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Irritancy and sensitisation

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Research Report

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Irritancy and sensitisation

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This study aimed to document key clinical differences between irritation and sensitisation in the workplace, with a view to potentially arming the clinician with new ways to assess cases of work-related respiratory disease. Hitherto, most clinical cases would be assessed on the nature and duration of symptoms alone, or perhaps in conjunction with simple measures of lung function and IgE testing where appropriate. The study was particularly interested in determining whether irritancy or sensitisation in the workplace was associated with the immune profile of a worker. Specifically, the expression of cell surface markers on T cells and monocytes, as well as the concentration of inflammatory cytokines, were investigated.

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EXECUTIVE SUMMARY

This study aimed to document key clinical differences between irritation and sensitisation in the workplace, with a view to potentially arming the clinician with new ways to assess cases of work-related respiratory disease. Hitherto, most clinical cases would be assessed on the nature and duration of symptoms alone, or perhaps in conjunction with simple measures of lung function and IgE testing where appropriate. The study was particularly interested in determining whether irritancy or sensitisation in the workplace was associated with the immune profile of a worker. Specifically, the expression of cell surface markers on T cells and monocytes, as well as the concentration of inflammatory cytokines, were investigated.

The study took place between 2001 and 2004 and recruited 172 workers across five worksites. The study aims were addressed by undertaking a detailed health assessment of a number of worker cohorts, including workers predominantly exposed to irritant chemicals (specifically, welders and metalworkers exposed to irritant gas, 55 workers in all), workers predominantly exposed to sensitisers (specifically, workers exposed to latex and insect allergens, 76 workers in all), and non-exposed workers (i.e. office workers, 41 workers in all). Clinical tests were carried out to allow immune profile, lung function, airway responsiveness and reported respiratory symptoms to be compared between worker groups. Health assessment of each worker commenced with the administering of the study questionnaire. This was followed by the taking of a whole and clotted blood sample, determination of baseline lung function and the testing of airway responsiveness. Data on workplace sensitisation and the reporting of work related upper and lower respiratory symptoms were used to define three worker sub-groups, an irritant group, made up of symptomatic steel workers and welders, a non-sensitised group, made up of symptomatic but non-sensitised insect and latex workers and a sensitised group, made up of symptomatic and sensitised insect and latex workers. Workers in each of the three groups were further classified on the basis of the nature of respiratory symptoms reported (i.e. upper or lower) producing six groups in total.

Eye irritation was the most commonly reported work related symptom in all worksites. Work related wheeze was reported most commonly in welders (2/10 or 20%), cough in insect workers (5/32 or 16%), phlegm in steel workers (2/45 or 6%) and chest tightness (7/44 or 16%), nasal irritation (13/44 or 30%) and eye irritation (24/44 or 55%) in latex workers. The prevalence of a positive atopic status varied between 28 and 36% in the worksites investigated, and was on the whole lower in the insect breeders and latex braiders, known to be occupationally exposed to allergens, and higher in the steel workers and welders, regarded to be more exposed to irritants. The prevalence of workplace sensitisation in the latex braiders and insect breeders was slightly lower than the prevalence of atopy in such worksites, being 26% in the latex braiders and 22% in the insect breeders. There was evidence that workers sensitised and reporting work related respiratory symptoms were more likely to have abnormal lung function than those in the irritant group reporting the same broad type of work related respiratory symptoms. The respiratory symptoms in sensitised workers were more likely to be manifested as cough, chest tightness, both hallmarks of asthma, and eye irritation, than the symptoms reported in irritant exposed workers. The irritant group, in contrast, tended to report wheeze, phlegm and nasal irritation, although the differences observed for these symptoms across the two groups were not statistically significant.

While differences in cell surface marker activation observed across worker groups on eyeballing were often relatively large, none of the differences reached statistical significance. Median CD4+/CD25+ in irritant workers reporting lower respiratory symptoms was over twice the median for sensitised workers reporting symptoms, while median CD54+ was 1.8 times higher. In contrast, median CD14+ was higher in sensitised than irritant workers reporting lower

respiratory symptoms by a factor of over 2. For workers reporting upper respiratory symptoms, differences were lesser. The largest difference was observed for CD4+/CD25+, the median in irritant workers reporting upper respiratory symptoms being 1.5 times higher than that for sensitised workers. Significant differences in cytokine profile in (latex and metal) exposed and non-exposed (office) workers, specifically for IL2, IL10, TNF-α, and IFN-γ, were observed. For both exposed worker groups, but particularly for metal workers, this was characterised by reduced cytokine concentrations relative to the office workers. When comparisons were made between metal workers and latex workers, significant differences were observed for the same set of cytokines, with concentrations being reduced in metal workers relative to latex workers. Within group comparisons were also made to determine whether there were any evidence supporting associations between cytokine concentrations and the reporting of any work related respiratory symptoms. No significant differences in cytokine concentrations across symptoms groups were observed for either worker group. It is inevitable that differences in non-occupational factors, for example, age, gender, smoking and atopy, across the worker groups, explain some of the apparent immune profile differences. Metal workers tended to be older and more were of male and current smokers than either latex workers or office workers. In addition, metal workers tended to have worked at the worksite for longer and test positive for atopy than both office workers and latex workers.

Study findings suggest that workers may exhibit a change in immune profile, particularly with regards the number of monocytes expressing the LPS receptor and the number of activated T helper cells circulating in peripheral blood, when exposed to certain workplace exposures. However, any changes in immune profile appear to be small and to largely occur in the absence of any discernable change in lung function or symptoms status. In addition, results suggest that workers exposed and sensitised to allergens in the workplace and reporting work related respiratory symptoms may exhibit a different pattern in presentation of respiratory symptoms and also may be more likely to exhibit abnormal lung function than workers exposed to irritants and reporting work related respiratory symptoms. However, again, as before, these differences appear not to be paralleled by differences in immune profiling as documented on the basis of immune cell surface marker activation. In particular, it was observed that workers reporting respiratory symptoms potentially attributable to allergen exposure and sensitisation to the allergen were more likely to report cough, chest tightness and eye irritation than those irritant exposed and reporting symptoms, while the most common symptoms reported by symptomatic irritant exposed workers were wheeze and nasal irritation.

1 INTRODUCTION

Occupational lung disease remains common. Reports to the SWORD scheme still frequently detail individuals with occupational asthma thought to arise due to allergic sensitisation to workplace allergen, and also those who have developed occupational asthma via irritant exposure (Meyer, 2001). The exact diagnostic criteria required for individuals to be reported in these categories are not laid down proscriptively. It is left to the individual respiratory physician (or occupational physician) to decide whether the asthma symptoms observed are primarily attributable to allergic sensitization or the symptoms constitute irritant asthma. Current understanding of the pathological differences between these two categories of asthma is still very much lacking. Indeed, there may be considerable overlap between these two groups (Tarlo *et al* 2000).

Allergic sensitisation typically involves a specific immunological response, and normally in the case of high molecular weight agents, the production of specific IgE antibody. Specific IgG_4 antibody may be a precursor of this event, subsequently diverting to specific IgE production. A typical example of such an exposure would be a laboratory animal worker exposed to urinary proteins, developing a specific IgE response (as measured by RAST or skin prick testing) and subsequent allergic sensitisation in the airway. The implication for a sensitised worker is that once rendered sensitive, subsequent further re-exposure in the workplace to airborne allergen, or during a specific bronchial challenge, will reproduce symptoms and a characteristic pattern of lung function change. In addition, individuals with specific bronchial responsiveness to occupationally encountered agents also normally exhibit non-specific airway hyper-responsiveness, measured either with physical stimuli such as cold air or exercise, or with agents such as histamine or methacholine.

Airway irritation attributable to irritant exposure, by contrast, exhibits different clinical features, and it is normally the difference in symptoms presentation that allows the physician to make a provisional separation between these two categories (Banks 2001). By definition, respiratory irritants do not cause sensitisation. A typical example would be a worker exposed to sulphur dioxide or ammonia, developing respiratory symptoms on first exposure to a respiratory irritant, with fairly rapid resolution of symptoms on cessation of exposure. Non-specific bronchial responsiveness may be present, as exhibited in chlorine pulp workers, although these workers are far less studied than those with classic sensitisation. The exact mechanisms by which irritants exert their effect are unknown, but clearly involve a complex set of airway receptors (Nowak 2002, Widdicombe 2001). Clearly, the dose of irritant exposure is important. A condition known as reactive airway dysfunction syndrome (RADS) is described (Alberts and Brooks 1996), that appears to be an asthma-like state associated with a very high (normally single) exposure to a respiratory irritant gas. Individuals such exposed can develop typical asthmatic symptoms, and typical increase in diurnal variation of peak expiratory flow. Implicit in the definition of reactive airway dysfunction syndrome is the presence of bronchial hyper-responsiveness, measured by one of the methods described above. There is really very little data on the natural history of RADS, although it appears that on cessation of exposure certain individuals may subsequently improve. Measured improvement in bronchial responsiveness over a number of years following exposure may also be seen. Typical examples of RADS have been described in the Sacramento River disaster (Cone 1994) and the Manchester air disaster (O'Hickey 1987). There is considerable debate relating to the presence of RADS in individuals exposed to continuous or lower doses of irritant gases, where the irritant exposure is not deemed to be sudden and of high concentration (Tarlo *et al* 2000). However, it is not the remit of this report to address this issue further.

Practically, the main clinical problem relates to differential diagnosis in the workplace between individuals with respiratory symptoms who are exposed both to potentially irritant substances in addition to those potentially causing sensitisation. Furthermore, certain agents (a good example being flour dust and additives) that are capable of causing sensitisation can also cause irritant symptoms in high concentrations. The current diagnostic toolkit available to investigate these individuals is lacking. For example, work related symptoms have relatively low specificity and although are relatively sensitive markers of the development of occupational asthma, they are clearly also present in individuals exposed to irritants. Serial peak expiratory flow rates are relatively sensitive (75%) and specific (96%) for the diagnosis of asthma (Newman-Taylor *et al.* 2004), although individuals exposed to irritants in the workplace (particularly if they have airway hyper-responsiveness) can also develop typical changes of asthma. There are no current algorithms to separate asthma due to sensitisation and asthma due to irritants by peak flow monitoring alone. It has been suggested that the magnitude of peak flow change between work and rest is a surrogate marker of sensitisation versus irritation, with greater magnitudes of diurnal variation change between work and rest being seen in those who are sensitised (D Fishwick, personal comm.). However, this has yet to be proven. Again, measurement of sequential bronchial responsiveness between periods of work and rest has been suggested as a diagnostic criterion for occupational asthma due to sensitisation, but no data exists allowing the separation of asthma due to sensitisation and irritant exposure (D Fishwick, personal comm.). Measurement of specific IgE to workplace allergens again is lacking in terms of its ability to separate those with symptoms due to sensitisation and symptoms due to irritation. One would be persuaded that asthma due to sensitisation is more likely, for example, in a baker with specific IgE measurable to flour or fungal alpha-amylase. However, specific IgE to flour and amylase occurs in individuals who are exposed only, who don't have symptoms directly as a result of sensitisation. Further compounding this problem is that the sensitivity and specificity of positive specific IgE measures or skin prick tests varies widely between agents (Newman-Taylor *et al.* 2004).

The main objective of the current study was to add to the knowledge base regarding the relationship between the pathological mechanisms underlying sensitiser-induced and irritant-induced asthma and the nature of associated symptoms. This was addressed through undertaking detailed respiratory health assessments of a number of worker cohorts. The cohorts included workers predominantly exposed to irritant chemicals (specifically, welders and metalworkers exposed to irritant gas, 55 workers in all), workers predominantly exposed to sensitisers (specifically, workers exposed to latex and insect allergens, 76 workers in all), and non-exposed workers (i.e. office workers, 41 workers in all). Clinical tests were carried out to allow immune cell surface marker activation, lung function, airway responsiveness and reported respiratory symptoms to be compared between worker groups.

Respiratory health assessments of each worker commenced with the administering of the study questionnaire, which was followed by the taking of a whole and clotted blood sample, determination of baseline lung function and ended with the testing of airway responsiveness. The study questionnaire, among other information, detailed occupational exposure history and respiratory and allergic symptoms experienced. The blood samples taken allowed the determination of atopic status, specific IgE to workplace allergens and blood immune cell surface marker activation. Baseline lung function testing involved the determination of standard pulmonary physiological endpoints including PEF, FEV_1 and FVC. The latter information was then used as baseline data to determine airway responsiveness in airway histamine challenge testing.

2 METHODS AND MATERIALS

2.1 SUBJECTS

Three groups of workers were investigated in this study. A cohort of 76 workers exposed to known respiratory sensitisers (32 from an insect breeding facility and 44 from a latex braiding factory), a second group of (55) workers exposed to a range of low molecular weight irritant chemicals, (10 from a steel foundry and 45 from a steel facility manufacturing fencing), and a group of 41 office-based workers recruited from a local warehouse to act as controls.

All volunteers provided informed consent. Approval for the study to proceed was obtained from the Health and Safety Executive's Research Ethics Committee (ref: *ETHCOM/REG/01/10*).

2.2 QUESTIONNAIRE

A copy of the questionnaire used in the study is included in Appendix 1. The questionnaire was designed using questions from a previously validated questionnaire used to diagnose work-related irritant and allergic symptoms in textile workers (Fishwick *et al.*, 1994) and welders working with irritant gases (Fishwick *et al.*, 1997; 1998).

These questions had originally been adapted from the MRC respiratory questions, with extra questions aimed at determining work-related exacerbation of symptoms. Symptoms were taken to be work-related if they were worse at work, better on days off, or better on holidays. The demographic details were altered to allow coding for current and previous workplaces, and total time spent working at current and previous workplaces.

Workers answering "yes" to the question "Have you ever suffered from eczema, hay fever, or other allergies" were judged as having self-reported atopy. Ex-smokers were taken as workers who had ever smoked as much as 1 cigarette per day, or 1 cigar per week, or 1 ounce of tobacco a month for as long as a year.

