by the appearance of pathogenic autoantibodies to a specific epitope on the non-collagenous domain (NC1) of the α3 chain of collagen IV, a matrix protein found in lung and glomerular basement membranes (anti-GBM antibodies) (Bombassei and Kaplan 1992). These antibodies may occur following injury to lung or glomerular basement membrane resulting in alterations to basement membrane components and subsequent antibody-mediated disease (Salant 1987).

A. J. Stevenson (*author for correspondence*), **H. J. Mason** *are in the Biomedical Sciences Group, Health and Safety Laboratory, Broad Lane, Sheffield S3 7HQ, UK,* **P. Pai**, **G. M. Bell** *are at the Royal Liverpool University Hospital, Prescot Street, Liverpool, UK; and* **M. Yaqoob** *is at the Royal London Hospital, Whitechapel, London E1 1BB, UK.*

Other less characterized and possibly non-pathogenic antibodies to basement membrane components such as laminin and collagen IV can be produced following chemical exposure. Increased serum concentrations of these less characterized autoantibodies may be useful markers of general basement membrane disturbances in populations exposed to potentially nephrotoxic chemicals.

We report here serum concentrations of general, non-epitope characterized auto-antibodies to collagen IV and the specific antibody to the collagen IV epitope implicated in Goodpasture's syndrome (anti-GBM) in a cross-sectional study of workers occupationally exposed to a range of HCs and VOCs commonly found in spray paints and mineral oils.

POPULATION AND METHODS

Two groups of healthy males occupationally exposed to various HCs and VOCs were studied. Group 1 comprised 111 paint sprayers exposed mainly by inhalation to a range of volatile chemicals including xylene, toluene, butanol, naphthas and glycol ethers. Group 2 comprised 100 transmission shop workers exposed both dermally and by inhalation to HCs in petroleum-based oil mists. The mean duration of employment in these two groups was 21 years and 22 years respectively. Cumulative hydrocarbon exposure scores were estimated for each worker by means of a validated questionnaire (Hotz *et al.* 1989, Yaqoob *et al.* 1993), and were calculated as the product of the appropriate intensity factor and the total number of hours' exposure to hydrocarbons. Atmospheric levels of VOCs and oil mist at the time of this study were within UK permissible limits. The limits at that time were: 100 ppm—xylene; 100 ppm—toluene; 50 ppm—*n*-butyl alcohol; 5 mg m^{-3}—mineral oil mist. Reports on subclinical renal alterations and other basement membrane disturbances in these workers have already been published (Yaqoob *et al.* 1993, Stevenson *et al.* 1995). The control group (Group 3) was made up of 108 males with no known occupational exposure to HCs or VOCs. Some characteristics of the three groups are shown in Table 1. All three groups were comparable in terms of age and numbers of smokers. The two exposed groups were comparable in terms of alcohol consumption, however the paint sprayers had a significantly higher mean exposure score than the transmission shop workers ($p < 0.001$).

Samples were randomized before analysis and quality control samples were run with each batch of samples for each assay. Serum anti-GBM antibodies (to the NC1 region of the α3 chain of collagen IV) were measured using a commercial immunoassay kit which is widely used in the clinical diagnosis and management of Goodpasture's syndrome (BioDiagnostics, UK). Uncharacterized antibodies to collagen IV were measured using an immunoassay developed in-house. Briefly, samples diluted 10-fold in phosphate buffered saline (PBS), containing 0.1% bovine serum albumin, were incubated overnight at 4 °C in microtitre plates previously coated with 1 μg ml^{-1} human collagen IV (Gibco BRL, UK). After four washes in PBS (0.02% Tween) the plates were incubated with a peroxidase-labelled rabbit antibody to human IgG, A, M (Serotec, UK). After washing and incubation with the substrate *ortho*phenylenediamine (OPD) the plates were read kinetically at 450 nm. Units are quoted as the rate of change in absorbance.

