

Irritancy and sensitisation

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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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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 IgG₄ 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, FEV₁ and FVC. The latter information was then used as baseline data to determine airway responsiveness in airway histamine challenge testing.

activation (CD25) markers. Normal ranges had already been established "in-house" for these markers, using a normal volunteer population of people with FEV₁/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.

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.

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
<hr/>					
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	Latex Worker n=44	Insect Worker n=32	Steel Worker n=45	Welder n=10
	No. %	No. %	No. %	No. %	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 5
Prevalence of Symptoms 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. %
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	Irritant- exposed* n=55	OR** (95% CI)
	No. %	No. %	
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	Irritant- exposed* n=55	OR** (95% CI)
	No. %	No. %	
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)

¹Allergen exposed = latex and insect workers, irritant exposed = steel workers and welders

²Odds 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

⁴Mean 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

⁴Mean 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

⁴Mean 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

⁴Mean linear fluorescence (MLF)

Table 15
Lung Function 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
	No.	No.	No.		
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	Non-sensitised n=9	Sensitised n=5	Sensitised versus Irritant	Sensitised versus non-sensitised
	No.	No.	No.		
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	Non-sensitised n=15	Sensitised n=7	Sensitised versus Irritant	Sensitised versus non-sensitised
	No.	No.	No.		
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	Non-sensitised	Sensitised	P Value
	n=10	n=9	n=5	
	No.	No.	No.	
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	Non-sensitised	Sensitised	P Value
	n=13	n=15	n=7	
	No.	No.	No.	
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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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 (FEV₁) 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 μ l of whole blood was incubated for 30 minutes at 37°C with 5 μ l 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 μ g/ml.

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

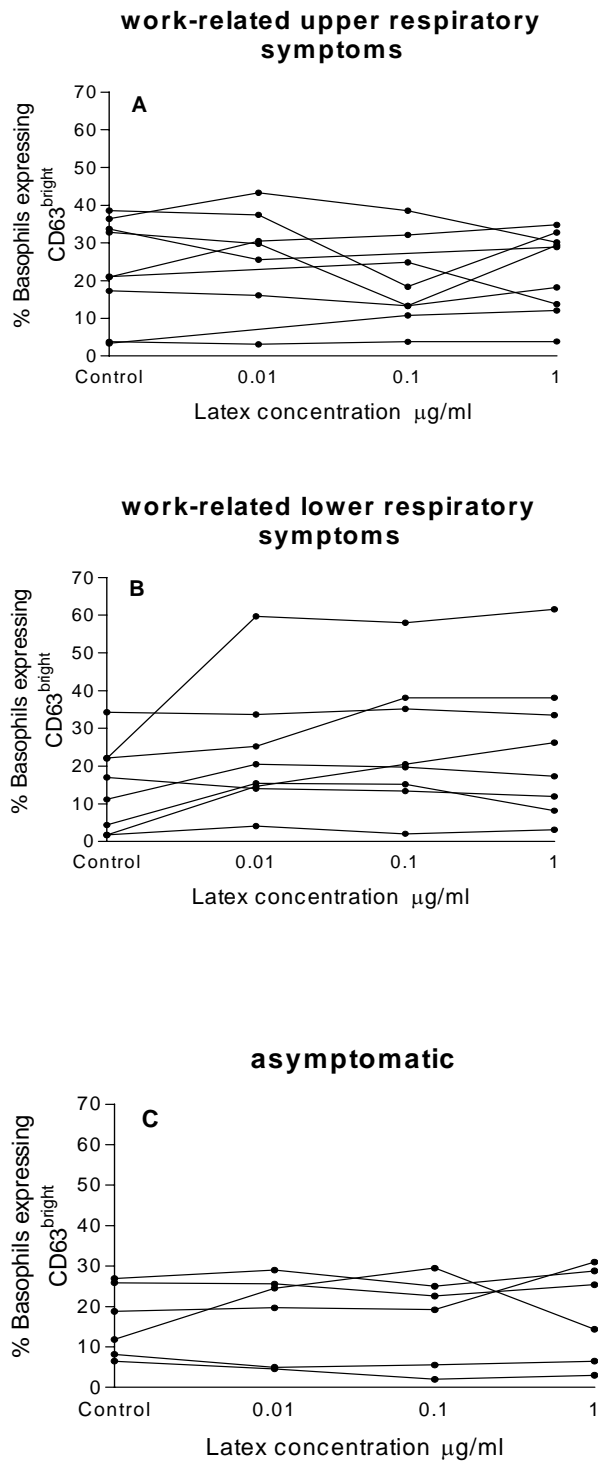
	All volunteers N=44	Basophil group N=23
Male (%)	50	44
Mean age in years (range)	37 (19-59)	41 (19-59)
Mean months employed at this worksite	61 (1-156)	67 (1-156)
Current or ex smokers (%)	73	75

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 0.1 $\mu\text{g/ml}$ 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 $\mu\text{g/ml}$ 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.

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

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/m³ (range 0.13 - 19.75). Total protein estimates for this sampling day were measured at a mean of 0.076mg/m³ (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/m³ (range 0.09-1.93). Total protein estimation for these days noted means respectively of 60.4µg/m³ (range 3.29 - 442.3) and 10.26µg/m³ (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 completed 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/m³.

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/m³.

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