For the questions on cough and phlegm, clearing the throat and a single cough were excluded. Coughing with the first cigarette or on going outdoors was taken as positive responses. The interviewer demonstrated wheeze if the questions on wheeze were not understood. Questions on work-related mucous membrane irritation were included in the questionnaire to help identify workers with rhinitis and conjunctivitis.

Each data point was coded separately to facilitate data entry, and the responses used throughout were: 1= YES; 2= NO; 3= NOT APPLICABLE. A database was created for each data point in SPSS Version 12. Code data was entered by the research nurse and physician in tandem, and cross checked in a random sample.

2.2.1 QUESTIONNAIRE REPEATABILITY

The questionnaire was repeated in a random sample of individuals. The repeat responses for key questions (relating to persistent cough, chronic bronchitis, phlegm, chest tightness, wheeze, shortness of breathe, nasal irritation and eye irritation) were compared.

The responses were compared in two ways:

First, an exact match implies that the two responses were identical (i.e. they both fell into one of three categories; (i) no symptom present, (ii) non work related symptom present or (iii) work related symptom present.

A second method of comparing the responses used was that of a 'work-related match' by combining the categories of 'no symptom present' and 'non-work related symptom present' and comparing this to work related symptom present. The reproducibility for determining work-related symptoms is thus estimated. This would seem more relevant in the setting of the present study that is particularly interested in the characterisation of work related symptoms. Data for the reproducibility of the eight main questions is shown in Appendix 2.

2.3 PULMONARY FUNCTION MEASUREMENT

On the same visit as employees were asked to answer the questionnaire, they were also asked to perform a reproducible measurement of their lung function. All recordings were made using the same spirometer (Alpha 2, Vitalograph, Buckinghamshire, UK), and following a standard protocol. A new disposable mouthpiece was used for each worker, and the temperature of the room was monitored throughout lung function measurement. The procedure was explained to each worker, and they were then asked if they consented to participate.

Measurement followed ERS and ATS guidelines. Two forced expiratory manoeuvres were performed initially, and the forced expiratory volumes in one second (FEV_1) compared. If the two measurements were within 5% of each other, the higher value was accepted and recorded. If they varied by more than 5%, further forced expiratory manoeuvres were performed until two reproducible blows were attained. Again, the higher value was recorded. Workers with poor technique were given further instruction, and all workers were encouraged to produce a maximal forced vital capacity. As well as FEV_1 (litres), the other lung function parameters measured were forced vital capacity (FVC in litres), FEV1/FVC ratio (as a percentage), forced expiratory flow at 25-75% of vital capacity (FEF_{25-75} in litres per second), and peak expiratory flow rate (PEFR in litres per minute). The spirometer produced a printed record of absolute values, and of percentage predicted for age, sex, height and ethnic group for each lung function parameter.

2.3.1 SPIROMETER CALIBRATION

The calibration of the spirometer was checked before and after each visit, as per manufacturer's instructions. This was performed with a three-litre calibration syringe, and was accepted if within 5%. To ensure accurate calibration, the spirometer and syringe were left to stand in the room for one hour prior to testing. Air at room temperature was drawn through the syringe twenty times prior to pushing it through the spirometer.

2.4 NEBULISER OUTPUT

2.4.1 DEVILBISS 40 GLASS HAND-HELD NEBULISERS

GRAVIMETRIC METHOD

Bronchial hyperreactivity was assessed by the method described by Yan et al (1983), administering histamine from DeVilbiss 40 glass hand-held nebulisers. These nebulisers were also used for cough challenge. The output of five nebulisers was assessed gravimetrically as described by Yan (1983).

One millilitre of normal saline was placed in each nebuliser, the rubber bungs replaced, and then weighed. In turn, each nebuliser was squeezed ten times (with the stoppers removed), the stoppers were then replaced, and the nebuliser reweighed. This was repeated ten times for each nebuliser, and the mean output per squeeze calculated.

FLUORIDE TRACER METHOD

The accuracy of the gravimetric assessment of output was assessed by a fluoride tracer method (Dennis et al 1990). It has been recognised that for jet nebulisers, weight loss overestimates true aerosol output due to concomitant loss of water vapour. It has been suggested that measurement of output by a direct chemical tracer method gives a more accurate result. Indeed for the DeVilbiss 40 nebulisers, previous work has shown that mean output as assessed by weight loss, overestimated true aerosol output by 321% (Hartley-Sharpe et al 1995). This is clearly important for epidemiological studies, and the assessment of airway responses to doses of histamine. The method is described in detail elsewhere, both in general terms (Dennis et al, 1992) and specifically for the DeVilbiss 40 nebuliser (Hartley-Sharpe et al 1995).

Briefly, one millilitre of 1% weight per volume sodium fluoride was placed inside each nebuliser. The tip of the throat tube was held just inside a plastic tube which had air drawn through it by an air pump at a flow rate of 15 litres per minute (designed to represent *in vivo* operating conditions), The nebuliser was then activated, and the aerosol was then collected onto a 25mm Whatman glass fibre filter paper, held 5 centimetres away at the end of the plastic tube. Each nebuliser was activated twice, separated by a thirty second time interval. The filter paper was then removed and stored overnight in a buffer solution, and the fluoride content measured using a standard fluoride electrode. This was repeated twice for each nebuliser, and mean true aerosol outputs calculated for each of the five nebulisers.

2.4.2 MEFAR MB3 NEBULISER

Cough challenges were carried out by administering citric acid from a Mefar MB3 nebuliser, based on the method described previously (Morice et al, 1992). Ten nebuliser pots were calibrated both gravimetrically, and by a fluoride tracer technique, in the same way as for the DeVilbiss 40 nebulisers. Calibration of Mefar nebulisers by weight loss has also been shown to overestimate true aerosol output, the overestimate varying between sets of nebulisers. Dennis et al (1992) found true aerosol output to be as low as 43%, and as high as 83%, of that predicted by weight loss.

2.5 ASSESSMENT OF BRONCHIAL RESPONSIVENESS

2.5.1 CHOICE OF PROTOCOL

Assessment of bronchial responsiveness was performed by an existing rapid, simple method (Yan et al, 1983). This protocol was developed specifically for use in epidemiological studies, and to be more rapid, and more convenient to administer than other bronchial challenges. It has since been extensively used for workplace studies, where ease of transportation, lack of requirement of electricity, and speed of administration are important. This method has been shown to compare favourably with a well-established existing technique developed by Cockcroft et al in 1977.

2.5.2 CHALLENGE PREPARATION

Histamine solution of 50 mg/ml was obtained from the Royal Hallamshire Hospital pharmacy. On each challenge day, this was serially diluted to produce concentrations of 25 mg/ml, 6.25 mg/ml, and 3.13 mg/ml. The different strengths of solution were then entered into one of four numbered De Vilbiss No. 40 glass handheld nebulisers (previously calibrated). The fifth nebuliser contained normal saline.

2.5.3 CHALLENGE

Bronchial challenges were not performed in workers with unstable or brittle asthma, or who had had a chest infection in the previous four weeks. The informed consent of each worker was gained, and baseline lung function recorded. Challenges were not performed in workers with a baseline FEV_1 below 1.5 litres, or 80% predicted. Each worker then inhaled three breaths of normal saline from the first nebuliser. The worker was asked to exhale to slightly below functional residual capacity, and the mouthpiece of the nebuliser held between the teeth of the worker. The worker was then asked to inhale towards total lung capacity over two seconds, and then to breath hold for three seconds. At the start of the inspiration, the operator gave the bulb of the nebuliser a firm squeeze.

One minute later the spirometry was repeated, and the higher of the two reproducible FEV_1s recorded. The workers then inhaled sequential doubling doses of histamine (doses ranging from 0.0009 mg to 1.2mg) each dose being followed after one minute by repeat spirometry. The test was terminated and recorded as positive if baseline FEV_1 fell by 20% or more. Any worker experiencing a significant fall in FEV_1 was administered 200mcg of inhaled Salbutamol and kept until it returned to baseline levels. The dose of histamine causing a 20% fall in FEV_1 over the post-saline value (PD_{20}) was calculated.

2.6 COUGH CHALLENGE

2.6.1 CHOICE OF AGENT AND METHOD OF ADMINISTRATION

Assessment of cough thresholds were carried out using citric acid, and delivered by a previously described method (Morice et al, 1992). This technique had previously been widely used for assessing cough thresholds both experimentally and in hospital outpatient cough clinics. This method of administering tussive stimuli has been used for citric acid and capsaicin challenges, and investigators often use both agents in the same study. As cough challenges were being performed in the workplace, limited time was available to assess cough thresholds. Therefore, a single tussive agent was chosen.

Citric acid was selected as it was easier to obtain and prepare than capsaicin. Comparative studies have also shown slightly greater reproducibility with citric acid (Di Franco et al, 2001).

2.6.2 PROTOCOL

Workers were asked to inhale half log incremental concentrations of citric acid (range 10-1000mM) from a calibrated breath activated Mefar MB3 dosimeter. The challenge commenced with four one-second inhalations of normal saline, each inhalation separated by one minute intervals. This was followed by four inhalations of 10, 30, 100, 0 (normal saline), 300, and 1000 mM solutions of citric acid.

The workers were blinded to the citric acid concentration, and extra saline inhalations were used in an attempt to prevent workers recognizing the incremental nature of the challenge. If

coughing occurred, the number of coughs in the ten seconds immediately post-inhalation were counted and recorded. The challenge was terminated once an average of two or more coughs at one concentration had occurred, or the fourth inhalation of 1000 mM solution inhaled.

The concentration of citric acid causing two coughs was taken as the cough D_2 threshold, the exact concentration being calculated by linear interpolation of log transformed data. Those not attaining a cough threshold at the highest concentration of citric acid were arbitarily ascribed a D_2 threshold of 1000 mM.

2.7 IMMUNOLOGY

2.7.1 RAST

Each worker provided a 10ml sample of clotted blood. This was used for the measurement (by RAST) of atopic status and specific IgE to relevant workplace allergens.

Latex products were collected from the factory. Extracts were made for each by (i) extracting in 10% w/v ammonium bicarbonate, (ii) filtering the mixture, (iii) centrifuging the filtrate at 10000rpm for 30mins and (iv) then removing the supernatant and freeze drying to concentrate the protein. This was then re suspended in 15mls of PBS.

Locusts, mealworms and crickets were collected from the factory. Extracts were made for each species by (i) homogenising the insects in PBS, (ii) filtering the mixture, (iii) centrifuging the filtrate at 10000rpm for 30mins and (iv) then removing the supernatant

BCA protein estimation was performed to determine the amount of protein in each extract in order to calculate the correct weight of activated discs to be used.

The RAST discs were prepared from cyanogen-activated discs. 1mg of each extract per 100mgs of activated discs were rotated overnight in 0.1M sodium bicarbonate buffer. The next day the discs were washed, blocked with ethanolamine, washed in acetate buffer, washed in bicarbonate buffer and stored in PBS/0.1% Tween 20 at -20°C.

200µl of test or control serum (diluted in PBS) was incubated with a disc overnight at room temperature. All the supernatant was then removed and three washes performed using 1ml of PBS/0.1% Tween 20. After washing, 100µl of ^{125}I labeled anti-human IgE was added to each disc and incubated at room temperature for 24 hours. Washing to remove unbound anti-human IgE was carried out as for the previous washing stage.

The radioactivity of the bound anti-human IgE was measured using a gamma counter (Packard). All assays were performed in duplicate and results were expressed as a RAST score. Samples were rejected if the duplicate results differed by more than 10% for samples binding 1% or more of the added I^{125} (15% for samples binding less than 1% of the added I^{125}). The corrected RAST score was calculated as the percentage score for the test serum disc minus that of the negative control disc. A RAST score of equal to or greater than 2 was considered positive.

2.7.2 FLOW CYTOMETRY

Each worker also supplied a 5 ml sample of EDTA treated blood. This sample was used for the measurement of phenotypic and induced cell surface markers and intracellular proteins. We used a flow-cytometry technique to measure phenotypic (CD3, CD4, CD8 and CD45) and

activation (CD25) markers. Normal ranges had already been established "in-house" for these markers, using a normal volunteer population of people with FEV_1/FVC greater than 70%.

The EDTA treated blood was added to LP4 tubes containing the appropriate amount of each labeled antibody. Appropriate isotype control antibody conjugates were included to establish background fluorescence. Each tube was then incubated for 25 minutes at room temperature in the dark. These were then fixed and the red cells lysed with the Coulter immunoprep system on the 35-second cycle. Samples were analysed on a calibrated Epics-XL flow cytometer (Coulter Electronics, UK).

Prior to analysis, the instrument was calibrated for optical alignment and fluorescence intensity using Immunocheck and Immunobrite fluorescent microspheres (Beckman Coulter, UK). Lymphocytes and monocytes were distinguished from cell debris and other cell types by $CD14^+/CD45^+$ back-gating. Ten thousand events were collected for each lymphocyte and 5000 for each monocyte sample. Various combinations of fluorescently labelled monoclonal antibodies were used to identify and quantify specific populations of cells. Data for CD3, 4, 8, and 25 were reported as the percentage of lymphocytes expressing the particular marker and the mean linear fluorescence of the marker.

3 RESULTS

3.1 STUDY POPULATION

The study took place between 2001 and 2004 and recruited 172 workers across five worksites. Data on the demographics of the workers recruited from the five worksites are summarised in Table 1. The mean ages of workers from each of the five worksites were broadly similar. However, the mean duration of employment for the welders and steel workers tended to be longer than for the workers working in the insect breeding and latex braiding plant. The exposed workers investigated were predominantly male except for those from the latex braiding plant, where there was a 50:50 split of males to females. However, workers in the control group (i.e. office workers) were predominantly female. Trends in smoking habits on the whole reflected the gender splits in the workforces (assuming a current smoking habit generally to be more common in males), with the steel workers and insect workers in particular, having a predominance of current smokers, whereas latex workers having an even split of current to never smokers. However, the prevalence of current smoking in the welders was perhaps lower than expected considering the predominance of males over females in the workforce.