The prevalence of increased concentrations of serum GBM and collagen IV antibodies in exposed and control groups was compared using one-tailed Fisher's exact tests. Group means were compared using Dunnet's test on one-way ANOVA. Multiple regression analyses were used to investigate the effect of possible confounding factors such as exposure score, age, smoking or alcohol intake in exposed subjects.

	Paint sprayers $n = 111$	Transmission shop $n = 100$	Controls $n = 108$
Age (years) (mean ± SD)	45 ± 7.7	49 ± 7.0	42 ± 6.4
Current smoker (yes/no)	27/84	23/70	22/32
Alcohol (units per week) (mean ± SD)	21.3 ± 9.0	20.2 ± 10.4	–
Exposure score (geometric mean) (range)	72508 9440–156300	49354 755–108000	–

Table 1. Age (mean, sd), smoking status, alcohol consumption and exposure score (geometric mean, range) data are shown where available.

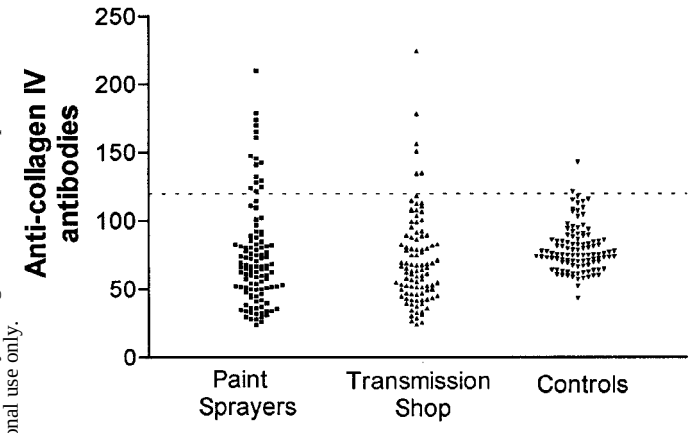

Figure 1. The distribution of anti-collagen IV antibodies in serum is shown for each group. The dotted line indicates the 97.5 percentile derived from the control group.

Results

The 97.5 percentile in the control group was used to define abnormality in the two exposed groups for antibodies to collagen IV. Abnormality for the anti-GBM assay was defined as greater than 20 ELISA units (EU), with 10–20 EU being defined as 'borderline', quoted by the manufacturer of this clinically validated assay. Figures 1 and 2 show the distribution of results in each group. Among subjects exposed to VOCs commonly found in spray paints, 14% had elevated serum concentrations of autoantibodies to collagen IV ($p = 0.0005$). A significant number of subjects in this group also had anti-GBM results defined as 'borderline' by the assay manufacturer (7%, $p = 0.0038$). In the group exposed mainly to petroleum-based oil mists, 5% had serum anti-GBM antibodies greater than 20EU ($p = 0.0244$). In addition, a significantly increased proportion of this group had 'borderline' anti-GBM results (10%, $p = 0.0005$).

Group 1 had a significantly higher HC exposure score than group 2 when compared using two-tailed Student's *t*-test (Table 1). Subjects in group 1 with 'borderline' anti-GBM results had a significantly increased mean HC exposure score than those with normal anti-GBM results (103012 ± 36013) vs

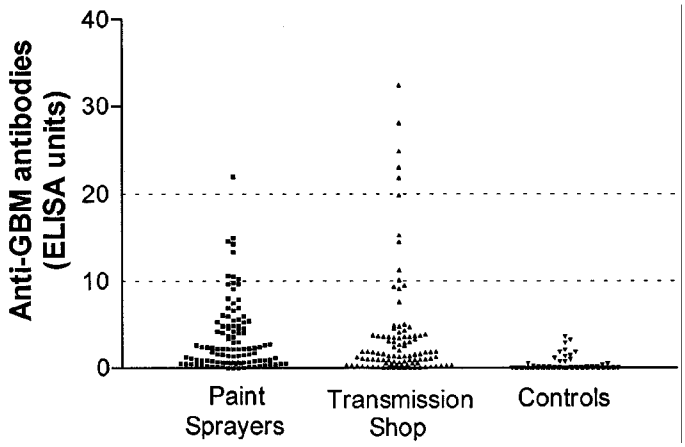

Figure 2. The distribution of anti-GBM antibodies in serum for each group is shown. Dotted lines indicate 'borderline' positive results at 10 ELISA units and positive results at 20 ELISA units as defined by the assay manufacturer.