3.2 RESPIRATORY SYMPTOMS AND SENSITISATION STATUS

The prevalence of symptoms by worksite is summarised in Table 2. Even in the office workers, supposedly non-exposed to allergens and irritants in the workplace, work related respiratory symptoms were reported. The number reporting upper respiratory symptoms was particularly marked (10/41 or 24%), although the number reporting lower symptoms was much less (1/41 or 2%). Eye irritation was the most commonly reported work related symptom in all worksites. Work related wheeze was reported most commonly in welders (2/10 or 20%), cough in insect workers (5/32 or 16%), phlegm in steel workers (2/45 or 6%) and chest tightness (7/44 or 16%), nasal irritation (13/44 or 30%) and eye irritation (24/44 or 55%) in latex workers. The prevalence of a positive atopic status varied between 28 and 36% in the worksites investigated (see Table 4), and was on the whole lower in the insect breeders and latex braiders (known to be occupationally exposed to allergens), and higher in the steel workers and welders (regarded to be more exposed to irritants). The lower prevalence of atopy in the insect breeders and latex braiders is likely to be attributable to such worksites reflecting survivor populations, i.e. worksites where atopic workers have either left employment or have avoided employment from the outset. The prevalence of workplace sensitisation in the latex braiders and insect breeders was slightly lower than the prevalence of atopy in such worksites, being 26% in the latex braiders and 22% in the insect breeders (see Table 4). Data on cell surface marker activation for the individual worksites are presented in Table 6 for information. The results of tests of statistical significance in relation to such data are summarised in a later section.

3.3 PREVALENCE OF RESPIRATORY SYMPTOMS BY WORKSITE AND EXPOSURE GROUP

Data in Table 5 summarise rates of work related respiratory symptoms by worksite. Rates of both upper and lower respiratory symptoms were broadly similar in steel workers and welders. In addition, rates of workplace sensitisation and lower respiratory symptoms were broadly similar in latex and insect workers; rates of upper respiratory symptoms differed more, being much higher in latex workers. The respiratory symptoms, both upper and lower, reported in latex workers tended not to be paralleled by sensitisation to latex. However, there was a more even split in irritant symptoms and symptoms potentially attributable to workplace sensitisation in insect workers. Unadjusted odds ratios illustrating the risks of work related respiratory symptoms in those exposed to allergens relative to irritants, and in those testing positive for

workplace sensitisation and atopy, are reported in Tables 7, 8 and 13. Also shown are the risks of abnormal lung function in those exposed to allergens relative to irritants. No significant differences in risk of either work related upper or lower respiratory symptoms in workers exposed to allergens compared to irritants were apparent. However, there was evidence to suggest that allergen exposed workers were more likely to exhibit abnormal PEF.

3.4 CELL SURFACE MARKER ACTIVATION BY EXPOSURE STATUS

Unadjusted odds ratios were derived summarising the predicted change in odds of being irritant as opposed to allergen exposed (regardless of symptoms status), for a unit increase in % cells expressing a certain cell marker (see Table 9). The odds ratios provide an indication of the strength of association between cell surface marker activation and exposure status for the workers in the study cohort. A significant association was found between being exposed to irritant rather than allergen and a higher % of cells expressing CD4+/CD25+. Additionally, there was an association between being exposed to allergen rather than irritant and a higher mean linear fluorescence for CD14. Associations between exposure and all other cell surface markers investigated were insignificant. According to the regression models constructed, the odds of a worker from the study cohort being exposed to irritant rather than allergen increased by 4.4 per unit increase in the % of cells expressing CD4+/CD25+ (and by 2.1 per half unit increase), while the odds of being exposed to allergen rather than irritant increased by 1.3 per unit increase in mean linear fluorescence for CD14 (and by 1.7 per 2 unit increase). It is worth noting that the median and ranges for CD4+/CD25+ and CD14 for allergen exposed and irritant exposed workers were 0.76, 0.11-2.09 (CD4+/CD25+, allergen), 1.15, 0.28-2.89 (CD4+/CD25+, irritant), 6.35, 1.68-10.70 (CD14, allergen), and 4.06, 1.90-10.10 (CD14, irritant) respectively (see Table 9). Mean linear fluorescence for CD14 (as well as being associated with allergen exposure), was also found to be associated with the reporting of work related upper respiratory symptoms (see Table 11). However, no associations were found between the % of cells expressing CD4+/CD25+ and the reporting of either upper or lower respiratory symptoms (see Tables 11 and 12). In addition, neither CD4+/CD25+ or CD14 varied with sensitisation status (see Table 10).

3.5 RELATIONSHIP BETWEEN IRRITANT, SENSITISED AND NON-SENSITISED GROUPS; AND RESPIRATORY SYMPTOMS AND LUNG FUNCTION MEASUREMENTS

It was further hypothesised that the nature of clinical presentation of symptoms, as well as the immune and physiological mechanisms underlying symptoms, may differ in those exposed to allergens rather than irritants. As a consequence of the low numbers in the groups compared (i.e. 10 and 13 in the irritant groups, 5 and 7 in the sensitised groups, and 9 and 15 in the non-sensitised groups), P-values were derived using a combination of Fisher's Exact Chi-Square Tests and non-parametric tests (in particular the Mann-Whitney U Test and the Kruskal-Wallis H Test). The data in Table 5 on workplace sensitisation and the reporting of work related upper and lower respiratory symptoms were used to define three worker sub-groups, an irritant group, made up of symptomatic steel workers and welders, a non-sensitised group, made up of symptomatic but non-sensitised insect and latex workers and a sensitised group, made up of symptomatic and sensitised insect and latex workers. Workers in each of the three groups were further classified on the basis of the nature of respiratory symptoms reported (i.e. upper or lower) producing six groups in total. The demographics of these groups are compared in Tables 18 and 19. The irritant, non-sensitised and sensitised groups reporting lower respiratory symptoms were broadly similar ($P>0.05$) with regards smoking habits, gender, atopy and age, but significantly differed ($P<0.05$) with respect to employment duration, the medians for the latter being 16 years, 4 years and 2 years for the groups respectively. For the same groups reporting upper respiratory symptoms, age and smoking habits were again broadly similar

(P>0.05), but, as for the groups reporting lower respiratory symptoms, the groups significantly differed with regards employment duration (P<0.05).

Tables 14 and 15 compare the prevalence of abnormal lung function in the irritant, non-sensitised and sensitised groups. Table 14 summarises the results of significance tests in those reporting work related lower respiratory symptoms and Table 15 the same for those reporting work related upper respiratory symptoms. There was evidence, although not statistically significant in all cases, that workers sensitised and reporting work related respiratory symptoms were more likely to have abnormal lung function than those in the irritant group reporting the same broad type of work related respiratory symptoms. This was manifested both as an FEV_1 and PEF less than 80% of predicted and was true both for those sensitised and reporting upper respiratory symptoms (abnormal PEF only, P=0.031), as well as those sensitised and reporting lower respiratory symptoms (abnormal FEV_1, P=0.077 and PEF, P=0.095). In addition, the respiratory symptoms in sensitised workers were more likely to be manifested as cough (P=0.047), chest tightness (P=0.041), both hallmarks of asthma, and eye irritation (P=0.051) than the symptoms reported in irritant exposed workers (See Tables 16 and 17). The irritant group, in contrast, tended to report wheeze, phlegm and nasal irritation, although the differences observed for these symptoms across the two groups were not statistically significant.

3.6 Immune Profiles of Sensitised, Non-sensitised and Irritant Groups

Data on the immune profiles of the various worker groups are summarised and statistically compared in Tables 20 and 21. Unfortunately, owing to the small group sizes, the relationships could not be modelled by multiple regression as before and therefore are investigated using simple non-parametric statistical tests. While differences in cell surface marker activation observed across worker groups were often relatively large, none of the differences reached statistical significance. Median CD4+/CD25+ in irritant workers reporting lower respiratory symptoms (see Table 20) was over twice the median for sensitised workers reporting symptoms, while median CD54+ was 1.8 times higher. In contrast, median CD14+ was higher in sensitised than irritant workers reporting lower respiratory symptoms by a factor of over 2. For workers reporting upper respiratory symptoms, differences were lesser (see Table 21). The largest difference was observed for CD4+/CD25+, the median in irritant workers reporting upper respiratory symptoms being 1.5 times higher than that for sensitised workers.

As an extension to this study, the blood sera of a sub-sample of allergen-exposed (a sample of latex workers), irritant exposed (a sample of metal workers) and non-exposed office workers from the study cohort were further investigated to determine cytokine concentrations in peripheral blood. The reader is referred to Annex 4 of this report for more detailed information on the background and aims to this investigation, study methodology and overall results. Significant positive correlations (CC>0.500, P<0.05) between levels of IL2 and IL10 and IFN-γ, IL6 and IL10 and TNF-α, IL10 and IFN-γ, and TNF-α and IFN-γ were observed. The main findings of worker group comparisons were that the cytokine profile in the peripheral blood of metal workers and latex workers differed to that observed for office workers (P<0.05), specifically for IL2, IL10, TNF-α, and IFN-γ. For both exposed worker groups, but particularly for metal workers, this was characterised by reduced cytokine concentrations relative to the office workers. When comparisons were made between metal workers and latex workers, significant differences were observed for the same set of cytokines, with concentrations being reduced in metal workers relative to latex workers. Within group comparisons were also made to determine whether there were any evidence supporting associations between cytokine concentrations and the reporting of any work related respiratory symptoms. No significant differences in cytokine concentrations across symptoms groups were observed for either worker group. It is inevitable that differences in non-occupational factors, for example, age, gender, smoking and atopy, across the worker groups, may explain some of the apparent differences in

cytokine concentrations. Metal workers tended to be older (mean age = 44 years, 34 years [office], 37 years [latex]) and more were of male sex (% = 100%, 37% [office], 50% [latex]) and current smokers (% = 50%, 17% [office], 39% [latex]) than either latex workers or office workers. In addition, metal workers tended to have worked at the worksite for longer (mean employment duration = 19 years, 1 year [office], 5 years [latex]) and test positive for atopy (% = 36%, 33% [office], 28% [latex]) than both office workers and latex workers. Cytokine levels were also compared across groups with the study cohort categorised according to gender, atopic status and current smoking habits. Concentrations of IL2, IL10, TNF-α, and IFN-γ were all significantly lower in males than females. No other significant demographic trends were observed.

4 DISCUSSION

This study assessed a variety of markers of respiratory ill health in a diverse set of occupations. The main aim was to document key clinical differences between respiratory symptoms associated with irritant exposure and symptoms occurring in parallel with workplace sensitisation, ultimately with a view to potentially arming the clinician with new ways to investigate causes of respiratory symptoms in the workplace. Hitherto, most clinical cases would be assessed on the nature and duration of symptoms alone, or perhaps in conjunction with simple measures of lung function and IgE testing where appropriate. This study aimed to determine whether irritancy or sensitisation in the workplace was associated with certain immune profiles, specifically, the expression of certain cell surface markers on T cells and monocytes, and therefore whether such information could potentially contribute in investigation of the causes of occupational respiratory disease. The study employed flow cytometry to measure phenotypic and induced cell surface markers and intracellular proteins in the peripheral blood of workers who volunteered a blood sample. This analytical method measures alterations to antigens expressed by specific sets of circulating T-lymphocytes and monocytes in the samples of blood provided by workers. The following phenotypic markers were measured as part of the study (including relevant combinations): CD3 on T-cells, CD25 on activated T-cells, CD4 on T-helper cells, CD8 on cytotoxic T-cells and CD14 and CD54 on monocytes (i.e. markers for LPS receptor and ICAM-1 respectively).

The final numbers constituting each work exposure group were lower than anticipated, which resulted from the genuine difficulty in recruiting for studies from workplaces, and perhaps the relatively complex nature of volunteer involvement in the study. No significant side effects from any of the investigations were seen, and no adverse issues arose in the workplaces. The final study population consisted of 76 workers exposed to known respiratory sensitisers (32 from an insect breeding facility and 44 from a latex braiding factory), a second group of (55) workers exposed to a range of low molecular weight irritant chemicals, (10 from a steel foundry and 45 from a steel facility manufacturing fencing), and a group of office-based workers recruited from a local warehouse to act as controls.

The mean age of the workers recruited to the study (37 years, range 17-65) was similar to other workplace-based studies, suggesting that the populations identified were broadly representative of working groups. Workers had been employed at the current site of employment for a mean of 2.5 years, with a large range (<1-40 years). It was decided *a priori* not to censor those with short work duration, particularly as latency was hypothesised to be a relevant issue for the separation of likely irritant related and sensitisation effects. Approximately 63% of workers were male, and 37% were current cigarette smokers. The latter figure is reasonably consistent with current national figures, although smoking habit did vary between work sites. Certain demographic differences between actively exposed work groups and the control office workers were present. It was not possible to totally match the control population for the actively exposed groups, and a decision had been made *a priori* not to "over match" the population with controls, as some of the standard factors used to match (e.g. age, sex, smoking) were regarded to be potential independent risk factors for the development of the disease under investigation.

The results of statistical analysis suggest that workers may exhibit a change in immune profile, particularly with regards the number of monocytes expressing the LPS receptor and the number of activated T helper cells circulating in peripheral blood, when exposed to certain workplace exposures. However, any changes in immune profile appear to largely occur in the absence of any discernable change in lung function or symptoms status. It is reasonable to hypothesise from these findings that the changes in immune profile observed may reflect early signs of the onset of disease, before the onset of symptoms. In addition, results suggest that workers exposed

and sensitised to allergens in the workplace and reporting work related respiratory symptoms may exhibit a different pattern in presentation of respiratory symptoms and also may be more likely to exhibit abnormal lung function than workers exposed to irritants and reporting work related respiratory symptoms. However, again, as before, these differences appear not to be paralleled by differences in immune profiling as documented on the basis of immune cell surface marker activation. In particular, it was observed that workers reporting respiratory symptoms potentially attributable to allergen exposure and sensitisation to the allergen were more likely to report cough, chest tightness and eye irritation than those irritant exposed and reporting symptoms, while the most common symptoms reported by symptomatic irritant exposed workers were wheeze and nasal irritation. Taken collectively, results suggest that data on cell surface markers collected as part of the study appear to be no more informative as more traditional clinical data on the reporting of symptoms, lung function and sensitisation for the investigation of irritant induced or sensitiser induced occupational respiratory disease.

The results of cytokine profiling of a sub-sample of workers from the study cohort (see Annex 4) support the results of this study, in that differences in cytokine concentrations were observed across worker categories but these differences appeared to bare little if any association with more traditionally investigated clinical endpoints such as the reporting of symptoms, lung function or sensitisation status. Specifically, statistically significant differences in cytokine concentrations, in particular, IL2, IL10, TNF-α, and IFN-γ, in peripheral blood in workers exposed to different agents, for example, irritants and allergens in the workplace, relative to office workers, were observed. Perhaps rather spuriously, concentrations of these cytokines were found to be higher in non-exposed office workers than both the exposed worker groups, while comparison of the two exposed groups showed levels to be higher in latex workers. It is inevitable that differences in non-occupational factors, for example, age, gender, smoking and atopy, across the worker groups, may explain some of the apparent differences in cytokine concentrations, given that these factors differed across worker groups (with metal workers tending to be to be older, of male sex, current smokers and to have worked at the worksite for longer than either latex workers or office workers). Group comparison of cytokine levels with the study cohort stratified by demographic characteristics suggested levels of all cytokines to be lower in males than females. Given males were much more predominant in both metal workers (100% male) and latex workers (50% male) than office workers (37% male), and in metal workers than latex workers, it may be that the differences in cytokine levels across worker groups are attributable in a large part to the gender differences across the groups. However, the confounding effects of such factors could not be adequately controlled for owing to insufficient data on cytokine levels for the study cohort. In conclusion, this work has demonstrated contrasting cytokine concentrations in workers exposed and non-exposed to latex allergens and irritants in the workplace suggesting that certain workers may exhibit a change in immune profile attributable to the agents they are exposed to in the workplace. However, any changes in immune profile appear to largely occur in the absence of any discernable change in lung function or symptoms status. It is reasonable to hypothesise from these findings that the changes in immune profile observed may reflect early signs of the onset of disease, prior to the onset of symptoms. An alternative explanation is that the differences in cytokine levels across the worker groups are entirely due to gender differences across the groups. Further investigative work on a larger work cohort would need to be undertaken to determine the any occupationally driven trends in cytokine levels in demographically diverse worker groups.

5 RESULTS TABLES

Table 1
Demographics by Worksite

	Office Worker n=41	Latex Worker n=44	Insect Worker n=32	Steel Worker n=45	Welder n=10
Age					
Mean (years)	34.44	37.16	33.72	44.20	39.70
(Range)	(21-60)	(19-59)	(17-56)	(28-60)	(17-65)
Duration of work at site					
Mean (months)	14.59	61.16	28.67	233.27	170.70
(Range)	(4-24)	(1-156)	(2-108)	(9-480)	(9-372)
Duration of work in industry					
Mean (months)	88.59	126.11	34.30	255.93	183.90
(Range)	(6-216)	(1-504)	(2-144)	(9-516)	(9-444)
Gender					
Male (%)	36.6	50.0	71.9	100.0	80.0
Female (%)	63.4	50.0	28.1	0.0	20.0
Smoking					
Current (%)	17.1	38.6	65.6	50.0	25.0
Never (%)	61.0	36.4	34.4	37.5	20.0

Table 2
Prevalence of Symptoms by Worksite

	Office Worker n=41 No. %	Latex Worker n=44 No. %	Insect Worker n=32 No. %	Steel Worker n=45 No. %	Welder n=10 No. %
Wheeze ever	13 31.7	13 29.5	11 34.4	14 43.8	2 20
Work related wheeze	1 2.4	4 9.1	2 6.3	2 6.3	2 20
Cough ever	2 4.9	7 15.9	7 21.9	4 12.5	0 0
Work related cough	0 0	4 9.1	5 15.6	2 6.3	0 0
Chest tightness ever	10 24.4	12 27.3	15 46.9	5 15.6	5 50
Work related chest tightness	1 2.4	7 15.9	4 12.5	2 6.3	1 10
Shortness of breath ever (MRC grade2)	3 7.3	2 4.5	3 9.4	4 12.5	0 0
Work related shortness of breath	0 0	1 2.3	1 3.1	0 0	0 0
Phlegm ever	1 2.4	5 11.4	1 3.1	2 6.3	0 0
Work related phlegm	0 0	2 4.5	0 0	2 6.3	0 0
Nasal irritation ever	10 24.4	19 43.2	10 31.3	10 31.3	2 20
Work related nasal irritation	3 7.3	13 29.5	4 12.5	8 25	2 20
Eye irritation ever	11 26.8	24 54.5	7 21.9	5 15.6	3 30
Work related eye irritation	7 17.1	14 31.8	5 15.6	3 9.4	2 20

Table 3
IgE RAST by Worksite: Tests Carried Out

	Office Worker n=41	Latex Worker n=44	Insect Worker n=32	Steel Worker n=45	Welder n=10
Atopy	✓	✓	✓	✓	×
Latex	✓	✓	×	×	×
Insect	✓	×	✓	×	×

RAST tests:
Atopy – specific IgE to common environmental allergens including house dust mite, cat dander, cocksfoot, timothy grass and meadow grass
Latex – specific IgE to "silica" latex, "talco" latex and natural rubber latex and dyed cotton
Insect – specific IgE to extract of whole locust, meal worm and meal work feed (bran)

Table 4
Prevalence (%) of + IgE RAST by Worksite

	Office Worker n=41	Latex Worker n=44	Insect Worker n=32	Steel Worker n=45	Welder n=10
	No. %	No. %	No. %	No. %	No. %
Atopy +	6 33.3	11 28.2	5 27.8	16 36.4	- -
Latex +	0 0	10 25.6	- -	- -	- -
Insect +	1 5.9	- -	4 22.2	- -	- -

Positive test taken as positive to at least one of allergens tested for
Percentages expressed relative to total no. giving blood (n=18 for office workers, 18 for insect workers, 39 for latex workers, 0 for welders and 44 for steel workers)

Table 5
Prevalence of Symptoms by Worksite

	Office Worker n=41 No. %	Latex Worker n=44 No. %	Insect Worker n=32 No. %	Steel Worker n=45 No. %	Welder n=10 No. %
Workplace sensitisation*	0 0	10 25.6	4 22.2	0 0	0 0
Work related upper respiratory symptoms**	10 24.4	21 47.7	6 18.8	11 24.4	2 20
Work related lower respiratory symptoms***	1 2.4	10 22.2	7 21.9	8 17.8	2 20
Workplace sensitisation and work related upper respiratory symptoms	- -	5 12.8	2 11.1	0 0	0 0
Work related upper respiratory symptoms but no workplace sensitisation	- -	13 33.3	2 11.1	11 24.4	2 20
Workplace sensitisation and work related lower respiratory symptoms	- -	3 7.7	2 11.1	0 0	0 0
Work related lower respiratory symptoms but no workplace sensitisation	- -	6 15.4	3 16.7	8 17.8	2 20

*Office workers, steel workers and welders assumed not to be sensitised to latex and insect allergens therefore rates of workplace sensitisation are zero
**Upper respiratory symptoms include nasal and eye symptoms
***Lower respiratory symptoms include cough, phlegm, wheeze, shortness of breath and chest tightness

Table 6
Cell Surface Marker Activation by Worksite

	Office Worker n=41	Latex Worker n=44	Insect Worker n=32	Steel Worker n=45	Welder n=10
Valid cases	n=26	n=37	n=18	n=42	n=8
CD3+*					
Mean	73.86	74.19	70.51	72.42	71.57
Range	59.91-90.07	60.80-87.79	41.05-83.22	12.43-83.70	51.67-79.07
CD3+/CD4+*					
Mean	47.07	47.41	44.14	47.87	45.33
Range	38.50-56.70	24.60-66.10	24.90-57.40	8.66-62.50	28.20-56.80
CD3+/CD8+*					
Mean	25.05	25.14	23.96	22.92	24.66
Range	15.20-34.10	10.50-41.10	15.00-33.70	3.51-47.90	19.50-30.70
CD4+/CD25+*					
Mean	1.09	0.68	1.20	1.30	0.87
Range	0.46-2.51	0.11-1.96	0.23-2.09	0.28-2.89	0.71-1.22
CD14+**					
Mean	3.60	7.28	5.39	5.23	2.90
Range	2.05-6.87	4.66-10.70	1.68-8.94	1.95-10.10	1.90-5.77
CD54+**					
Mean	0.47	0.22	0.33	0.28	0.68
Range	0.16-0.87	0.14-0.35	0.15-0.67	0.13-0.57	0.25-1.13

*% of cells expressing cell surface marker
**Mean linear fluorescence

Table 7
Unadjusted Risk of Symptoms by Exposure

	Allergen-exposed* n=76 No. %	Irritant-exposed* n=55 No. %	OR** (95% CI)
Work related upper respiratory symptoms	27 35.5	13 23.6	1.78 (0.82-3.88)
Work related lower respiratory symptoms	17 22.4	10 18.2	1.30 (0.54-3.10)

*Allergen exposed = latex and insect workers, irritant exposed = steel workers and welders
**Odds ratios derived using symptoms odds in irritant exposed as reference category

Table 8
Unadjusted Risk of Abnormal Lung Function by Exposure

	Allergen-exposed* n=76 No. %	Irritant-exposed* n=55 No. %	OR** (95% CI)
Abnormal FVC (<80% predicted)	5 6.8	0 0	-
Abnormal FEV_1 (<80% predicted)	8 11.0	2 3.8	3.14 (0.64-15.43)
Abnormal PEF (<80% predicted)	17 23.3	2 5.7	5.06 (1.40-18.29)
Abnormal MEF (<40% predicted)	2 2.7	3 3.8	0.72 (0.10-5.27)
FEV_1:FVC ratio <0.7	4 5.5	4 7.3	0.74 (0.18-3.10)

*Allergen exposed = latex and insect workers, irritant exposed = steel workers and welders
**Odds ratios derived using odds in irritant exposed as reference category

Table 9
Cell Surface Marker Activation by Exposure Status

	Allergen-exposed[1] n=76	Irritant-exposed[1] n=55	OR[2] (95% CI)
CD3+[3]			
Median	75.63	74.73	0.99
Range	41.05-87.79	12.43-83.70	(0.95-1.03)
CD3+/CD4+[3]			
Median	46.70	47.80	1.01
Range	24.60-66.10	8.66-62.50	(0.97-1.06)
CD3+/CD8+[3]			
Median	24.50	22.40	0.97
Range	10.50-41.10	3.51-47.90	(0.92-1.02)
CD4+/CD25+[3]			
Median	0.76	1.15	4.43
Range	0.11-2.09	0.28-2.89	(1.84-10.65)
CD14+[4]			
Median	6.35	4.06	0.77
Range	1.68-10.70	1.90-10.10	(0.63-0.95)
CD54+[4]			
Median	0.26	0.30	7.38
Range	0.14-0.67	0.13-1.13	(0.45-119.80)

[1]Allergen exposed = latex and insect workers, irritant exposed = steel workers and welders
[2]Odds ratios derived using odds in irritant exposed as reference category
Odds ratios represent change in odds of being irritant as opposed to allergen exposed for a unit increase in % cells expressing marker/MLF
[3]% of cells expressing cell surface marker
[4]Mean linear fluorescence (MLF)

Table 10
Cell Surface Marker Activation by Sensitisation Status

	Sensitised n=14	Non-sensitised n=98	OR (95% CI)
CD3+[3]			
Median	71.00	75.63	0.98
Range	59.17-82.92	12.43-87.79	(0.93-1.03)
CD3+/CD4+[3]			
Median	43.65	47.80	0.98
Range	24.60-58.10	8.66-66.10	(0.92-1.03)
CD3+/CD8+[3]			
Median	23.55	23.40	1.02
Range	12.40-41.10	3.51-47.90	(0.95-1.10)
CD4+/CD25+[3]			
Median	0.80	0.98	0.79
Range	0.11-2.09	0.13-2.89	(0.27-2.34)
CD14+[4]			
Median	6.52	4.99	1.13
Range	3.12-9.39	1.68-10.70	(0.84-1.52)
CD54+[4]			
Median	0.21	0.28	0.11
Range	0.17-0.55	0.13-1.13	(0.00-17.78)

Odds ratios represent change in odds of being sensitised as opposed to non-sensitised for a unit increase in % cells expressing marker/MLF
[3]% of cells expressing cell surface marker
[4]Mean linear fluorescence (MLF)

Table 11
Cell Surface Marker Activation by Symptoms Status

	Upper Symptoms n=40	No Upper Symptoms n=91	OR[2] (95% CI)
CD3+[3]			
Median	75.32	74.97	0.99
Range	12.43-82.92	41.05-87.79	(0.95-1.04)
CD3+/CD4+[3]			
Median	46.30	47.80	0.99
Range	8.66-62.50	24.60-66.10	(0.95-1.04)
CD3+/CD8+[3]			
Median	23.60	22.60	0.99
Range	3.51-41.10	7.96-47.90	(0.94-1.05)
CD4+/CD25+[3]			
Median	0.88	0.96	0.96
Range	0.11-2.89	0.23-2.13	(0.45-2.08)
CD14+[4]			
Median	7.11	4.21	1.37
Range	1.94-10.70	1.68-10.10	(1.09-1.71)
CD54+[4]			
Median	0.20	0.30	0.03
Range	0.14-0.57	0.13-1.13	(0.00-1.20)