77050 ± 29563, mean ± SD, $p = 0.0202$), using two-tailed Student's *t*-test.

Following appropriate normalization of data, multiple regression analysis in the exposed subjects was used to investigate the influence of exposure score, age, alcohol intake (units per week) and smoking (pack years) as possible explanatory factors for the abnormalities. No relationship was found between these variables and the level of autoantibodies measured here (data not shown).

Discussion

In a previous study we demonstrated some evidence of endothelial cell damage and basement membrane alterations in the same group of workers exposed to VOCs and HCs studied here (Stevenson *et al.* 1995). This report shows further evidence of basement membrane disturbances in workers exposed to spray paints, indicated by an increased prevalence of subjects with abnormally high serum concentrations of uncharacterized autoantibodies to collagen IV and increased numbers of subjects with 'borderline' anti-GBM antibody results in the same group. This pattern of damage appears distinct to that found in workers exposed to petroleum-based oil mists where increased numbers of subjects with abnormally high serum levels of anti-GBM antibodies to the specific collagen IV epitope implicated in Goodpasture's syndrome, were found.

Bombassei *et al.* (1992) have recently reviewed the literature on the association between HC/VOC exposure and anti-GBM mediated renal disease and suggested a causal relationship between exposure to HCs/VOCs and development of Goodpasture's syndrome. A recent study of chronic perchloroethylene exposure in dry cleaners by Mutti *et al.* (1992) did not find an increase in numbers of subjects with raised anti-GBM antibodies, although the mean serum anti-GBM level was increased compared with controls. Mutti suggested that this finding may indicate autoimmune glomerular disturbances, following episodes of acute peak

exposure to perchloroethylene, in susceptible individuals. The group of transmission shop workers in our study, where significant numbers of subjects with positive anti-GBM titres were found, had been chronically exposed, both dermally and by inhalation, to oil mists for approximately 22 years. However, higher 'peak' exposures may have occurred during this time resulting in the production of these autoantibodies. It should be noted that workers with any history of clinical renal disease had been excluded from this study.

Chemically-induced vascular damage may expose sub-endothelial basement membranes to the immune system with subsequent formation of autoantibodies to basement membrane components. Increased serum concentrations of uncharacterized antibodies to collagen IV have been reported in clinical studies of patients with scleroderma, Raynaud's disease and systemic vasculitis (Petty *et al.* 1986, Gabrielli *et al.* 1988, Direskeneli *et al.* 1994). In a study by Kefalides *et al.* (1986) autoantibodies to regions of the collagen IV molecule different to that implicated in Goodpasture's syndrome were detected in patients with poststreptococcal glomerulonephritis. Antibodies to laminin were also reported in these patients. Kefalides suggests that, following tissue damage, the autoantibodies produced may be directed against all the integral basement membrane components, rather than a single component or a single epitope on an antigen. Another author suggests that antibodies directed against other subunits of type IV collagen, including the NC1 region of the α1 chain, may be less pathogenic than antibodies against the NC1 of the α3 chain (anti-GBM) (Johansson *et al.* 1993). Our finding of significantly increased levels of uncharacterized collagen IV-antibodies in the paint sprayers would seem to support this idea. It is possible that the finding of positive or 'borderline' anti-GBM antibody concentration in significant numbers of subjects indicates a transient alteration to basement membranes, perhaps due to an episode of acute chemical exposure, rather than any permanent pathological event.