Odds ratios represent change in odds of being symptomatic for a unit increase in % cells expressing marker/MLF
[3]% of cells expressing cell surface marker
[4]Mean linear fluorescence (MLF)

Table 12
Cell Surface Marker Activation by Symptoms Status

	Lower Symptoms n=27	No Lower Symptoms n=104	OR (95% CI)
CD3+[3]	75.14	75.21	1.01
Median	41.05-82.92	12.43-87.79	(0.96-1.06)
Range			
CD3+/CD4+[3]			
Median	48.60	47.00	1.02
Range	24.90-62.50	8.66-66.10	(0.97-1.08)
CD3+/CD8+[3]			
Median	22.75	23.50	1.00
Range	13.60-41.10	3.51-47.90	(0.94-1.06)
CD4+/CD25+[3]			
Median	0.76	0.98	0.95
Range	0.11-2.89	0.13-2.58	(0.40-2.28)
CD14+[4]			
Median	4.37	5.24	0.98
Range	1.94-9.62	1.68-10.70	(0.77-1.23)
CD54+[4]			
Median	0.31	0.27	1.90
Range	0.15-0.57	0.13-1.13	(0.13-27.00)

Odds ratios represent change in odds of being symptomatic for a unit increase in % cells expressing marker/MLF
[3]% of cells expressing cell surface marker
[4]Mean linear fluorescence (MLF)

Table 13
Unadjusted Risk of Symptoms by Workplace Sensitisation and Atopy

	Workplace sensitised* n=14 No. %	OR** (95% CI)	Atopic*** n=32 No. %	OR**** (95% CI)
Work related upper respiratory symptoms	7 50	2.50 (0.80-7.78)	9 28.1	1.37 (0.57-3.31)
Work related lower respiratory symptoms	5 35.7	2.31 (0.69-7.69)	12 37.5	1.86 (0.69-5.01)

*in allergen exposed workers only (n=57)
** Odds ratios derived using symptoms odds in non-sensitised workers as reference category
***in allergen and irritant exposed workers (n=101)
**** Odds ratios derived using symptoms odds in non-atopic workers as reference category

Table 14
Lung Function by Worker Group in those with Work Related Lower Respiratory Symptoms

	Irritant* n=10 No.	Non-sensitised* n=9 No.	Sensitised* n=5 No.	Sensitised versus Irritant	Sensitised versus non-sensitised
Abnormal FVC (<80% predicted)	0	0	1	P=0.333	P=0.357
Abnormal FEV_1 (<80% predicted)	1	1	3	P=0.077	P=0.095
Abnormal PEF (<80% predicted)	0	2	2	P=0.095	P=0.455
Abnormal MEF (<40% predicted)	3	0	1	P=0.571	P=0.357
FEV_1:FVC ratio <0.7	2	0	1	P=0.571	P=0.357

*Irritant group = steel workers and welders with work related respiratory symptoms; Non-sensitised group = insect workers and latex workers with work related respiratory symptoms but no workplace sensitisation, Sensitised group = insect workers and latex workers with work related respiratory symptoms and workplace sensitisation
Worker groups compared via Fisher's Exact Chi-Square Tests

Table 15
Lung Function by Worker Group in those with Work Related Upper Respiratory Symptoms

	Irritant n=13 No.	Non-sensitised n=15 No.	Sensitised n=7 No.	Sensitised versus Irritant	Sensitised versus non-sensitised
Abnormal FVC (<80% predicted)	0	1	1	P=0.350	P=0.545
Abnormal FEV_1 (<80% predicted)	1	3	2	P=0.270	P=0.523
Abnormal PEF (<80% predicted)	2	4	3	P=0.031	P=0.387
Abnormal MEF (<40% predicted)	3	1	1	P=0.589	P=0.545
FEV_1:FVC ratio <0.7	2	1	2	P=0.270	P=0.227

Worker groups compared via Fisher's Exact Chi-Square Tests

Table 16
Patterns of Symptoms Presentation by Worker Group in those with Work Related Lower Respiratory Symptoms

	Irritant n=10 No.	Non-sensitised n=9 No.	Sensitised n=5 No.	Sensitised versus Irritant	Sensitised versus non-sensitised
Work related wheeze	5	4	2	P=1.000	P=1.000
Work related cough	2	3	4	P=0.047	P=0.133
Work related shortness of breath	0	2	1	P=0.521	P=0.725
Work related phlegm	3	1	1	P=1.000	P=1.000
Work related chest tightness	4	4	5	P=0.041	P=0.086

Worker groups compared via Fisher's Exact Chi-Square Tests

Table 17
Patterns of Symptoms Presentation by Worker Group in those with Work Related Upper Respiratory Symptoms

	Irritant n=13 No.	Non-sensitised n=15 No.	Sensitised n=7 No.	Sensitised versus Irritant	Sensitised versus non-sensitised
Work related eye irritation	7	8	7	P=0.051	P=0.051
Work related nasal irritation	10	9	5	P=1.000	P=1.000

Worker groups compared via Fisher's Exact Chi-Square Tests

Table 18
Demographics by Work Group (Work Related Lower Respiratory Symptoms)

	Irritant n=10 No.	Non-sensitised n=9 No.	Sensitised n=5 No.	P Value
Age				P=0.387
Median (years)	42	33	47	
(Range)	32-56	28-59	28-52	
Duration of work at site				P=<0.001
Median (months)	186	48	24	
(Range)	156-300	18-156	2-84	
Duration of work in industry				P=0.015
Median (months)	186	72	24	
(Range)	156-360	18-504	2-240	
Gender				P=0.100
Male (%)	10 (100)	6 (66.7)	3 (60)	
Female (%)	0 (0)	3 (33.3)	2 (40)	
Smoking*				P=0.547
Current (%)	6 (60.0)	3 (33.3)	3 (60.0)	
Never (%)	2 (20.0)	3 (33.3)	2 (40.0)	
Atopy				
Positive	3 (42.9)**	2 (22.2)	4 (80.0)	P=0.112

Work sites compared via one way Kruskal Wallis H Test (for age and work durations) and Pearson's Chi-Square tests (for gender, smoking and atopy)
*Data for ex smoking not presented
**Only 7 of irritant group gave blood for a test for atopy to be carried out

Table 19
Demographics by Work Group (Work Related Upper Respiratory Symptoms)

	Irritant n=13 No.	Non-sensitised n=15 No.	Sensitised n=7 No.	P Value
Age				P=0.172
Median (years)	46	34	41	
(Range)	32-57	23-52	31-52	
Duration of work at site				P=<0.001
Median (months)	204	60	84	
(Range)	18-360	16-156	12-144	
Duration of work in industry				P=0.009
Median (months)	288	100	144	
(Range)	18-360	18-228	12-240	
Gender				P=0.003
Male (%)	13 (100.0)	6 (40.0)	5 (71.4)	
Female (%)	0 (0)	9 (60.0)	2 (28.6)	
Smoking*				P=0.955
Current (%)	4 (30.8)	6 (40.0)	3 (42.9)	
Never (%)	7 (53.8)	6 (40.0)	3 (42.9)	
Atopy				
Positive	4 (36.4)**	3 (20.0)	5 (71.4)	P=0.065

Work sites compared via one way Kruskal Wallis H Test (for age and work durations) and Pearson's Chi-Square tests (for gender, smoking and atopy)
*Data for ex smoking not presented
**Only 11 of irritant group gave blood for a test for atopy to be carried out

Table 21
Cell Surface Marker Activation by Worker Group in those with Work Related Upper Respiratory Symptoms

	Irritant n=13	Non-sensitised n=15	Sensitised n=7	Sensitised versus Irritant	Sensitised versus non-sensitised
Valid cases	n=12	n=13	n=7		
CD3+**				P=0.384	P=0.241
Median	74.87	75.92	69.88		
Range	12.43-80.91	66.19-81.65	63.31-82.92		
CD3+/CD4+**				P=0.384	P=0.351
Median	47.67	46.90	44.30		
Range	8.66-62.50	41.20-53.50	38.90-54.90		
CD3+/CD8+**				P=0.902	P=0.588
Median	23.35	26.10	23.50		
Range	3.51-30.70	15.30-32.00	18.00-41.10		
CD4+/CD25+**				P=0.340	P=0.817
Median	1.22	0.74	0.80		
Range	0.28-2.89	0.13-1.96	0.11-2.09		
CD14+***				P=0.884	P=0.181
Median	5.95	7.75	5.97		
Range	1.94-9.62	4.96-10.70	3.12-9.39		
CD54+***				P=0.404	P=0.295
Median	0.19	0.18	0.23		
Range	0.14-0.57	0.14-0.33	0.17-0.55		

**% of cells expressing cell surface marker
***Mean linear fluorescence
Worker groups compared via Mann-Whitney U Tests

Table 20
Cell Surface Marker Activation by Worker Group in those with Work Related Lower Respiratory Symptoms

	Irritant n=10	Non-sensitised n=9	Sensitised n=5	Sensitised versus Irritant	Sensitised versus non-sensitised
Valid cases	n=8	n=9	n=5		
CD3+**				P=0.622	P=0.699
Median	75.40	73.98	76.30		
Range	64.21-79.95	41.05-80.53	64.35-82.90		
CD3+/CD4+**				P=0.171	P=0.797
Median	48.60	49.40	41.20		
Range	45.10-62.50	24.90-58.00	37.40-55.40		
CD3+/CD8+**				P=0.065	P=0.518
Median	19.40	25.70	24.50		
Range	13.60-30.00	15.00-33.70	21.30-41.10		
CD4+/CD25+**				P=0.524	P=1.000
Median	1.39	0.71	0.63		
Range	0.38-2.89	0.25-1.15	0.11-2.07		
CD14+***				P=0.214	P=0.686
Median	3.38	5.68	7.04		
Range	1.94-9.62	3.98-7.39	3.12-9.39		
CD54+***				P=0.368	P=0.686
Median	0.38	0.32	0.21		
Range	0.15-0.57	0.18-0.48	0.17-0.55		

**% of cells expressing cell surface marker
***Mean linear fluorescence
Worker groups compared via Mann-Whitney U Tests

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7 APPENDICES

APPENDIX 1 – STUDY QUESTIONNAIRE

APPENDIX 2 – QUESTIONNAIRE REPEATABILITY

Tables A1 and A2 show data in relation to repeatability of the questionnaire.

Table A1

Reproducibility of the questionnaire: all results

SYMPTOM	EXACT MATCH	WORK-RELATED MATCH	NUMBER ANSWERED
PERSISTENT COUGH	8 (80%)	9 (90%)	10
PHLEGM	10 (100%)	10 (100%)	10
CHRONIC BRONCHITIS	10 (100%)	10 (100%)	10
CHEST TIGHTNESS	8 (80%)	8 (80%)	10
WHEEZE	7 (70%)	8 (80%)	10
EYE IRRITATION	8 (80%)	9 (90%)	10
NASAL IRRITATION	9 (90%)	9 (90%)	10
SHORTNESS OF BREATH	8 (80%)	10 (100%)	10
TOTAL	**68**	**73**	**80**
PERCENT	**85%**	**91%**	**100%**

Table A2

Latex Workers; inter interviewer results

SYMPTOM	EXACT MATCH	WORK-RELATED MATCH	NUMBER ANSWERED
PERSISTENT COUGH	5 (71%)	6 (86%)	7
PHLEGM	7 (100%)	7 (100%)	7
CHRONIC BRONCHITIS	7 (100%)	7 (100%)	7
CHEST TIGHTNESS	6 (86%)	6 (86%)	7
WHEEZE	5 (71%)	5 (71%)	7
EYE IRRITATION	5 (71%)	6 (86%)	7
NASAL IRRITATION	6 (86%)	6 (86%)	7
SHORTNESS OF BREATH	5 (71%)	7 (100%)	7
TOTAL	**46**	**50**	**56**
PERCENT	**82**	**89**	**100**

8 ANNEXES

ANNEX 1 – BASOPHIL STIMULATION DATA

This annex details the results of an in depth study carried out at the braiding factory. Whilst some of the methods are common to the main study, they are described in this section in detail.

CLINICAL ASSESSMENT

RECRUITMENT

Each employee at the braiding factory was invited to participate in the study. Employees attended meetings at which the study team described the investigation and the need for volunteers. Calling notices were also placed on notice boards within the factory. Ethical approval had been obtained as part of a larger study (ETHCOM/REG/01/10). Volunteers completed a consent form after having read the study information sheet and had the opportunity to discuss details with a doctor.

QUESTIONNAIRE

A physician-led questionnaire was administered to all consenting participants (appendix 1). This is an adaptation of the Medical Research Council (Minette 1989) and European Community Respiratory Health Survey (Burney 1994) designs. It focused on the presence (or absence) of a work relationship for each particular symptom and records the full work history.

Work related symptoms:
Work related symptoms were defined as those described by the individual as worse at work or alternatively as improving on rest days or during holidays.

Work-related lower respiratory symptoms (lower WRRS):
Symptoms of work-related cough, shortness of breathe, chest tightness or wheeze are categorised as lower respiratory symptoms for the purpose of this study.

Work-related upper respiratory symptoms (upper WRRS):
Work–related nose, eye, mouth or throat irritation (mucous membrane irritation) are categorized as upper respiratory symptoms for the purpose of this study.

Atopy:
This was defined as a positive RAST test to one or more of five common environmental aeroallergens including pollens, house dust mite and cat dander.

LUNG FUNCTION ASSESSMENT

All lung function assessments were carried out using a Fleisch type pneumotachograph spirometer (Vitalograph Alpha III, Vitalograph, UK). This machine has previously undergone an “in house” reproducibility exercise and found to be accurate. The spirometer was calibrated daily, and adjusted for ambient temperature. The flow head was warmed each day by passing air from the test room repeatedly over the head.

All consenting workers were asked to perform reproducible forced expiratory manoeuvres, so as to measure the forced expiratory volume in one second (FEV_1) and the forced vital capacity (FVC). Standard acceptance criteria were used to determine the validity of the spirometry values

obtained (best two; within 200mls: the American Thoracic Society criteria). Those workers with technically unsatisfactory results were encouraged to produce satisfactory results, and if this was not possible the data was not used in subsequent analysis.

VENEPUNCTURE

This was performed using a standard technique in the antecubital fossa. All blood specimens were collected into:

1. Lithium heparin vacutainer bottles (BD U.K. Ltd.) for the basophil stimulation study

2. Plain vacutainer bottles (BD UK Ltd.) for serum RAST analysis (to identify the presence of specific IgE to latex and/or cotton), and to common environmental allergens.

RAST ANALYSIS

RAST analysis was performed as according to section 2.2.2. Each serum sample was tested against atopy discs, and discs prepared from extracts of latex, dyed cotton, latex braiding coated with silica or talc, provided by the braiding company.

FLOW CYTOMETRIC ANALYSIS OF BASOPHIL ACTIVATION

Whole blood samples from individuals were collected in Vacutainer (Becton Dickinson, Oxford, UK) heparinised tubes. 100 µl of whole blood was incubated for 30 minutes at 37°C with 5µl of *latex* (Allergon AB, Ängelholm, Sweden), dissolved in sterile Phosphate Buffered Saline (PBS), at a final concentration of 0.05, 0.1 or 0.5 µg/ml.

As a negative control, 100µl of whole blood from each subject was incubated with 5µl sterile PBS under the same conditions, or with scampi allergen at a final concentration of 0.5µg/ml. For the quantification of basophil activation following LPS challenge, 100µl of whole blood was either incubated with 5µl of allergen and LPS (*Escherichia coli* serotype 055:B5, Sigma, UK) mix, at a final concentration of either 0.05µg/ml *latex* with 0.01, 0.05 or 0.1µg/ml LPS, 0.5µg/ml *latex* with 0.01, 0.05 or 0.1µg/ml LPS, or LPS alone.

The samples were then stained with a combination of the monoclonal antibody CDw123 (PE-labelled, Becton Dickinson Ltd, London, UK), HLA-DR (Pc5-labelled) and CD63 (FITC-labelled) (Beckman Coulter, High Wycombe, UK) for 25 minutes at room temperature in the dark. Isotype control antibody conjugates were included with each sample.

The red cells were lysed, and the remaining cells were fixed using the Coulter Immunoprep system (Beckman Coulter, High Wycombe, UK). The lysed whole blood was analysed using a Coulter Epic XL flow cytometer. Basophils were identified with CDw123+ve/HLA-DR-ve backgating (Heinemann *et al* 2000) and at least 1000 events within this gate were analysed.

STATISTICAL ANALYSIS

Group results were expressed as mean ± standard deviation and initially One-Way Analysis of Variance test for linear trend between column means and number was undertaken. T-test were used to determine significant differences between population means. All analysis was undertaken using GraphPad Instat (version 3.0 for Windows 95, GraphPad software, California, USA).

RESULTS

RECRUITMENT

44 individuals, from a total workforce of 86, consented to take part in the study (22 male and 22 female). Employees were recruited from all work areas of the site (i.e. office, machine operators, quality control, packing, mechanics and stores). All 44 individuals are part of a larger study which included the analysis of T-cell activation markers. 23 individuals, from the 44 volunteers, were randomly selected for the *in vitro* basophil stimulation assay.

QUESTIONNAIRE DATA

All 44 volunteers completed the questionnaire. The group selected for the basophil stimulation assay (n=23) was compared against the group as a whole in terms of age, gender and duration of employment at the worksite. Table 1AN details these descriptive statistics and it can be seen that the basophil stimulation group appear to be representative of the volunteer population as a whole.

Table 1AN

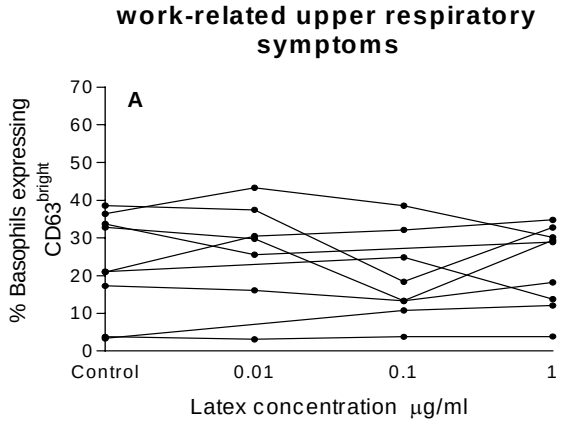
	All volunteers N=44	**Basophil group N=23**
Male (%)	50	44
Mean age in years (range)	37 (19-59)	41 (19-59)
Mean months employed at this worksite	61 (1-156)	67 (1-156)
Current or ex smokers (%)	73	75

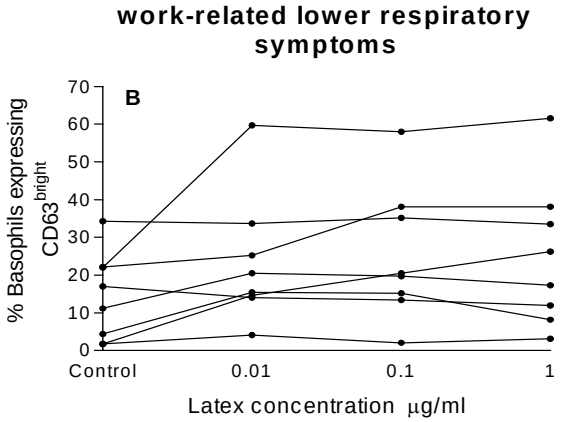
Of the 23 chosen for further analysis, 6 had no respiratory symptoms, 9 had work related upper respiratory symptoms and 8 complained of work related lower respiratory symptoms.

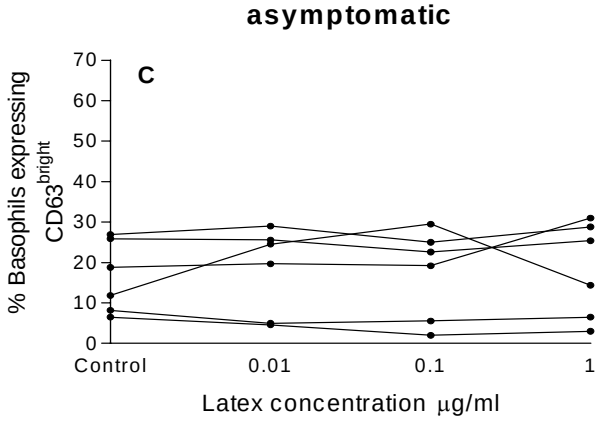
BASOPHIL STIMULATION ASSAY

Repeated measures ANOVA for those with lower WRRS demonstrated a significant trend of increasing basophil stimulation (percentage of cells expressing $CD63^{bright}$) across the concentrations of latex used for *in vitro* stimulation ($p=0.021$). This was not the case for the asymptomatic and upper WRRS groups. [For upper WRRS and asymptomatic individuals the values were $p=0.719$ and $p=0.902$ respectively]. The dose response to latex stimulation for each individual grouped according to symptoms is shown in fig.1AN.

Statistical analysis was utilised to determine a significant difference in $CD63^{bright}$ expression



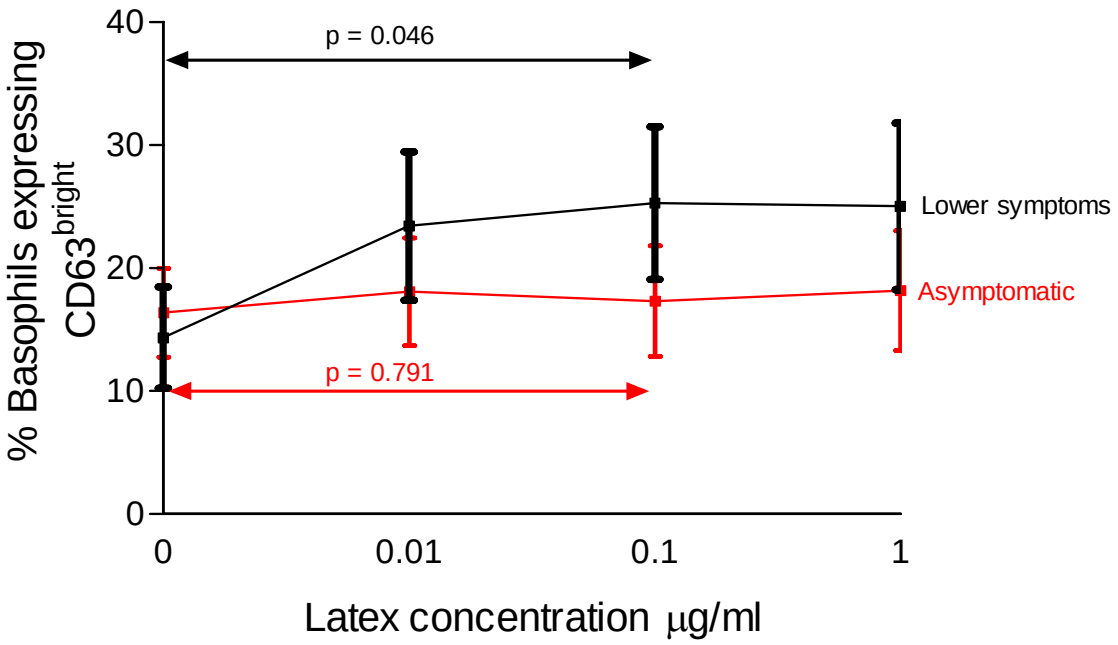




between the asymptomatic group, and the group with lower WRRS. A paired t test (2-tailed) comparing basophil stimulation at 0 and 0.1ug/ml latex for the two groups is shown in figure 1AN. From this it can be seen that in the lower WRRS group, there was a significant increase in $CD63^{bright}$ expression following stimulation with 0.1 µg/ml latex ($p=0.046$).

Figure 2AN Percentage of basophils expressing $CD63^{bright}$ following latex stimulation. Individuals are grouped according to the presence or absence of lower WRRS.

Figure 1AN. Percentage of basophils expressing CD63^{bright} following latex stimulation



DETERMINATION OF SIGNIFICANT BASOPHIL STIMULATION

The pooled data of the asymptomatic individuals was used as a reference group. At a latex concentration of 1 μg/ml, the mean increase in the percentage of activated basophils (compared to control) was 1.82 (S.D. 5.56). To assign a value to the upregulation of CD63 (basophils stimulation);

Basophil stimulation = mean % of basophils expressing CD63^{bright} at latex 1μg/ml - mean % of basophils expressing CD63^{bright} at control

Significant basophil stimulation was arbitrarily defined as being an increase of 15.70 percent of basophils expressing CD63^{bright}. This represented the increase in percentage of basophils from the asymptomatic reference group expressing CD63^{bright} + 2.5 S.D. 1.82 + (2.5x5.56) = 15.70

Using this value as a threshold to determine significant basophil stimulation:

- no individuals with work-related upper respiratory symptoms demonstrated significant basophil stimulation
- 3 individuals with work-related lower respiratory symptoms demonstrated significant basophil stimulation

SYMPTOMS, SPECIFIC IGE AND BASOPHIL STIMULATION

Each group of workers was assessed for specific IgE to NRL, and a positive basophil stimulation response. The individual results of RAST analysis for specific IgE and the basophil stimulation assay are presented in table 2AN.

WORK-RELATED LOWER RESPIRATORY SYMPTOMS

Of 8 individuals with work-related lower respiratory symptoms (lower WRRS): 4 were atopy IgE positive and of these 2 were NRL IgE positive. Both NRL IgE positive individuals demonstrated significant *in vitro* basophil stimulation to NRL. A third individual also demonstrated basophil stimulation.

WORK-RELATED UPPER RESPIRATORY SYMPTOMS

All 9 of the individuals with upper WRRS were NRL IgE negative (2 were atopy IgE positive). None demonstrated *in vitro* basophil stimulation to NRL.

ASYMPTOMATIC GROUP

All 6 asymptomatic workers were negative for NRL IgE and atopy IgE. None demonstrated *in vitro* basophil stimulation.

Table 2AN. Individual RAST and basophil stimulation results

ID	RAST score >2 is positive		
	ATOPY	LATEX	Increased expression of CD63
ASYMPTOMATIC 209	0.6	1.2	No
ASYMPTOMATIC 217	0.5	0.9	No
ASYMPTOMATIC 218	1.2	1.1	No
ASYMPTOMATIC 219	0.6	1.1	No
ASYMPTOMATIC 234	1.1	1.0	No
ASYMPTOMATIC 236	1.9	0.9	No
UPPER WRRS 213	**106.2**	1.9	No
UPPER WRRS 226	1.0	1.0	No
UPPER WRRS 241	0.7	1.0	No
UPPER WRRS 237	0.5	0.9	No
UPPER WRRS 208	0.7	1.0	No
UPPER WRRS 210	**8.4**	1.0	No
UPPER WRRS 203	0.6	0.9	No
UPPER WRRS 239	1.3	1.3	No
UPPER WRRS 212	0.9	1.1	No
Lower WRRS 216	1.6	1.7	No
Lower WRRS 244	**16.0**	**21.8**	**Yes**
Lower WRRS 205	**101.4**	**3.1**	**Yes**
Lower WRRS 214	0.6	1.0	**Yes**
Lower WRRS 233	0.5	1.1	No
Lower WRRS 222	0.8	1.0	No
Lower WRRS 235	**24.3**	1.0	No
Lower WRRS 232	**8.8**	1.1	No

DISCUSSION

The clinical diagnosis of work-related respiratory health effects must include a clear history of work-related symptoms. In addition, a putative causal agent must be present in the workplace and some evidence is usually sought to demonstrate individual susceptibility to that agent.