We would suggest that the measurement of uncharacterized antibodies to collagen IV may be useful in detecting vascular basement membrane effects in VOC exposed workers. However the pathogenicity of these autoantibodies is unclear.

References

Beirne, G. J. and Brennan, J. T. (1972) Glomerulonephritis associated with hydrocarbon solvents. *Archives of Environmental Health*, **25**, 365–369.

Bombassei, G. J. and Kaplan, A. A. (1992) The association between hydrocarbon exposure and anti-glomerular basement membrane antibody mediated disease (Goodpasture's syndrome). *American Journal of Industrial Medicine*, **21**, 141–153.

Direskeneli, H., D'Cruz, D., Khamashta, M. A. and Hughes, G. R. V. (1994) Autoantibodies against endothelial cells, extracellular matrix and human collagen type IV in patients with systemic vasculitis. *Clinical Immunology and Immunopathology*, **70**, 206–210.

Gabrielli, A., Montroni, M., Rupoli, S., Caniglia, M. L., DeLustro, F. and Danielli, G. (1988) A retrospective study of antibodies against basement membrane antigens (type IV collagen and laminin) in patients with primary and secondary Raynaud's phenomenon. *Arthritis and Rheumatology*, **31**, 1432–1436.

Hotz, P., Pilliod, J., Soderstrom, D., Frey, F., Boillat, M. A. and Savolainen, H. (1989) Relation between renal function tests and a retrospective organic solvent exposure score. *British Journal of Industrial Medicine*, **46**, 815–819.

Johansson, C., Butkowski, R., Swedenborg, P., Alm, P. and Weislander, J. (1993) Characterisation of a non-Goodpasture autoantibody to type IV collagen. *Nephrology, Dialysis and Transplantation*, **8**, 1205-1210.

Kefalides, N. A., Pegg, M. T., Ohno, N., Poon-King, T., Zabriskie, J. and Fillit, H. (1986) Antibodies to basement membrane collagen and to laminin are present in sera from patients with poststreptococcal glomerulonephritis. *Journal of Experimental Medicine*, **163**, 588–602.

Mutti, A., Alinovi, R., Bergamaschi, E., Biagini, C., Cavazzini, S., Franchini, I., Lauwerys, R. R., Bernard, A. M., Roels, H., Gelpi, E., Rosello, J., Ramis, I., Price, R. G., Taylor, S. a., De Broe, M., Nuyts, G. D., Stolte, H., Fels, L. M. and Herbort, C. (1992) Nephropathies and exposure to perchloroethylene in dry-cleaners. *Lancet*, **340**, 189–193.

Nelson, N., Robins, T. G. and Port, F. K. (1990) Solvent nephrotoxicity in humans and experimental animals. *American Journal of Nephrology*, **10**, 10–20.

Petty, R. E., Hunt, D. W. C. and Rosenberg, A. M. (1986) Antibodies to type IV collagen in rheumatic diseases. *Journal of Rheumatology*, **13**, 236–253.

Roy, A. T., Brautbar, N. and Lee, D. B. N. (1991) Hydrocarbons and renal failure. *Nephron*, **58**, 385–392.

Salant, D. J. (1987) Immunopathogenesis of crescentic glomerulonephritis and lung purpura. *Kidney International*, **32**, 408–425.

Stevenson, A., Yaqoob, M., Mason, H., Pai, P. and Bell, G. (1995) Biochemical markers of basement membrane disturbances and occupational exposure to hydrocarbons and mixed solvents. *Quarterly Journal of Medicine*, **88**, 23–28.

Yaqoob, M., Bell, G. M., Stevenson, A., Mason, H and Percy, D. F. (1993) Renal impairment with chronic hydrocarbon exposure. *Quarterly Journal of Medicine*, **86**, 165–174.

Received 6 March 1996, revised form accepted 18 May 1996