RAST analysis, for the detection of specific IgE to the occupational allergen in question, is the most commonly used tool. However, the presence of work related symptoms often does not correlate with the presence of specific IgE. This can be seen in the current investigation of a textile-braiding factory. There were 8 individuals with lower WRRS but only 2 with specific IgE to latex. This discrepancy is not clearly understood, but may in part be due to the limited sensitivity of the RAST assay.

An alternative view is that the mixed exposure to allergens and other substances in the workplace is not accurately reflected in the production of RAST discs. This may be due to the presence of endotoxin in the workplace, or that individuals may be exposed to a highly complex mixture of allergens.

Further, the presence of specific IgE does not predict that an individual will have symptoms upon exposure to the allergen. It is sometimes necessary to consider specific allergen challenges e.g. skin prick tests or specific inhalation challenges that measure a functional outcome so as to determine an individual's allergic state. Specific inhalation challenges are time consuming, necessitate the volunteer being observed in a hospital setting and can be life-threatening. The *in vitro* basophil activation assay aims to be a specific allergen test that measures a functional outcome safely.

From the clinical analysis, it was observed that 23% (10/44) of the population of employees who volunteered to take part in this study reported lower work-related symptoms (7 of which also reported upper work-related symptoms), 50% (22/44) reported upper work-related symptoms, and that 43% (19/44) reported no symptoms at all. Unfortunately, no information was available regarding those individuals who did not take part in the study. It is possible that the high level of reported symptoms may in part be due to the fact that a higher proportion of individuals with symptoms volunteered for the study. It would be impossible to rule this out unless information regarding the non-participants was available.

A small subset of volunteers was randomly chosen to take part in the basophil study. Of the 6 individuals with no reported symptoms and the 9 individuals with upper work-related symptoms none demonstrated specific IgE to natural rubber latex, or a positive basophil response. However, 2 individuals with lower work-related symptoms demonstrated specific IgE to natural rubber latex and a positive basophil response, and a further individual demonstrated a positive basophil response only. The results from this analysis indicates the increased sensitivity of the basophil test in comparison to the traditional RAST assay.

During this project, we have developed a basophil activation assay, and demonstrated its use as a diagnostic tool in the assessment of respiratory health effects, both during a mixed exposure (to allergen and endotoxin) and in a workplace setting. The *in vitro* basophil assay was shown to be a more sensitive diagnostic tool for the prediction of work related respiratory effects of individuals working at a braiding factory, compared to the RAST analysis. It is a quick and simple tool, which has the potential to be utilised in the diagnosis of occupational asthma.

ANNEX 2 – AIRBORNE ALLERGEN ASSESSMENT

AIRBORNE DUST CONCENTRATIONS

METHODS

Personal dust sampling was carried out at 2 sites (insect breeders and the latex braiding). In each case, the following methods were used.

Personal inhalable sampler heads (SKC I.O.M. sampler, SKC Ltd., U.K.) were attached to the individual's clothing so that they were within the breathing zone of the subject. Air was drawn over the filters by means of a portable pump at 2 litres/min. The inhalable dust was collected by means of a filter medium and different fractions could be measured. Simple gravimetric analysis was used (after correction for change in control filter weight) to calculate total inhalable dust levels.

TOTAL PROTEIN ESTIMATION

Total protein estimation was performed using a bicinchoninic acid protein assay, (BCA) [Smith *et al* 1985] using the Cobas Fara. The BCA method has the advantage of being tolerant to detergents used in protein solubilisation, such as Tween 20. Protein determination reagent consists of 5ml bicinchoninic acid (Sigma) with 100µl of copper(II) sulphate (Sigma). The principle behind the assay is that the protein in the sample will reduce alkaline Cu(II) to Cu(I).The bicinchoninic acid is a chromogenic reagent for Cu(I), and produces a purple complex with maximum absorbance at a wavelength of 562nm. The protein standard used was bovine serum albumin (1mg/ml, Sigma) and quality control samples were prepared from human serum albumin (Sigma) in distilled water.

RESULTS

Data from the insect-breeding site were taken from one sampling visit (02.04.03). The mean total inhalable dust levels were measured at 3.65mg/m^3 (range 0.13 - 19.75). Total protein estimates for this sampling day were measured at a mean of 0.076mg/m^3 (range 0.01 - 0.138).

Data for the latex-braiding site were taken from a two sampling visits (10.12.02 and 16.01.03). The mean total inhalable dust was measured at 1.04 mg/m3 (range 0.34-1.44) on the first sampling day, and a mean on the second sampling day of 1.26mg/m^3 (range 0.09-1.93). Total protein estimation for these days noted means respectively of 60.4µg/m^3 (range 3.29 - 442.3) and 10.26µg/m^3 (range 4.07 – 15.54).

ANNEX 3 – AIRBORNE ALLERGEN ASSESSMENT

LATEX ASSAY

BACKGROUND

Enzyme linked immunosorbant assay (ELISA) is a precise and sensitive method for the estimation of biological parameters. The method involves linking an immunoreagent (antigen or antibody) to a plastic microtiter plate by passive absorption, commonly called 'coating' the plate. The test substance containing antigen or antibody is added to the solid phase in limiting amounts, so it can bind to the pre-bound protein.

The unbound material is washed away and the quantity of bound protein is determined by the addition of an enzyme-linked antibody specific for the bound species, which reacts with a chromogenic substance and causes a colour development. The colour developed for the test substance is compared against a standard curve generated from known amounts of test protein. In this study, the system was adapted using a FITkit™ (FIT Biotech Oyj Plc, Tampere, Finland) for the determination of hevein in natural rubber latex products.

At present four of the identified latex allergens have been unequivocally demonstrated in manufactured products (Hev b1, Hev b3, Hev b5, Hev b6.02), and FITkit™ provide assay kits for each of the allergens.

ASSAY METHOD

Microtiter plates were pre-coated with mouse monoclonal Hev b 6.02 antibody provided with the kit. 50µl per well of the assay buffer (containing phosphate, sodium chloride, EDTA, bovine plasma albumin, and mouse antibodies) were added to the plate, followed by 50µl of either standards (0-10ng/ml, diluted in PBS from a 200ng/ml standard provided with the kit), QCs or diluted test samples. The plate was incubated for 2 hours at room temperature on a plate shaker.

After washing four times, 100µl per well of enzyme conjugate (horse radish peroxidase conjugated monoclonal anti-Hev b 6.02) was added and incubated for one hour, again at room temperature on a plate shaker.

The plates were then washed and a TNB solution was added and the plates incubated for 30mins. The reaction was stopped by the addition of 2M sulphuric acid. The plates were read on a plate reader (Biotek) at 450nm. The method was repeated using the Hev b1, Hev b3, Hev b5 test kits.

RESULTS

Two sampling exercises were carried out. The first was competed on 10.12.02, and noted the following results. Total dust values in the personal breathing zone ranged between 0.34 and 1.44 mg/m3. Total protein levels varied between 3.29 and 442.3 ug/m3. Airborne Hevb6 varied between 0.30 and 3.29 ng/m^3.

The second sampling exercise was carried out on 16.01.03, and noted the following results. Total dust values in the personal breathing zone ranged between 0.09 and 1.93 (mean 1.26) mg/m3. Total protein levels varied between 4.06 and 15.5 (mean 10.26) ug/m3. Airborne Hevb6 varied between 0.04 and 0.92 ng/m^3.

ANNEX 4 – CYTOKINE ASSAYING

BACKGROUND AND AIMS

The respiratory system exhibits a limited ability to respond to an external insult. Therefore, the clinical presentation of lower respiratory symptoms in workers, whether exposed to allergens or irritants in the workplace, tends to be characterised by one or, more usually, a combination of work-related cough, phlegm, chest tightness, wheeze or shortness of breath. In a workplace where workers are exposed to a several different exposures, perhaps including both allergens and irritants, determining the predominant pathological mechanisms underlying symptoms in an individual is important as it allows exposure avoidance strategies to be optimally targeted. The documentation of workplace sensitisation to allergens by skin prick or specific serum IgE testing in workers reporting respiratory symptoms, provides an indication that the causative pathology underlying symptoms in such workers, such as airway inflammation or hyperresponsiveness, is IgE mediated and likely to be predominantly attributable to allergen exposure.. Alternatively, the absence of sensitisation is suggestive that the causative pathology is perhaps driven by a humoral response mediated by antibody other than IgE, or perhaps is induced more by exposure to irritant.

The characterisation of respiratory disease based on the documentation of sensitisation makes the assumption that the development of respiratory symptoms in those exposed to allergen necessarily follows the sensitisation process. However, a number of studies have documented sensitisation in individuals exposed to allergen reporting no respiratory symptoms, as well individuals exposed to allergen reporting respiratory symptoms in the absence of sensitisation. The association between occupational exposure, airway inflammation, airway hyperresponsiveness and resulting respiratory symptoms is clearly complex. Whether workers sensitised to workplace allergen subsequently go on to develop respiratory symptoms attributable to work is likely to be determined, in part, by personal susceptibility factors, the potency of the allergen exposed to, as well as the magnitude, frequency and duration of allergen exposure, which are all subject to inevitable variation. The absence of sensitisation in those exposed to allergen and reporting symptoms may be explained by the mechanism underlying symptoms perhaps being non-IgE mediated and therefore sensitisation not being demonstrable, or perhaps by the non-allergic effects of irritant exposures in workplaces where workers are also exposed to allergen. Whether sensitisation is demonstrated in those symptomatic is also likely to be influenced by the sensitivity of the test used for the documentation of sensitisation status. For example, validation studies suggest specific serum IgE measurement to be a less sensitive test for evaluation of allergic sensitisation than skin prick testing.

Concentrations and profiles of cytokines and chemokines in peripheral blood may provide an alternative surrogate of respiratory disease pathology to the documentation of sensitisation and in doing so may provide a greater insight into the causes and mechanisms of respiratory disease attributable to occupational exposures. Collectively, cytokines and chemokines coordinate immune responses, with particular sets coordinating either cellular based immunity (mediated by T lymphocytes) or humoral immunity (involving specific recognition and elimination of antigens, essentially mediated by B lymphocytes and antibodies, for example IgE). The roles of cytokines and chemokines are subtly different; cytokines are involved in directing the function of the immune cells whereas chemokines are involved in directing migration of specific immune cells to sites of inflammation. Cytokines derived from both $CD4^+$ T helper (Th) 2 cells and $CD8^+$ cytotoxic T cells are thought to play an important role in the pathology of respiratory diseases such as asthma. For example, elevated CD4 Th2-cell activation is now firmly established as a key defining characteristic of respiratory diseases with an underlying allergic cause, with certain Th-2 cytokines believed to co-ordinate key humoral responses such as B cell activation and IgE synthesis. In addition, evidence is also accumulating suggesting that Th1-cell

cytokines may actively suppress the release of Th2 cytokines and in doing so protect against the expression of allergic responses. The role of cytotoxic T cells in orchestrating airway immune responses is less well understood but it may be that such cells lyse host cells damaged by toxic exposures and the effects of localised inflammation, as well as foreign invading cells such as bacteria and viruses.

Several population based studies have investigated trends in cytokine and chemokine levels in peripheral blood, many studies focusing on trends apparent in certain diseased or allergic groups, such as asthmatics (Camilla *et al.* 2001), atopics (Wosinska-Becler *et al.* 2004), those suffering respiratory infections (Pitrez *et al.* 2004), or in groups exhibiting certain pathology, for example early/late phase allergic responses (Cieslewicz *et al.* 1999), airway hyperresponsiveness (Hakonarson *et al.* 1999) or airway inflammation (Makela *et al.* 2000). In addition, a few studies have investigated cytokine and chemokine levels in certain occupationally exposed groups, comparing trends, often for various sub-categories, for example Th2 and Th1 cytokines, in exposed groups relative to those non-exposed (Iavicoli *et al.* 2005, Saikai *et al.* 2004).

Numerous studies have documented infiltration and activation of an array of inflammatory cells, including Th-2 lymphocytes, mast cells, basophils and eosinophils, at sites of acute allergic inflammation, including the airways of allergic asthmatics (Greenfeder *et al.* 2001, Steinke *et al.* 2001). Related to these findings, Th-2 cytokines, including interleukin (IL)-4, IL-5 and IL-13, have been consistently shown to be upregulated, and Th-1 cytokines, interferon-γ (IFN-γ) in particular, downregulated, at sites of allergic inflammation ((Vladich *et al.* 2005, Herrick *et al.* 2000, Cieslewicz *et al.* 1999, Hakonarson *et al.* 1999, Doucet *et al.* 1998). Studies have also shown the activation of pro-inflammatory cells, such as Th-2 lymphocytes, to induce other inflammatory mediators and cytokines, such as IL-9, IL-10 and granulocyte-macrophage colony-stimulating factor (GM-CSF) (Yuhong Zhou *et al.* 2001, Stampfli *et al.* 1998). While IL-9 and GM-CSF have been shown to exhibit pro-inflammatory effects similar to IL-4, IL-5 and IL-13, the effects of IL-10 have been reported in a number of studies to be anti-inflammatory, which has been suggested to reflect a regulatory immune response mechanism (Yssel *et al.* 1992 see Laouini et al. 2003 ref 8).

Cytokines including interferon-γ (IFN-γ), tumour necrosis factor-α (TNF-α), IL-2 and IL-12, are widely regarded to characterise Th-1 type immune responses and several studies have reported upregulation of such cytokines in individuals with certain viral or bacterial infections, for example SARS (Li et al. 2003). In addition, IL-1 (both α and β types), predominantly expressed by monocytes, has been shown to be an important inflammatory mediator when present in combination with these cytokines, TNF α and IFN γ in particular (Dube et al. 2004). Legg *et al.* (2003), in contrast, implicated a deficient Th-1 response in the pathogenesis of RSV bronchiolitis. Similarly, several studies have observed downregulation of Th-1 type cytokines in individuals with allergic diseases, such as atopic asthma. Other cytokines, such as IL6, are often described as being multifunctional (Dube et al. 2004), exhibiting both pro- and anti-inflammatory effects as well as being involved in a variety of immune responses.

The ratios of levels of Th-2 to Th-1 cytokines is often used as an effect measure to characterise the immune profiles of individuals with certain disease types, or exposed to certain types of exposures. For example, Saikai *et al.* (2004) found the immune profile of mushroom workers to exhibit a shift toward a Th-2 dominant state characterised by increased IL-13 and decreased IFN-γ, which were attributed to innate immunity to spore allergen. In contrast, Iavicoli *et al.* 2005 compared levels of IL-2, IL-4 and IFN-γ in workers exposed to trichloroethylene, a volatile organic compound widely used as an industrial solvent, to those in non-exposed

controls. The study observed increases in IL-2 and IFN-γ and a decrease in IL-4 in the exposed workers, which were attributed to workplace exposure to trichloroethylene.

Chemotactic cytokines, otherwise known as chemokines, play a major role in regulating which specific leukocytes are recruited to areas of inflammation. As well as influencing cell migration, chemokines are also thought to influence immune responses by altering cytokine profile and by mediating the activation and degranulation of distinct leukocyte populations. IL-8 is one of a class of chemokines, known as CXC or α chemokines, considered the most important mediators of the accumulation of granulocytes, including neutrophils and eosinophils (Lukacs et al. 1999). ENA78 (or Epithelial cell derived neutrophil activating peptide) is an inflammatory chemokine produced concomitantly with IL-8 in response to stimulation with IL-1 or TNFα (Lukacs et al. 1999). The second main class of chemokines is the CC or β chemokines. RANTES is one such chemokine that has been shown to be a potent mononuclear cell chemoattractant (Lukacs et al. 1999).

The main objective of this study was to identify trends in the cytokine profile of different groups of workers exposed to allergens and irritants in the workplace and reporting work related respiratory symptoms, in particular, to determine whether workers sensitised to workplace allergen and reporting respiratory symptoms had a different cytokine profile to those reporting respiratory symptoms attributable to irritant exposures. A further objective was to investigate whether the trends in cytokine profile observed for different groups of workers provided any better a predictor of the reporting of work related symptoms than other more traditional clinical tests such as the documentation of workplace sensitisation to allergen.

RESEARCH METHODS

Data on a group of 17 office workers, 18 latex braiders and 35 metal workers were investigated. These worker groups had previously been investigated as part of a HSL study investigating the causes and clinical presentation of work related respiratory symptoms in workers exposed to irritants and allergens in the workplace. The reader is referred to the main sections of this report for more detailed information on study methodology. Respiratory symptoms, lung function and atopic and workplace specific IgE status were measured along with a range of workplace and demographic factors as part of the original study. This dataset was supplemented with measurements of cytokine levels in peripheral blood derived as part of the present study by reanalysing stored serum samples (where sufficient serum remained) that had been previously provided by the workers.

CYTOKINE PROFILING

Intracellular cytokines and chemokines in peripheral blood were assayed using a Luminex BioPlex bead reader. A Bio-Plex Cytokine Reagent kit and Bio-Plex Human Serum Diluent kit (Bio-Rad Laboratories Inc., Hemel Hempstead) were used. A pre-prepared Bio-Plex Human Cytokine panel (Bio-Rad) was also used containing beads conjugated to antibodies corresponding to the following TH1 cytokines: INFg, TNFa, IL2, Ila, IL8, TH2 cytokines: IL4, IL-5, IL-10, and chemokines: RANTES, ENA78. All samples/beads were added together in a 96 well MultiscreenTM-BV filter plate (Millipore, Watford). Serum samples were thawed and centrifuged for 5 minutes at 3000g, 100ml aliquots of the serum were then diluted 1 in 2 in the BioRad assay diluent buffer. 50ml of the multiplex bead solution was then added gently mixed and incubated at 200C for 45 minutes. The beads were washed by vacuum filtration and addition of excess BioPlex Wash Buffer wash solution according to the manufacturers instructions. The beads were then resuspended in 25ml of BioPlex Detection Antibody mixture (for the relevant cytokines) and incubated with mixing for 30 minutes at 200C. The beads were washed as previously described and resuspended in 50ul of BioPlex Streptavidin Phycoerythrin

and incubated for 20 minutes in the dark at 200C. The beads were then washed according to the manufacturers instructions and resuspended in 125ml of BioPlex Assay Buffer.

A calibration curve of standards for each of the cytokines was included in the assay with a range from 0.2 to 3,200 pg/ml. In addition to the standards, 3 replicates of 2 quality control samples were included. The quality control samples consisted of pooled serum containing a low level or a high level of cytokines. Quality control samples were produced by pooling the mononuclear fraction from two healthy adult volunteers and separating into two sets of cells. The mononuclear cells were isolated from 20ml peripheral blood samples from which the serum was recovered and stored at –200C and the mononuclear cell population recovered and counted. The mononuclear cells were divided into two sets of cells and resuspended at a concentration of 107 per ml in X-Vivo-15TM medium (BioWhittacker; Cambrex BioScience, Wokingham Ltd). 10 ng/ml of phorbol myristal acetate (PMA) was added to one set of cells to activate the release of inflammatory cytokines. The second sample was incubated in medium alone. Both sets of cells were incubated at 370C in a humidified 5% CO_2 incubator. After 24 hours the cells were pelleted by centrifugation at 200g for 5 minutes and the supernatant collected. The supernatant was then added back in an appropriate volume to original serum sample. The samples were aliquoted and stored at –400C.

The plate was analysed using a BioRad BioPlex (Luminex 100) system using a sample size of 50ml per well and 100 beads per region settings. Software from BioRad; BioPlex Manager 3.0, automatically calculated the concentration of each cytokine (pg/ml) from the standard curves.

STATISTICAL ANALYSIS

All data analyses were performed using SPSS (Statistical Package for Social Scientists v13.0, SPSS Inc., Chicago, USA). Data on cytokine levels were on the whole non-normally distributed and therefore non-parametric statistical techniques, including the Mann Whitney U Test (differences between 2 samples) and Kruskal-Wallis H Test (differences between >2 samples), were used to test for significant differences across worker groups. Correlations between cytokine and cell surface marker levels were investigated using both the Spearman's Rank and Kendal's tau-b test. Results are presented in the tables that follow.

RESULTS TABLES

Table 1: Cytokine Concentrations (pg/ml) by Worksite

	Office Workers n=17	Metal Workers n=35	Latex Workers n=18	Metal v Office P-value	Latex v Office P-value	Metal v Latex P-value
IL1-α median range	all<LOD	all<LOD	all<LOD	1.000	1.000	1.000
IL2 median range	2.26 1.39-4.49	1.06 0.06-3.16	1.84 0.90-2.40	<0.001	0.003	<0.001
IL4 median range	0.88 0.88-4.67	0.88 0.88-5.04	all<LOD	0.218	0.140	0.473
IL5 median range	all<LOD	0.17 0.04-2.26	0.17 0.15-0.51	0.191	0.310	0.654
IL6 median range	2.81 2.33-4.23	2.71 2.07-3.52	2.03 1.29-4.90	0.001	0.540	<0.001
IL8 median range	22.47 4.07-1345.85	14.76 2.77-1618.84	22.53 2.44-413.87	0.396	0.644	0.693
IL10 median range	1.59 1.26-2.49	0.55 0.13-1.83	1.45 0.55-2.13	P<0.001	0.042	<0.001
TNF-α median range	10.35 6.46-14.66	5.91 2.92-10.92	8.16 5.34-10.99	P<0.001	0.021	<0.001
IFN-γ median range	0.91 0.16-2.70	0.16 0.04-0.86	0.37 0.16-0.94	P<0.001	0.009	<0.001
ENA 78 median range	1160.53 360.81-2377.08	993.83 323.55-3312.76	911.74 536.90-2829.18	0.238	0.060	0.513
RANTES median range	3870.84 2212.98-5530.07	4192.90 1259.18-6588.77	5317.45 1995.73-8138.12	0.764	0.040	<0.001

Half LOD assumed for values <LOD

Table 2: Cytokine Concentrations (pg/ml) by Worksite for symptomatic and non-symptomatic groups

	Office Workers (No symptoms) n=12	Latex Workers (No symptoms) n=9	Latex Workers (Any WR symptoms) n=9	Metal Workers (No symptoms) n=23	Metal Workers (Any WR symptoms) n=12
IL1-α					
median	all<LOD	all<LOD	all<LOD	all<LOD	all<LOD
range					
IL2					
median	2.38	2.04	1.75	0.98	1.10
range	1.39-4.49	1.34-2.40	0.90-2.33	0.06-3.16	0.41-2.37
IL4					
median	0.88	all<LOD	all<LOD	0.88	all<LOD
range	0.88-4.67			0.88-5.04	
IL5					
median	all<LOD	0.17	0.17	0.17	0.21
range		0.17-0.34	0.15-0.51	0.04-0.73	0.04-2.26
IL6					
median	2.70	2.64	2.77	1.96	2.22
range	2.33-4.23	2.07-3.52	2.07-3.36	1.29-3.02	1.77-4.90
IL8					
median	14.57	21.07	24.01	14.77	13.16
range	4.07-736.46	2.44-61.09	8.56-413.87	2.77-1618.84	6.00-1382.81
IL10					
median	1.49	1.49	1.41		
range	1.26-2.49	0.55-2.13	0.55-1.62	0.53	0.57
				0.13-1.83	0.46-1.32
TNF-α					
median	10.89	8.99	6.63	5.91	5.44
range	7.54-12.34	5.90-10.99	5.34-10.42	2.92-8.82	2.92-10.92
IFN-γ					
median	0.94	0.35	0.48	0.16	0.16
range	0.16-1.23	0.16-0.94	0.16-0.76	0.04-0.86	0.04-0.47
ENA 78					
median	1192.78	951.74	844.94	1107.48	888.88
range	360.81-2377.08	536.90-1419.38	593.67-2829.18	323.55-3312.76	437.11-1669.28
RANTES					
median	3876.84	5317.45	5144.87	4158.43	4218.44
range	2508.52-5530.07	4685.42-8138.12	1995.73-6762.18	1259.18-6122.83	1892.14-6588.77

Half LOD assumed for values <LOD

Table 3: Cytokine Concentrations (pg/ml) by Worksite for symptomatic and non-symptomatic groups

	Latex Workers (No symptoms) n=9	Latex Workers (Any WR symptoms) n=9	Latex Workers (Symptoms versus No Symptoms) P-value	Metal Workers (No symptoms) n=23	Metal Workers (Any WR symptoms) n=12	Metal Workers (Symptoms versus No Symptoms) P-value
IL1-α median range	all<LOD	all<LOD	1.000	all<LOD	all<LOD	1.000
IL2 median range	2.04 1.34-2.40	1.75 0.90-2.33	0.269	0.98 0.06-3.16	1.10 0.41-2.37	0.702
IL4 median range	all<LOD	all<LOD	1.000	0.88 0.88-5.04	all<LOD	0.470
IL5 median range	0.17 0.17-0.34	0.17 0.15-0.51	0.396	0.17 0.04-0.73	0.21 0.04-2.26	0.408
IL6 median range	2.64 2.07-3.52	2.77 2.07-3.36	0.657	1.96 1.29-3.02	2.22 1.77-4.90	0.098
IL8 median range	21.07 2.44-61.09	24.01 8.56-413.87	0.310	14.77 2.77-1618.84	13.16 6.00-1382.81	0.602
IL10 median range	1.49 0.55-2.13	1.41 0.55-1.62	0.536	0.53 0.13-1.83	0.57 0.46-1.32	0.313
TNF-α median range	8.99 5.90-10.99	6.63 5.34-10.42	0.354	5.91 2.92-8.82	5.44 2.92-10.92	0.889
IFN-γ median range	0.35 0.16-0.94	0.48 0.16-0.76	0.894	0.16 0.04-0.86	0.16 0.04-0.47	0.320
ENA 78 median range	951.74 536.90-1419.38	844.94 593.67-2829.18	0.627	1107.48 323.55-3312.76	888.88 437.11-1669.28	0.914
RANTES median range	5317.45 4685.42-8138.12	5144.87 1995.73-6762.18	0.441	4158.43 1259.18-6122.83	4218.44 1892.14-6588.77	0.532

Table 4: Correlation between cytokine and cell surface marker concentrations for all workers

	IL2	IL6	IL10	TNF-alpha	IFN-gamma	CD14	CD54
IL2							
Spearman's	×	-	0.654	-	0.537	-	-
Kendall's	×	-	-	-	0.683	-	-
IL6							
Spearman's		×	0.662	0.642	-	-	-
Kendall's		×	-	-	-	-	-
IL10							
Spearman's			×	0.653	0.540	-	-
Kendall's			×	-	0.705	-	-
TNF-alpha							
Spearman's				×	0.525	-	-
Kendall's				×	-	-	-
IFN-gamma							
Spearman's					×	-	-
Kendall's					×	-	-
CD14							
Spearman's						×	-0.726
Kendall's						×	-0.900
CD54							
Spearman's							×
Kendall's							×

Only correlation coefficients >0.500 and significant at P<0.05 are illustrated

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Health and Safety Executive

Irritancy and sensitisation

This study aimed to document key clinical differences between irritation and sensitisation in the workplace, with a view to potentially arming the clinician with new ways to assess cases of work-related respiratory disease. Hitherto, most clinical cases would be assessed on the nature and duration of symptoms alone, or perhaps in conjunction with simple measures of lung function and IgE testing where appropriate. The study was particularly interested in determining whether irritancy or sensitisation in the workplace was associated with the immune profile of a worker. Specifically, the expression of cell surface markers on T cells and monocytes, as well as the concentration of inflammatory cytokines, were investigated.

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